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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Task Force on Endocrine Disrupters Testing and Assessment (EDTA) of the Test Guidelines Programme

OECD Draft Report of the Validation of the Rat Uterotrophic Bioassay: Phase 2. Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories

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This document provides a description and summary of Phase-2 of the OECD validation of the rat uterotrophic bioassay. It contains the background on how the validation study was organised and performed, the standardised protocols used, a comprehensive summary of test data, and the conclusions drawn from the studies. The study was performed in two parts: the testing of single, pre-selected doses of a number of coded test chemicals, and dose-response studies of uncoded potent and weak oestrogen agonists.

The Phase-1 results of this validation study were approved by the Validation Management Group for the Screening and Testing of Endocrine Disrupters for Mammalian Effects (VMG-mam), and subsequently approved by the Task Force on Endocrine Disrupters Testing and Assessment (EDTA). The results were formally reported in ENV/JM/TG/EDTA(2001)1 and were also published in the peer-reviewed scientific literature. Following discussion and approval of the Phase-2 results of the validation program by the VMG-mam, the Phase-2 results will be submitted to the EDTA for endorsement, and recommendations for development of an OECD Test Guideline. The results of Phase-2 have also been submitted for publication in the peer-reviewed literature.

ACTION REQUIRED: ***The Validation Management Group for Mammalian Effects (VMG-mammalian) is invited to consider the summary report of the Phase-2 validation of the uterotrophic bioassay and confirm it as sufficient to support a recommendation to develop an OECD Test Guideline for the rodent uterotrophic bioassay.***

FOREWORD

This document is the report of the second phase of the work on the OECD validation of the rodent uterotrophic bioassay. The laboratory-testing portion of this phase was conducted between June 2000 and June 2001. This document was written by the OECD Secretariat. Extensive input was contributed by the Lead Laboratory, Drs. T. Inoue and J. Kanno (National Institute of Health Sciences, Japan); the independent statisticians, Drs. J. Haseman and S. Peddada (National Institute of Environmental Health Sciences, US); and members of the Mammalian Validation Management Group, notably Drs. J. Ashby (Sygenta, CTL Laboratory, UK), A. Maciorowski and G. Timm (US Environmental Protection Agency, Washington, DC), and W. Owens (Procter & Gamble, US).

This report provides a comprehensive summary of the Phase-2 testing performed by the participating laboratories, including a detailed presentation and evaluation of their results. The individual reports of the work carried out by participating laboratories, and the raw data submitted, are available directly from the Secretariat upon request.

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SUMMARY

i) This report summarises the results from an OECD inter-laboratory study conducted in 2000-2001 to demonstrate that the uterotrophic bioassay can reliably detect strong and weak oestrogens, to demonstrate the transferability of standardised protocols amongst laboratories, and to quantitate the inter- and intralaboratory reproducibility of the assay. The uterotrophic bioassay was one of three *in vivo* assays selected for validation by the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA), which was established to develop and validate new and improved methods to identify and to assess substances acting through endocrine mechanisms (1).

ii) The principle of the rodent uterotrophic bioassay is that the growth phase of the uterus in the natural estrous cycle is under the control of oestrogens that are necessary to stimulate and maintain growth of the uterine tissues. This growth in the natural estrous cycle is rapid and easily measurable within two days. When the endogenous source of oestrogen is not available, either because of immaturity of the animals or because the animals have been ovariectomised, then the animal becomes sensitive to an exogenous source of oestrogen to initiate or restore growth of the uterus. Chemicals that act as oestrogen agonists may then be identified, if they cause a statistically significant increase in the weights of the oestrogen-dependent uterine tissues, or as an oestrogen antagonist if they decrease the uterine growth response when co-administered with a potent reference oestrogen.

iii) The first phase (Phase-1) of the validation of the rodent uterotrophic bioassay involved 19 laboratories and measured the animals' responses to a potent reference oestrogen, 17α-ethinyl oestradiol. Despite differences in rat strain used and different levels of experience among the participating laboratories, there was acceptable agreement among laboratories with respect to the magnitudes of the responses at the different dose levels and the doses at which significant responses were obtained with two versions of the uterotrophic bioassay using the intact, immature rat and the young adult ovariectomised rat, respectively (10)(11).

iv) This report represents the second phase of the validation of the rodent uterotrophic bioassay and involved 20 laboratories from Denmark, France, Germany, Italy, Japan, Korea, U.K., and the U.S. Laboratories from both the public and private sectors participated in the work. The participating laboratories and principal investigators are identified in Annex 1. The lead laboratory for this Phase-2 validation study was the National Institute of Health Sciences, Tokyo, Japan, with statistical support provided by the U.S. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

v) The Phase-2 work involved the testing of seven test substances. The potent oestrogen, 17α-ethinyl oestradiol (hereafter, EE), was again used as the reference agonist for the Phase-2 study. In Phase-2, five weak oestrogen agonists that were three or more orders of magnitude lower in oestrogen receptor binding affinity than EE (bisphenol A (BPA), *o,p'*-DDT (DDT), genistein (GEN), methoxychlor (MC), and nonylphenol (NP)) were tested in dose-response experiments and as coded chemicals in coded single-dose experiments. The coded single-dose experiments also included one chemical (dibutyl phthalate) considered to be negative and to have no oestrogenic binding affinity or biological activity in well-designed and well-conducted studies.

vi) Four protocol versions of the uterotrophic bioassay were used: intact, immature rats treated by oral gavage (Protocol A) and sub-cutaneous injection with a dosing regimen of three consecutive days (Protocol B), and mature, ovariectomised (OVX) rats treated by subcutaneous injection using two different dosing regimens of three (Protocol C) and seven consecutive days (Protocol D), respectively. Responses consistent with an oestrogen mode of action by the test chemicals were measured as a statistically significant increase in uterine weight versus vehicle treated controls. The weights of uterine

containing luminal fluid (wet weight) and with the luminal fluid removed (blotted weight) were examined to determine which procedure provided the most reliable data, or whether either approach could be used. The following approaches were used to investigate the reliability and sensitivity of these protocols:

- the reference positive control chemical, EE, was tested at low and high doses in both the coded single-dose (not under code) and dose-response studies to evaluate the inter-laboratory reproducibility in this Phase, and provide data for comparison with the Phase-1 results from this chemical in the same laboratories.
- a dose-response study used the five weak oestrogen agonists;
- a coded single-dose study used the same chemicals in the dose-response study and a non-oestrogenic chemical. The dose selected for each chemical in the coded single-dose study was also used in the dose-response study to make direct comparisons possible; and
- the chemicals were coded in the coded single-dose study so that the laboratories would not know the identities of the chemicals.

vii) Specific goals of Phase-2 were to:

- evaluate the reproducibility of the protocols for identifying weak oestrogen agonists;
- compare different routes of exposure;
- compare immature and OVX rats;
- determine if the laboratories in a blind study could distinguish between weak oestrogens and a chemical considered to be non-oestrogenic (dibutyl phthalate);
- evaluate the inter- and intra-laboratory variations using the EE responses from both Phase-1 and Phase-2;
- evaluate the inter- and intra-laboratory variations using the weak agonists responses from both the coded single-dose approach and the equivalent dose from the dose-response experiments in Phase-2;
- characterize protocol variability at high and low doses of weak agonist;
- thereby, provide an overall data set and report to support the reliability and relevance of the uterotrophic bioassay as a robust method for the detection of chemicals that may act like oestrogen agonists and antagonists and, consequently, may have the potential to interfere with endogenous female hormones; and
- therefore, recommend the development of an OECD Test Guideline for the rodent uterotrophic bioassay.

vii) A total of 17 laboratories participated in the dose-response study. Several laboratories reported animal deaths during the course of the experiment, primarily with Protocol A, where the immature animals are most vulnerable just after weaning. Similar rates of toxicity were observed in both the dose-response and the coded single-dose experiments with the same chemicals. The greatest rate of toxicity was observed with NP, followed by *o,p'*-DDT. In several instances, two or more animals within a group died, which would reduce the power of the bioassay. These technical findings and animal welfare concerns suggest that range finding studies be considered in the future to avoid overt toxicity, particularly with the immature animals.

viii) All Protocols were able to detect each of the five weak oestrogen agonists provided that

sufficient doses were administered. The minimal effective doses or MED (the lowest dose concentration in a series achieving statistical significance) for the five weak agonists were substantially higher than for the potent reference EE. In Phase-1, the MED for EE in Protocol A by oral gavage was 0.3-1.0 μg/kg/d and in Protocols B, C, and D by sc injection was 0.1-0.3 μg/kg/d. In Phase-2, the MEDs of the weak agonists by oral gavage ranged from < 20 mg/kg/d for MC to 600 mg/kg/d for BPA; the latter MED being approximately 600,000-fold higher than for EE by the same route of exposure. This demonstrates the capability of the uterotrophic bioassay to detect oestrogen agonists over a substantial concentration range. Furthermore, this range should be sufficient to encompass all substances of interest to the regulatory community.

ix) Overall, no animal model (i.e., immature or OVX), protocol or route of administration was clearly and consistently superior. The specific characteristics of the individual chemical were equally or more important. For BPA, Protocol C appeared to be somewhat more sensitive than B. However, with other chemicals, such as GEN and MC, Protocol B seemed somewhat more sensitive. Similarly, oral administration in Protocol A appeared to achieve higher and earlier responses with MC and DDT, subcutaneous administration achieved higher and earlier responses with GEN and BPA, and subcutaneous administration was only modestly better with NP. Extending administration to seven consecutive days (Protocol D) showed no significant advantage over the 3-day treatment. While increasing the relative response slightly with BPA, no advantage of extending dosing in the magnitude of the response or the MED was obvious with the other weak agonists. In addition, the seven-day treatment protocol would have a modest disadvantage of higher costs as a result of the additional dosing and additional animal maintenance time. The seven-day treatment protocol cannot be recommended for routine use, but may be useful for chemicals that require longer dosing times to reach effective body burden concentrations or to induce specific metabolic enzymes.

ix) A total of 86 chemical/laboratory/protocol dose-response combinations were performed. Three approaches were used to present and to analyse the data: a) an analysis of the dose response of the individual agonists, b) an analysis of the two reference EE doses generated in conjunction with the dose response experiments, and c) a comparison of the minimal effective dose achieving statistical significance within each protocol and dose series. For each of these approaches, there was good agreement among laboratories, and across protocols. The magnitude and shape of the dose response curve for each of the five individual weak agonists was similar within a Protocol. The response to the EE reference doses was also similar within a Protocol. An analysis of the MED doses (the lowest dose concentration in a series achieving statistical significance) reinforces the conclusion of agreement and reproducibility among the laboratories within a Protocol. In several protocols, there was no difference observed in the MED within a 3- to 4-fold range despite differences in rat strains, diets, and other variables. No protocol or route of administration was clearly superior in the dose response experiments. The specific characteristics of the individual test substances were equally or more important. For example, Protocol C appeared to be somewhat more sensitive than Protocol B with BPA, but Protocol B somewhat more sensitive with GEN and MC. Similarly, oral administration in Protocol A appeared to achieve higher and earlier responses with MC and DDT, subcutaneous administration achieved higher and earlier responses with GEN and BPA, and subcutaneous administration was only modestly better with NP.

ix) A total of 16 laboratories participated in the coded single-dose studies. The single-doses were selected based on presumed positions in a dose-response curve. In several cases, these judgments were not accurate and some selected doses were at or near the MED in the low region of the dose response curve. It would then be anticipated that some laboratories would not achieve statistical significance under these conditions, and this was the case. However, there was consistent agreement of the repeatability of the relative increase at the selected dose within the same protocol across laboratories in the coded single-dose studies. The putative non-oestrogen was marginally positive in three out of 36 studies (laboratories and protocol variations) in which it was run, indicating a possible false positive rate of about 8%.

x) Intra- and inter-laboratory reproducibility were assessed in four ways: the proportion of assays achieving statistical significance when considering the position of the selected dose on the dose response curve; analysis as pooled data across all laboratories in a global approach using linear mixed effect models for a given chemical and protocol; a restricted comparison to only those laboratories performing experiments on the same chemical in given protocol; and an examination of the EE data from Phase-1 and both the dose response and coded single-dose response of Phase-2. Each approach shows that the relative increase in uterine weights within and across laboratories were reproducible both with all five weak agonists and with the reference EE, taking into account that the magnitude of the relative increase was dose dependent.

xi) Several possible sources of variability were identified. Most are common to both the immature and the OVX models, but a few are unique to one or the other model. The foremost variable appears to be the expertise and care within a laboratory. An initial examination of vehicle control mean blotted weights and coefficients of variation points to a laboratory effect and leads to the recommendation for a second round of statistical analysis. The objective of the additional analyses would be to determine by a more thorough examination if there is a major effect on variability due to the laboratory, any difference between the immature and the OVX models, or any difference between Protocols.

An analysis of the phytoestrogen contents of the laboratory diets revealed significant levels in many diets. A review of food consumption indicates that this would lead to different dietary intakes on an approximate ratio for OVX adult rats:immature rats:OVX adult mice of 1:2:4. An examination of the vehicle control weights and the responses to the weak agonists in different laboratories was made relative to an estimated dietary intake of phytoestrogens. The data indicated that no effect was evident for the adult OVX model. However, the data were suggestive of an effect for the immature rat model when genistein intakes would exceed 50 mg/kg/d. This level is consistent with other toxicological studies showing a LOEL in this range as well as the MED values in this study when genistein was administered by oral gavage. However, the interpretation that an influence of dietary phytoestrogens relies on the results from a single laboratory. A close examination of those data reveals that these data are open to question, and any conclusions must be drawn with caution until controlled studies are done with defined diets, defined doses, and sufficient doses of phytoestrogens. However, as a precaution until such data are available, experiments with immature rats or OVX mice should limit the dietary content of phytoestrogens to about 350 μg phytoestrogens/g diet and 175 μg phytoestrogens/g diet, respectively.

xii) It can then be concluded from this second phase of the validation study that the protocols are robust and reliable for identifying oestrogen agonists and antagonists and are transferable across laboratories.

INTRODUCTION

1. In 1997, the OECD concluded that existing Test Guidelines were insufficient to identify certain endocrine mechanisms (oestrogen, androgen, and thyroid) and might not be adequate to fully characterize the hazards of these mechanisms. Therefore, a *Special Activity on the Testing and Assessment of Endocrine Disrupters* was initiated as part of the OECD Test Guidelines Programme. The purpose of this activity was to revise existing Guidelines and to develop new OECD Test Guidelines in order to, first, screen chemicals in order to identify substances that could interact with the endocrine system and, second, to ensure that tests could characterize their hazards (Further information concerning the OECD Endocrine Disruptor program can be found at http://www.oecd.org/EN/document/0,,EN-document-524-nodirectorate-no-24-6685-8,00.html An OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) was then established to provide a focal point within the OECD to identify and recommend priorities for the development and validation of new and improved methods to identify and to assess substances acting through endocrine mechanisms (1).

2. Beginning with the final report of the US-EPA Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC), the rodent uterotrophic bioassay has been proposed for use as a screen for identifying potential oestrogenic or anti-oestrogenic substances. The uterotrophic bioassay was given high priority for validation by several expert panels and workshops as a mechanistic screen for substances that would act through an oestrogenic or antioestrogenic mode of action (2)(3)(4)(5). EDSTAC and other panels recognised that although the uterotrophic bioassay has been in use since the 1930s for pharmaceutical discovery and evaluation of oestrogens, the bioassay would have to be validated for its use as a mechanistic screen for weak oestrogens.

3. The biologic principle underlying the rodent uterotrophic bioassay is that the growth phase of the uterus is under the control of oestrogens in the natural oestrous cycle. That is, oestrogen is necessary to stimulate and maintain growth of the uterus. Further, this growth in the natural oestrous cycle is rapid, occurring within two days and is easily and quantitatively measurable. When the endogenous source of this hormone is not available, either because of immaturity of the animals or because the animals have been ovariectomised, then the animal becomes sensitive an exogenous source of oestrogen to initiate or restore growth of the uterus. Chemicals that act as oestrogen agonists may then be identified, if they cause an increase in the weights of the oestrogen-dependent tissues, or as an oestrogen antagonist, if they decrease the uterine growth response when co-administered with a potent reference oestrogen. The results from the uterotrophic bioassay would be used in a weight of evidence assessment, along with results from other assays, if available, and other information about the test substance, to determine whether the substance should be tested further in other specific or definitive tests or that sufficient information is available for hazard assessment purposes. The uterotrophic bioassay is included in the OECD conceptual framework for endocrine disrupter testing and assessment as agreed by EDTA6 in June, 2002 (6).

4. The need to validate the rodent uterotrophic bioassay arises from the concerns that exist that ambient levels of natural and industrial chemicals may interact with the endocrine system and as a consequence possibly elicit reproductive, developmental, and other adverse effects in humans and wildlife (7)(8)(9)(10)(11)(12)(13)(14)(15)(16)(17)(18)(19).

5. The OECD initiative to develop and validate *in vitro* and *in vivo* assays for the detection of chemicals that may interfere with the endocrine response is based the recommendations of a number of national, regional and international workshops (2)(3)(4)(5)(20)(21) and followed a detailed OECD review of the status of both existing regulatory assays and available research methodologies (22).

6. The objective of the OECD efforts with the uterotrophic bioassay is to develop and validate a test protocol, or series of protocols, to support the development of a Test Guideline for the detection of

chemicals having the potential to act like endogenous oestrogen in rodents. The Test Guideline, once available, is intended to be used as part of an overall testing strategy for the detection and assessment of potential endocrine disrupters. The history and prior use of the uterotrophic bioassay have been previously reviewed (23)(24). For the validation program, a thorough and extensive review of the literature on the uterotrophic bioassay was performed to generate a supporting background review document. This document also examined the chemicals recommended for used in the validation study, and provided information that was useful for determining the doses to be used in the validation study. The Background Review Document was circulated to the VMG-mam for review in July, 2001. The final version, that addressed all comments received, was made available in January, 2003 (25). An abbreviated version of the document has been published in the peer-reviewed literature (26).

7. The first stage of the OECD validation study of the rodent uterotrophic bioassay, in which 19 laboratories in Europe, Japan, Korea, and the United States participated, was completed in January 2000 (27). A manuscript summarising the results of this study has been published in the peer-reviewed literature (28).

8. At its second meeting in January 2000, the OECD Validation Management Group on Screening and Testing for Endocrine Disrupters for Mammalian Effects (VMG-mam) agreed that sufficient information had been obtained in the first phase of the validation study to confirm the reliability of the different protocol options (29)(30). The results showed that the protocols were robust and produced comparable results among laboratories for the reference oestrogen 17α-ethinyl oestradiol (EE). Phase-1 also demonstrated that the protocol could be used for the detection of potent, reference oestrogen antagonist, ZM 198.154.

9. The protocols used for Phase-2 of the validation study include minor modifications that were recommended by the VMG-mam based on the results of the Phase-1 study and subsequently endorsed by EDTA 5 (29)(30). The first modifications was an increase in the age range of the immature animals so that they are a minimum of 18 days old and a maximum of 20 days old at the start of treatment (with the day of birth as day 0 to be consistent with OECD Test Guidelines). The second modification was a longer period of acclimatisation of the mature rats following ovariectomy from a minimum of 7 days used in Phase-1 to a minimum of 14 days.

10. The four protocol options used in Phase-1 were retained by the VMG-mam for Phase-2 of the validation study are:

- Protocol A. Intact (unovariectomised), sexually immature animals treated by gavage (three consecutive daily doses followed by necropsy 24 hours after the last administration).
- Protocol B. Intact, immature animals treated subcutaneously (three consecutive daily doses followed by necropsy 24 hours after the last administration).
- Protocol C. Ovariectomised (OVX) animals treated subcutaneously (three consecutive daily doses followed by necropsy 24 hours after the last administration).
- Protocol D. OVX animals treated sub-cutaneously (seven consecutive daily doses followed by necropsy 24 hours after the last administration).

11. The protocols are described in more detail in following sections of this document, and the full text of the model protocols used by the laboratories are contained in Annex 2.

12. The Phase-2 validation studies were designed to determine whether the four protocol options of the bioassay would be sufficiently robust to detect a variety of test substances with weak oestrogen

activities (relevance of the assay) and whether the same degree of reproducibility that was demonstrated with the strong agonist EE in Phase-1, would be found when testing substances with lower oestrogenic potencies (reliability of the assay). Consequently, the VMG-mam selected five weak oestrogen agonists and a chemical not expected to have any oestrogenic effects (negative control). The test chemicals selected for use and their CASR-numbers are in Table 1, and their structures are in Figure 1. The five oestrogen agonists (bisphenol A, *o,p'*-DDT, genistein, methoxychlor, and nonylphenol) were three or more orders of magnitude lower in rat uterine oestrogen receptor binding affinity than EE as reported in a single laboratory with a standardized protocol (33)(34).

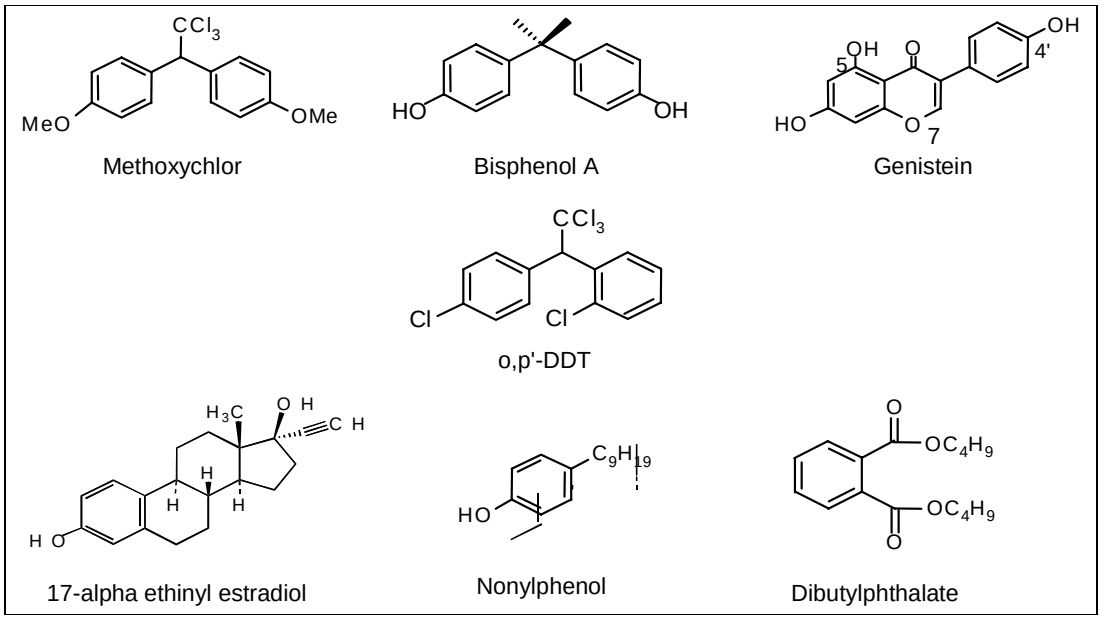
Table 1. Chemicals used in Phase-2 of the OECD validation of the rodent uterotrophic bioassay

Test Chemical	**CASRN**	**Oestrogenic Activity**
Bisphenol A (BPA)	80-05-7	Weak agonist
o,p'-DDT (DDT)	789-02-6	Weak agonist
Dibutyl phthalate (DBP)[1]	84-74-2	Non-oestrogenic
17α-Ethinyl oestradiol (EE)[2]	57-63-6	Potent reference agonist
Genistein (GEN)	446-72-0	Weak agonist
Methoxychlor (MC)	72-43-5	Weak agonist
Nonylphenol (NP)	25154-52-3	Weak agonist

[1] DBP was tested only in the coded single-dose procedures.

[2] EE was used as a positive control chemical in both the dose-response and coded single-dose procedures, and was tested at a high and low dose in each.

Figure 1. Structures of the Tested Chemicals



OBJECTIVES

13. The overall goal of the Phase-2 test validation study was to assess the intra- and inter-laboratory reproducibility of the uterotrophic bioassay in response to chemicals considered to be weak oestrogen agonists.

14. Specific goals of Phase-2 were to:

- evaluate the reproducibility of the OECD protocol for identifying weak oestrogen agonists;
- compare the increases in uterine weights induced by weak oestrogens when administered by different routes of exposure to immature and OVX rats;
- determine if the laboratories in a blind study could distinguish between weak oestrogens and a chemical considered to be non-oestrogenic (dibutyl phthalate) based on its lack of binding to oestrogen receptors *in vitro*, and an absence of effects in well-designed and well-conducted bioassays with sensitive life stages and oestrogen sensitive endpoints;
- compare the variability of the EE responses within the same laboratory, and with the results obtained in Phase-1;
- evaluate the variability within a laboratory by comparing the responses obtained from screening weak oestrogens in the coded single-dose approach and the equivalent dose from the dose-response experiment;
- provide information on the responses of the protocol variations at high and low doses of agonist; and
- identify potential refinements to the protocol to enhance its ability to identify substances of unknown oestrogenic activity; and,
- provide data to support a recommendation for the development of an OECD Test Guideline for the rodent uterotrophic bioassay.

15. In order to assess inter-laboratory variability, it was recommended that at least two laboratories test each chemical in each protocol variation. The following approaches were used to investigate the reliability and sensitivity of the protocols:

- the reference positive control chemical, EE, was tested at low and high doses in both the single-dose (not under code) and dose-response studies to evaluate the inter-laboratory reproducibility in this Phase, and provide data for comparison with the Phase-1 results from this chemical in the same laboratories.
- a dose-response study used five weak oestrogens;
- a coded single-dose study used the same chemicals as the dose-response study and a non-oestrogenic chemical. The dose selected for each chemical in the coded single-dose study was also used in the dose-response study to make direct comparisons possible; and
- the chemicals were coded in the coded single-dose study so that the laboratories would not know the identities of the chemicals.

16. Progress reports on the validation of the uterotrophic bioassay were presented and discussed at the fourth and fifth meetings of the Task Force on Endocrine Disrupter Testing and Assessment (31)(32).

TEST VALIDATION

17. *Validation* is a term that refers to the scientific process designed to characterise the operational characteristics and limitations of a test method, and to demonstrate its reliability and relevance for a particular purpose.

18. The Report of the OECD Workshop on Harmonisation of Validation and Acceptance Criteria for Alternative Test Methods (Solna Report) (35) provides the principles of test validation that are followed by OECD. Further practical guidance for the application of the validation and regulatory acceptance principles and criteria were discussed and agreed to by the Stockholm Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment (36). These principles are currently being incorporated into a revised OECD Guidance Document for the Preparation of Test Guidelines (Guidance Document No. 34). The OECD principles are consistent with approaches used in Europe, particularly those of the European Centre for Validation of Alternative Methods (ECVAM) (37) and are consistent with the approaches used in the US as stated by the Interagency Co-ordinating Committee on Validation of Alternative Methods (ICCVAM) (38).

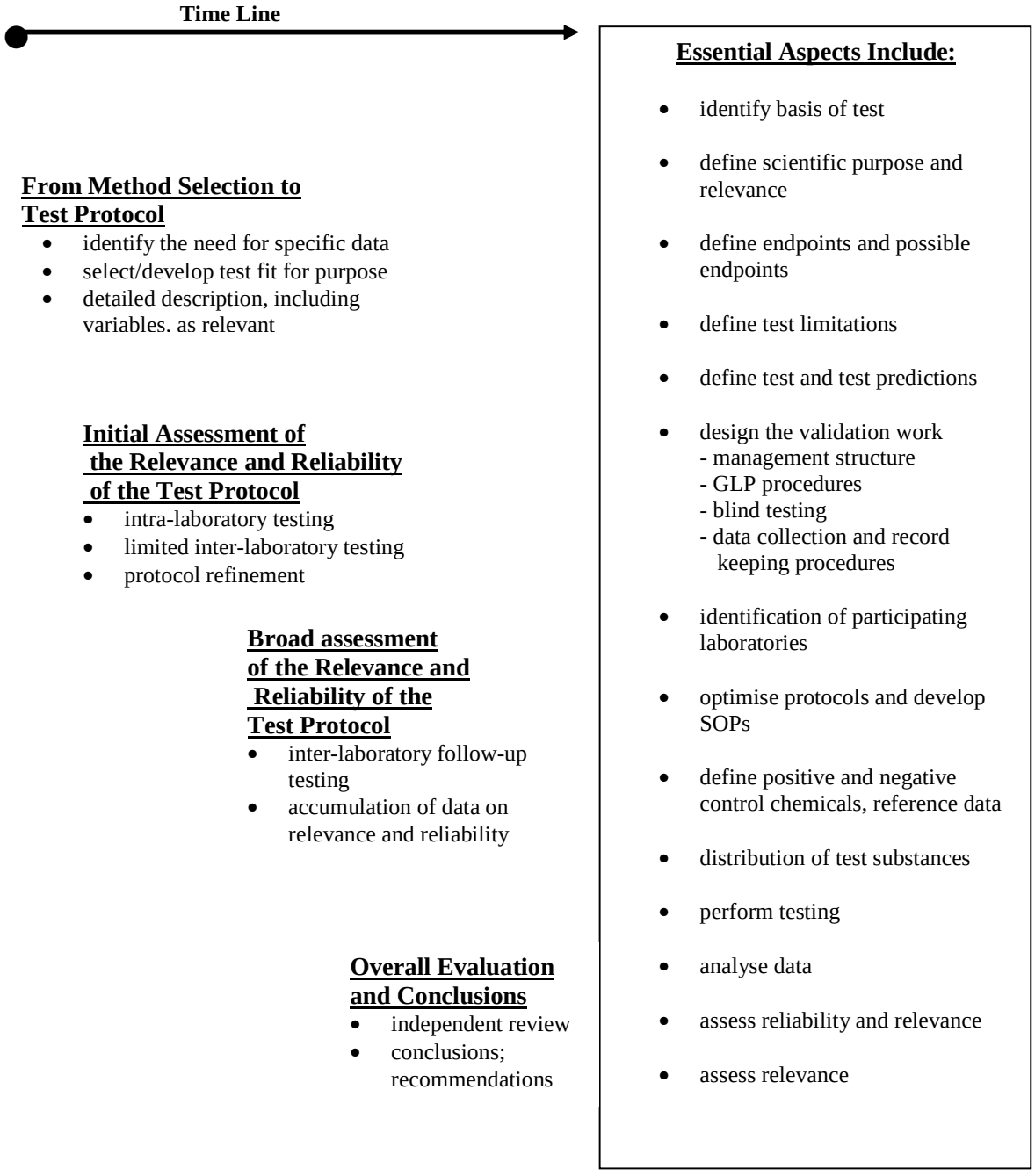
19. In 1998, the Joint Meeting of the OECD Chemicals Group and Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) decided that the criteria and approaches for the validation of test methods should apply equally to the development of all toxicology tests *in vitro* and *in vivo*, and to tests for ecotoxicological effects. The Joint Meeting agreed that flexibility should be shown in designing validation studies so that they would be appropriate for the specific test and its proposed purpose. Most importantly, all decisions on the extent and design of the validation study should be fully transparent and documented.

20. Rodent models to measure the response of the uterus in OVX or immature rodents to administered oestrogens have been in use since 1935 (39). There have been a wide number of protocol variations reported, which vary with regard to whether sexually immature or OVX rats or mice are used, the dosing regimen, and the tissues examined (25)(26). The rodent uterotrophic bioassay has traditionally been accepted by testing laboratories, industry, and regulatory authorities for measuring oestrogenic and anti-oestrogenic effects of pharmaceuticals.

21. For the uterotrophic bioassay, the VMG-mam recommended that the OECD validation procedures be performed in phases, taking into consideration the long use of the assay and its many variants. The first phase of the validation procedure would be to define a protocol that would be expected to identify potent oestrogenic and anti-oestrogenic substances. The second phase would be to measure the assay's intra- and inter-laboratory reproducibility with a variety of potent and weakly acting substances, and to determine the relative effectiveness of four protocol variations for measuring the effects. This approach is represented in Figure 2, which shows how the assessment process of the relevance and reliability of a test method can be undertaken in a stepwise, yet flexible, manner while still providing the information necessary to address the Solna criteria and principles.

22. This report of the second phase of the OECD validation of the rodent uterotrophic bioassay provides the data needed for determining its usefulness for the identification of oestrogenic and anti-oestrogenic substances among chemicals of interest and in the environment. This report, together with the report of Phase-1 (27) and the Detailed Background Review Document (25), are considered adequate justification for the method to be developed as an OECD Test Guideline. However, the development of an OECD Rodent Uterotrophic bioassay Test Guideline will only commence upon approval and affirmation of the results of this Phase-2 study, and a recommendation by the EDTA and the National Co-ordinators. Simultaneously, the Phase-2 studies are being published in the peer-reviewed literature (40)(41)(42).

Figure 2. Assessment Process of the Relevance and Reliability of New or Significantly Revised Testing Methods for Hazard Characterisation



TEST PROTOCOLS USED

23. The test protocols used for the dose-response and coded single-dose experiments were the same as those used for Phase-1 of the study, with minor changes. The model protocols used are in Annex 2. The participation of the laboratories in the coded single-dose and dose response studies, the protocols the laboratories performed, and the actual chemicals tested in each protocol by each laboratory are summarised in Table 2.

Table 2. Studies performed, protocols used, and chemicals tested by each laboratory in Phase-2

Lab. #[1]	Coded Single-Dose Studies*					Dose-Response Studies**				
Protocol	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
1	●	●	●	●		GEN; MC	GEN; MC	GEN; MC	GEN; MC	
2	●	●	●	●		BPA	BPA	BPA	BPA	
3	●	●	●	●		DDT; MC	DDT; MC	DDT; MC	DDT; MC	
4	●	●				NP	NP			
5	●	●				DDT	DDT			
6							BPA; NP	BPA; NP		
7						BPA; NP	BPA; NP	BPA; NP	BPA; NP	
8	●	●	●			GEN	GEN; BPA; NP	BPA; NP		
9						GEN; NP	GEN; NP	GEN; NP	GEN; NP	
11	●	●	●	●		DDT	DDT	DDT	DDT	
12	●	●	●		●	BPA; DDT; GEN; MC; NP	BPA; DDT; GEN; MC; NP	BPA; DDT; GEN; MC; NP		BPA; DDT; GEN; MC; NP
13	●	●				BPA	BPA			
14	●	●				MC	MC			
15							BPA; NP			
16		●								
17		●[2]								
18		●					BPA; NP			
19		●[3]	●[3]							
20		●					BPA; NP			
21		●					BPA; NP			
Totals	**10**	**16**	**7**	**4**	**1**	**12**	**17**	**9**	**6**	**1**

* Chemicals tested were, BPA, DDT, DBP, EE (2 doses), GEN, MC, and NP. All chemicals in the single-dose studies, except EE, were tested under code.

** In addition to the stated chemicals, two doses of EE were used as the positive reference control in these studies.

[1] For consistency, laboratories are numbered according to the sequence used in statistical reports for Phase-1 and literature publications for Phase-1 and Phase-2 (11)(32)(33)(34).

[2] GEN was not tested in Protocol B by Laboratory #17 due to formulation of dose preparation issues.

[3] Due to formulation of dose preparation issues, BP and GEN were not tested in Protocol B; GEN and MC were not tested in Protocol C by Laboratory #19.

24. Protocol A. Immature (18-20 day old) female rats were treated by oral gavage once daily, for 3 consecutive days. The animals were weighed and sacrificed 24 hrs after the final dose, and the uteri were removed. Wet weights (uteri with luminal contents) were obtained, after which the uterine wall was pierced and the luminal contents were expressed, and the uteri were blotted and weighed again.

25. Protocol B. Immature (18-20 day old) female rats were treated by subcutaneous (s.c.) injection once daily, for 3 consecutive days. The animals were weighed and sacrificed 24 hrs after the final dose, and the uteri were removed. Wet weights (uteri with luminal contents) were obtained, after which uterine wall was pierced and the luminal contents were expressed, and the uteri were blotted and weighed again.

26. Protocol C. Adult (6 weeks, or older) female rats were ovariectomised (OVX), and allowed to recover for 14-28 days. They were then treated by s.c. injection once daily, for 3 consecutive days. The animals were weighed and sacrificed 24 hrs after the final dose, and the uteri were removed. Wet weights (uteri with luminal contents) were obtained, after which the uterine wall was pierced and the luminal contents were expressed, and the uteri were blotted and weighed again.

27. Protocol D. In addition to performing Protocol C, a number of laboratories used a modified protocol wherein 7 consecutive daily s.c. injections were administered prior to animal sacrifice. Four laboratories (Laboratories # 1, 2, 3, 11) used Protocol D in the coded single-dose experiments, and 6 (Laboratories #1, 2, 3,7, 11) used it in the dose-response experiments.

28. One laboratory (Laboratory #12) added an additional protocol modification, and exposed the mature, OVX rats to the test chemicals by oral gavage (3 consecutive daily doses). This was done in addition to Protocol C. Although this protocol variation was not developed by the VMG-mam and the Lead Laboratory, the results are included in this report for comparison with the other protocol variations. This protocol variation is designated as Protocol E.

29. Immature animals were to be received from the supplier with dams or foster dams on approximately post-natal day (pnd) 14, or as weanlings on pnd 17, and reacclimatized (the day of birth was counted as day 0). Animals were randomly assigned to treatment groups of six animals so that all groups of animals had mean weights within ± 5%, and treatment was by a randomised block approach, rather than by complete randomisation. Chemical dosing was to begin on pnd 18, 19, or 20. All laboratories, except one, used group housing, 2-6 animals per cage, with most having 3 animals per cage.

30. Animals were ovariectomised at 42-56 days of age, and allowed to recover for a minimum of 14 days before dosing in order to allow the uterus weight to regress. Animals were randomly assigned to treatment groups of six animals each so that all groups of animals had mean weights within ± 5%.

31. Twenty-four hours after the last treatment, the animals were humanely killed using the method routinely used by the participating laboratory. The animals were humanely killed in the same sequence as the test substance was administered. The uterus and cervix were removed by incision at the vaginal fornix in order to preserve the luminal fluid contents. The uterus was trimmed of fascia and fat with care to avoid loss of luminal contents. Ovaries were removed in the case of the intact, immature females. The laboratories were also instructed to take care to avoid desiccation of the small organs. The uterus and cervix, including the luminal fluid contents, was weighed to the nearest 0.1 mg, and this wet weight recorded. Each uterus was then opened by piercing or by a longitudinal cut into each uterine horn wall, and the luminal fluid was expressed using moistened filter paper and gentle pressure. The uteri were then reweighed to the nearest 0.1 mg, and this blotted weight recorded.

SELECTION OF CHEMICALS AND DOSES TO BE TESTED

Background

32. Phase-1 of the validation study demonstrated the reliability of the protocol(s) for evaluating the uterotrophic response of the reference agonist 17α-ethinyl oestradiol (EE; CASRN 57-63-6) (27)(28).

Phase-2 of the validation study was initiated to evaluate the performance and standardised protocol(s) using test substances of three or more orders of magnitude lesser oestrogenic potency than EE, as measured by their receptor-binding affinities and prior knowledge of their uterotrophic effects.

33. A working group was established to review the scientific literature and contact researchers familiar with the assay, and to develop a list of test chemicals to be used in Phase-2. The test substances proposed, the principles behind the dose selection, and the recommended doses themselves, were subsequently discussed, and agreed upon, by the Validation Management Group for the Screening and Testing of Endocrine Disrupters (VMG-mam) in two teleconferences in July 2000.

34. The document, *Background Review of the Uterotrophic Assay: Summary of the Available Literature in Support of the OECD Programme to Standardise and Validate the Uterotrophic Assay* (25) was prepared in support of the validation study. This document contains an extensive review of scientific literature on each of the test substances, including *in vitro* assays of the test substances, summaries of prior uterotrophic test results for the various chemicals, and summaries of prior tests using sensitive life stages and oestrogen-sensitive endpoints.

35. Validation is based on the quantification of an assay's correct identification of positives and negatives or sensitivity and specificity, respectively. Sensitivity is defined as the proportion of active or positive chemicals correctly classified as positive. Specificity is defined as the proportion of inactive or negative substances correctly identified as negative substance (35)(38). To address specificity, the VMG agreed to include dibutyl phthalate as the negative test substance based on two lines of evidence. First, DBP does not display binding affinity for the rat uterine oestrogen receptor, i.e., there is no displacement of bound [^{3}H]17β-estradiol at concentrations up to 1 mM concentrations *in vitro* (33). Second, *in vivo* toxicological studies, with some including gene activation profiles, indicate that DBP does not elicit responses indicative of an oestrogen mode of action (43)(44)(45)(46)(47)(48). The VMG judged that a data set for a single negative chemical that included data for all four standardised protocols was adequate in order to conserve resources and animals.

Selection of test substances

36. The following criteria were used to select weak agonist test substances for the Phase-2 studies:

- the chemical binds to oestrogen receptors *in vitro*;
- the chemical has shown oestrogenic activity in other *in vitro* test systems;
- the chemical shown positive responses in an *in vivo* uterotrophic bioassay prior to the OECD validation program;
- information was available about the pharmacodynamic and pharmacokinetic behaviour of the test substance or structurally related substances; and
- the chemical produced oestrogenic responses in subchronic or chronic *in vivo* test systems that could be used to assess the predictive capacity of the uterotrophic bioassay, e.g., a comparison of the LOEL observed for an oestrogenic response in a subchronic or chronic test with the minimal effective dose observed in the uterotrophic bioassay.

37. Another practical criterion was that the test substances be available commercially or from chemical synthesis houses, so that sufficient quantities could be obtained for the centralised chemical repository.

38. Based on these criteria, as noted in previous sections, the five weak oestrogen agonists selected were methoxychlor (MC), bisphenol A (BPA), genistein (GEN), *o,p'*-DDT (DDT), and nonylphenol (NP). The negative substance selected was dibutyl phthalate (DBP). The positive reference substance, EE, was continued from Phase-1 to further address the reproducibility of the protocol(s) over time. The chemicals selected are listed in Table 1, and their structures are shown in Figure 1.

Dose selection

39. The published and unpublished information relevant to the appropriate doses to be used for the test chemicals varies in quantity and quality with respect to the individual chemicals, animal models, and routes of administration. The available information often did not adequately describe the protocols used to generate the data, and the statistical significance of the results frequently was not stated.

40. Because of these factors, it was not possible to confidently identify the doses that would best cover the full uterine weight response, or the lowest dose causing a response. Therefore, the approach taken was to select the information believed to be most reliable and consistent from the literature sources. Data on *in vitro* receptor binding affinity (RBA) was not used in the setting of doses, because the binding affinity does not integrate pharmacokinetic considerations. Caution was also taken to review the available literature in order to avoid selecting dose levels that approached the LD_{50} or the Maximal Tolerated Dose (MTD), as these doses could result in morbidity and/or mortality of the test animals and a reduction in the size of the animal groups. As judged appropriate, an upper limit doses of 1000 mg/kg was selected for consistency with other OECD Test Guidelines.

41. Different doses were selected for the oral and s.c. studies, and these are summarised in Tables 3 and 4, respectively. The selection of test doses for the dose-response approach was based on an effort to approximate the full dose-response curve, e.g., a No Effect Level (NOEL), an ED_{20}, ED_{50}, ED_{80}, and ED_{100} or maximum plateau. Apart from DBP, which was not included in the dose-response experiments, the same chemicals were tested using both routes of administration.

42. Two doses were selected for EE, based on the Phase-1 results. The first dose was to be in the lower portion of the ascending part of the dose response curve (~ED_{20}), and the second dose was to be near the maximum of the dose response curve (~ED_{80}). For oral gavage, the selected EE doses were 1 and 3 μg/kg/d, and, for subcutaneous injection, the selected EE doses were 0.3 and 1 μg/kg/d.

43. As some participating laboratories indicated that they were unable to commit resources to test all five dose levels for the selected weak agonist chemicals, the VMG-mam decided that in these cases:

- at least three identical doses should be tested by all laboratories for the specific chemical so that the data would be comparable; and
- the three intermediate doses of the five (expected to be on the ascending part of the dose-response curve) were chosen as these were judged to provide the most useful information about inter-laboratory variability, i.e., doses 2, 3, and 4 in Tables 3 and 4 (note: only Laboratories #6 and 12 eventually exercised the option to test only three doses).

Table 3. Oral gavage doses selected for the dose-response experiments (Protocols A and E)

Chemical	CASRN	Dose (mg/kg/d)				
		1	2	3	4	5
Bisphenol A	80-05-7	60	200	375	600	1000
o,p'-DDT	789-02-6	10	50	125	300	600
17α-Ethinyl oestradiol*	57-63-6	1*	3*	-	-	-
Genistein	446-72-0	20	60	120	300	500
Methoxychlor	72-43-5	20	50	120	300	500
Nonylphenol	25154-52-3	15	75	125	250	350

* Ethinyl oestradiol doses were in µg/kg/day

Table 4. Subcutaneous doses selected for the dose-response experiments (Protocols B, C, and D)

Chemical	CASRN	Dose (mg/kg/d)				
		1	2	3	4	5
Bisphenol A	80-05-7	10	100	300	600	800
o,p'-DDT	789-02-6	5	25	50	100	200
17α-Ethinyl oestradiol *	57-63-6	0.3*	1*	-	-	-
Genistein	446-72-0	1	15	35	50	80
Methoxychlor	72-43-5	20	100	250	500	800
Nonylphenol	25154-52-3	5	15	35	80	100

* Ethinyl oestradiol doses were in µg/kg/day

44. After selection of the five dose levels for the dose-response approach, one of the five doses for each chemical and route of administration was selected for use in the coded single-dose experiments. These selections are shown in Table 5. In general, the selected dose was the third or fourth dose in the series where a putative ED_{50} or ED_{80} would be expected to provide a robust uterotrophic response.

Table 5. Doses (mg/kg) selected for the coded single-dose experiments

Chemical	CASRN	Protocol				
		A	B	C	D	E
Bisphenol A	80-05-7	600	300	300	300	600
o,p'-DDT	789-02-6	300	100	100	100	300
Dibutyl phthalate	84-74-2	1000	500	500	500	1000
Genistein	446-72-0	300	35	35	35	300
Methoxychlor	72-43-5	300	500	500	500	300
Nonylphenol	25154-52-3	250	80	80	80	250
17α-Ethinyl oestradiol (low dose) *	57-63-6	1	0.3	0.3	0.3	1
17α-Ethinyl oestradiol (high dose) *	57-63-6	3	1	1	1	3

* Ethinyl oestradiol doses were in µg/kg/day

Test Chemical Supply

45. The European Chemical Industry Association (CEFIC) volunteered financial and managerial responsibility for the establishment of a centralised chemical repository to be used for all OECD validation programs, including both mammalian and ecotoxicity programs. TNO in the Netherlands was then selected and contracted as the centralised chemical repository. Chemicals were purchased, donated,

or acquired by synthesis to support not only the uterotrophic validation program, but also the Hershberger and the enhanced TG 407 validation programs. Where chemicals were included in more than one program, sufficient quantities of test substances were acquired so that the same batch would be used for current and future use. This would permit other subsequent studies to be performed with the same lots used here.

46. After the participation of each laboratory was confirmed, the quantities of test substance that would be needed by each laboratory were calculated. Chemicals were weighed, packaged and then shipped in compliance with regulatory and customs requirements of each nation where participating laboratories were located. Shipments were timed to arrive before the experimental animals in order to avoid wastage, e.g., expiration of the time window for using immature animals. Special arrangements were needed for the coded single-dose study because the chemicals were to be tested as unknowns:

- The amounts of test chemical needed by each laboratory were calculated and weighed into individually coded, opaque vials. Individualised instructions were given to each laboratory, including the volume of test vehicle to be added to provide a test dose solution that could be administered at 4 ml/kg (s.c. injection) or 5 ml/kg (oral gavage) to give the prescribed doses.
- The lead laboratory confirmed that the instructions for making up the solutions were suitable for all the test substances before sending them to individual laboratories.
- Participating laboratories were asked to have separate personnel preparing and administering the dosing solutions from those performing the necropsies, so that the personnel evaluating the animals would not know the identity of the test substances.
- Generic Material Safety Data Sheets were prepared and supplied with for to all test chemicals so that the health and safety of personnel at the laboratory would not be compromised. These Safety Data Sheets were provided in sealed envelopes to a specified individual at each laboratory who agreed to keep this envelope sealed unless in cases of emergency.

47. Separate vials of test chemical were supplied for the dose-response study. The laboratories were provided instructions in order to weigh out the correct amounts to make up the necessary dosage solutions. Otherwise, they were permitted to use their normal standard operating procedures for dose preparation.

Participating laboratories

48. The participating laboratories, and lead investigators, are identified in Annex 1.

49. All the laboratories that participated in the Phase-1 study, except one (Laboratory #10), volunteered to participate in the Phase-2 studies. Two new laboratories (Laboratories #20, 21) volunteered to join the study approximately two months prior to the start of Phase-2. Although the VMG-mam originally requested that new laboratories should repeat the Phase-1 work, this request was ultimately waived because of time constraints.

50. The participation of the laboratories in the coded single-dose and dose response studies, the protocols the laboratories performed, and the actual chemicals tested in each protocol by each laboratory are described in Table 2. The details of laboratory animal strains used, diets, animal husbandry and maintenance conditions, and dosage vehicles are described in Table 6.

Table 6. Laboratory animal strains, housing, test, and treatment conditions in Phase-2

				Vehicles	
Lab.	**Rat strain**	**Diet**	**Bedding**	**oral**	**s.c.**
1	Crj:CD(SD)IGS SPF/VAF	CRF-1, Oriental Yeast Co.	ALPHA-dri (immature) None for OVX	Corn oil	Corn oil
2	IGS	CRF-1; Oriental Yeast Co.	Arufa-dry (immature) None for OVX	Corn oil Ethanol	Corn oil Ethanol
3	Crj:CD(SD)IGS	Pelleted diet, MF; Oriental Yeast Co.	Autoclaved hardwood chips, Beta Chips	99.5% EtOH Sesame oil	99.5% EtOH Sesame oil
4	CRL:WI (GLX/BRL/HAN) IGS BR	Pelleted Kliba diet rat/mouse/hamster	SSNIFF (type 3/4)	Olive oil EP/DAB	n/a
5	CRL:CD (SD) IGSBR	PMI certified rodent diet meal 5002	ALPHA DRI	95% EtOH Corn oil	n/a
6	Crl;CD® (SD) IGS BR	UAR A04 C pellet maintenance diet	Autoclaved sawdust	n/a	Corn oil
7	Crj:CD(SD)IGS	CE-2; Clea, Japan	No bedding used	Corn oil	Corn oil
8	Alpk:APfSD	R&M3 to weaning R&M1 post weaning	Shredded paper	Peanut oil	Peanut oil
9	Crj:CD(SD)IGS	MF, Oriental Yeast Co.	Sunflake®, Oriental Yeast Co.	Olive oil 99.5% EtOH	Olive oil 99.5% EtOH
11	Wistar (BrlHan:WIST@Jcl)	CE-2; Clea, Japan	Sunflake (immature) None for OVX	Corn oil	Corn oil
12	Crl:CD®(SD)IGS BR	PMI Certified Rodent LabDiet® 5002	Ground corncob bedding "Bed-O'Cobs"	Corn oil	Corn oil
13	SPF-bred Wistar, HSD/Cpb: WU	Altromin 1324	Low-dust wood granules Type BK 8/15	Corn oil with min. EtOH	Corn oil with min. EtOH
14	SD ICO:OFA SD (IOPS Caw)	Pellet AO4C 10 UAR Certified rodent meal	UAR bedding	Corn oil	Corn oil
15	Wistar Hsd Cpb:WU	Rat & mouse no.3 breeding diet RM3 from SDS	No bedding used	n/a	Corn oil + min. EtOH
16	Wistar (HsdCpb:Wu)	Altromin 1324 FORTII	Wood chip - low dust	n/a	Peanut oil
17	Wistar (mol:wistar/han)	Altromin 1324	Tapvei	n/a	Peanut oil
18	Sprague-Dawley	PMI Lab Diet	Elm tree (autoclaved)	n/a	95% EtOH Corn oil
19	CD Sprague-Dawley	Special Diet Services RM1(E) SQC expanded pellet	Lignocel grade 4/4 woodflakes (immature) None for OVX	n/a	Corn oil EtOH + corn oil
20	Hsd: Sprague Dawley	Altromin MT	Nesting material	Corn oil	n/a
21	Crl:CD(SD)BR	GLP4RF25 Top certificate; Mucedola S.r.	Dust-free poplar/fir wood chips, heat processed	n/a	Corn oil

SD, Sprague-Dawley
n/a, non-applicable
EtOH, Ethanol

DATA RECORDING AND BASE STATISTICAL ANALYSES

51. Three measurements were recorded as data: body weight; wet uterine weight with imbibed, luminal fluid; and blotted uterine weight less imbibed, luminal fluid. Body weights were recorded each day in order to adjust dosing volumes and administer specific doses on a kg body weight per day basis, and body weights were recorded immediately prior to necropsy. The exception was Laboratory #21, where animals were weighed prior to all three daily doses, but the necropsy body weights were not recorded. The body weights, wet uterine weights, and blotted uterine weights along with other laboratory information, e.g., diet, animal strain, food consumption, and clinical signs, were recorded on standardised Excel spreadsheets provided by the OECD Secretariat. After receipt of the spreadsheets containing the data from the laboratories, the data were sent to the independent consulting statisticians (National Institute of Environmental Health Sciences, US) for analysis.

52. Data from Laboratory #21 were analysed separately, because the terminal body weights were not recorded by the laboratory. Instead, the statistical analysis was carried out on uterine weight data adjusted for body weights from day 3 of dosing, rather than necropsy weights in other laboratories.

Statistical analyses and methods used

53. The base statistical analyses determined whether the administration of a test substance resulted in a statistically significant increase in the uterine weight. This was determined by the independent consulting statisticians using the raw data for uterine weights and body weights from each participating laboratory that had been recorded on a standardised electronic spreadsheet. The uterine data were evaluated by an analysis of covariance (ANCOVA) approach with body weight at necropsy as the covariable. A variance-stabilising logarithmic transformation was carried out on the uterine data prior to the data analysis. Dunnett and Hsu's test was used for making pairwise comparisons of each dosed group to vehicle controls and to calculate the confidence intervals. Studentised residual plots were used to detect possible outliers and to assess homogeneity of variances. The data were analysed using the PROC GLM in the Statistical Analysis System (SAS Institute, Cary, NC), version 8. In addition to the absolute ratio of the mean uterine weights (the treated groups relative to the vehicle control groups) in Tables 2-26, the ratio of the geometric means of the uterine weights (treated relative to the vehicle control) after adjusting for the body weight of the individual animals at necropsy along with respective upper and lower 95% confidence levels were also calculated. Outliers were observed (as defined as Studentised Residuals > 3.75 or < -3.75), but they were not excluded from the statistical analyses. Only one data point, a recorded blotted uterine weight of only 3.8 grams (approximately one order of magnitude less than the other recorder weights, suggesting an error in the decimal place) was removed, and the analysis was run both before and after the removal.

54. In order to draw inferences across laboratories about the reproducibility of results at a given dose for each protocol, the data for the studies was pooled and analysed. Mixed effects linear models were used for the pooled analyses where the laboratories were treated as the random effects. This analysis took into consideration both "between labs" variability and "within lab" variability and provided an overall summary of the results. Thus, the analysis enabled the computation of a mean response to a chemical across labs and the lower and the upper 95% confidence limits under each protocol. Where mixed effects linear models are used, the analysis is referred to as a "Global analysis."

DIET ANALYSIS

55. Absolute uterine weight differences among the laboratories may be the result of many factors, including body weight, strain, age and initiation of ovarian secretion of endogenous oestrogens, and the

time of uterine regression after OVX. Those factors that would impact the responsiveness of the uterus to oestrogen stimulus, and thereby the relative weight increase of the treated uterus, were controlled, i.e., the age of the immature animals was set so that necropsy occurred prior to increases in uterine weight and the coefficient of variation in the animal groups (reviewed in 25, 26). In addition, there is evidence that, when high levels of phytoestrogens are present in the animal diet, uterine control weights could potentially be increased and thereby reduce the dynamic range of the assay by decreasing uterine responsiveness (49)(50)(reviewed in (25)(26)).

56. Therefore, the VMG requested that all laboratories retain and submit diet samples for phytoestrogen analyses. The American Chemistry Council volunteered to financially support the phytoestrogen analyses, and the Syngenta CTL laboratory received the diet samples and coded the samples before submission to the analytical laboratory, Bioclinical Services, Ltd., Cardiff, UK. The diet samples after hydrolysis of the glycosides were analysed for their daidzein, genistein, and coumestrol content. The methodological details of the dietary phytoestrogen analyses, the results of the analyses, and the comparison of the dietary phytoestrogen levels to uterotrophic bioassays with weak oestrogen agonists are contained in Annex 3.

RESULTS

57. The results from the coded single-dose and dose-response studies are presented below in separate sections. Based on the findings in Phase-1 of this validation study, the analyses of the uterine weights are based on the blotted weights, because the blotted weights were slightly less variable among the animals.

58. The protocols allowed variations in a number of experimental conditions, including the choice of rat strain, the laboratory diet, housing and husbandry practices such as the use of cage bedding, the vehicle employed, and, to a modest degree, the age of both immature and OVX animals. Because the intended purpose of the uterotrophic bioassay is for the rapid screening of a potentially large number of chemicals, the judgement was that rigorous and detailed standardisation of all of these variables would constrain the ability to widely and easily practice the uterotrophic bioassay in many of the OECD member countries. As a result, there is a relatively wide variation in the absolute wet and blotted uterine weights. This is particularly true in the immature animals where vehicle control means across the coded single-dose and dose-response studies ranged from a minimum of 14.8 mg to a maximum of 58.0 mg for blotted uterine weights (see Figures 5 and 6 in a later section). However, the bioassay is not directly based on absolute weights. Rather, the uterine weight increase in the test substance groups relative to the vehicle controls and its achieving statistical significance are the essential measurements. Therefore, the data are presented as, and primarily analysed as, the ratio of treated to vehicle control uterine weights, adjusted for the final body weight, including the upper and lower 95% confidence levels of that ratio and whether the results are statistically significant. The absolute mean wet and blotted uterine weights, and body weights, from the dose-response and coded single-dose studies are in Annex 4.

59. During the data audit, in addition to several transcription errors, it was discovered that several laboratories incorrectly prepared the two dilutions of the test solutions of the EE positive reference substance. In several instances they used a different dilution than was called for in the protocol. However, the most common error was the interchange of the dilutions where the sample intended to be the high reference dose concentration was actually the high dilution (*i.e.*, the low reference dose concentration). The audit was able to resolve these issues in all except one laboratory.

Test Chemical Toxicity

60. Several laboratories reported animal deaths during the course of the experiment, and these instances are reported in Table 7. The great majority of animal deaths occurred in Protocol A, where the animals are very young and just weaned, making them vulnerable to insult. LC_{50} and other data for maximum tolerated doses used to select the doses are rarely available for immature animals, and data from adult animals was used. Further, gavage errors and irritation of the esophagus and forestomach inhibiting feeding are also most likely to occur in these animals. In most cases, the animals exhibited clinical signs and, in several cases not only failed to gain body weight, but actually lost body weight during dosing. Similar rates of toxicity were observed in both the dose-response and the coded single-dose experiments with the same chemicals. The greatest rate of toxicity was observed with nonylphenol, followed by *o,p'*-DDT. In several instances, two or more animals within a group died, which would impair the power of the bioassay. These technical findings and animal welfare concerns suggest that range finding studies be considered with less well-characterised substances to avoid overt toxicity, particularly with the immature animals.

Table 7. Animal Mortality – Number of Animals Euthanized or Found Dead in Phase-2 (Group Starting Size was 6)

	Protocol	Coded single-dose		Dose-response		
Lab		**Chemical** [1]	**Deaths**	**Chemical**	**Dose**	**Deaths**
2	A	GEN NP	1 1	BP	1000 mg/kg	1
3	A			DDT	600 mg/kg	4
4	A	NP	4	NP	250 mg/kg 350 mg/kg	2 6
5	A	NP	2	DDT	300 mg/kg 600 mg/kg	1 4
6	B			BPA EE	600 mg/kg 0.3 µg/kg	1 1
7	A			NP	250 mg/kg 350 mg/kg	1 3
7	C			BPA	300 mg/g	1
8	A	NP	1			
9	A			NP	350 mg/kg	3
11	A	NP	2	DDT	600 mg/kg	4
12	A	BPA DBP DDT MC NP	3 3 6 3 4	BPA DDT NP	600 mg/kg 300 mg/kg 250 mg/kg	1 3 4
13	A	BPA NP	1 1]	BPA	600 mg/kg	1
14	A	BPA DDT GEN NP EE (3 µg)	2 2 1 4 1	MC	0 120 mg/kg 300 mg/kg 500 mg/kg	1 1 1 3
14	B			MC	800	1

[1] See Table 5 for coded single-dose dosages

Dose-Response Studies

61. The chemical doses and the rationale for their selection were reported in previous sections, and the absolute mean wet and blotted uterine weights and the body weights are in Annex 4. The number of laboratories performing a protocol with which of the five chemicals (BP, DDT, GEN, MC, NP) are shown in Table 8. With the exception of Protocol E (OVX, oral gavage; Laboratory #12) all chemical/protocol combinations were performed in two (Protocol D) or more laboratories (Protocols A, B, C).

Table 8. Numbers of laboratories testing each chemical in the dose-response studies

Chemical	Protocol A	Protocol B	Protocol C	Protocol D	Protocol E
BPA	4	10	5	2	1
DDT	4	4	3	2	1
GEN	4	4	3	2	1
MC	4	4	3	2	1
NP	4	10	5	2	1

62. The summary dose-response data for all laboratories and chemicals are in Tables 9 - 13. The results are reported as the ratio of the blotted uterine weight mean for the test substance groups relative to the blotted uterine weight mean of the vehicle group adjusted using body weight as a covariable in the ANOVA analysis. The 95% upper and lower confidence levels for this ratio are reported, respectively, in parentheses. If statistical significance was achieved, the data are marked with an asterisk. Other considerations such as animal mortality are noted. One laboratory (Laboratory #21) using Protocol B did not weigh the animals at the time of necropsy, but only on the days of dosing. Because the terminal weights of the animals were used as a covariable in the statistical analyses of the data, the results from this laboratory were subjected to a separate analysis. In this modified analysis, the animal weights at the time of the third dosing were used for Laboratory #21.

63. After the summary data with the five weak agonists, additional dose response data are summarised and analysed. This includes the EE reference doses and an analysis of the minimal effect dose or the first and lowest dose in the series at which statistical significance was achieved.

Summary Data for Five Weak Agonists

64. Bisphenol A (Tables 9a-d). BPA produced statistically significant dose-related increases in blotted uterine weight gains in at least one dose group in all protocols in all laboratories, with the exception of Protocol E (Laboratory #12) (Table 9d). Laboratory #12 tested the three intermediate doses, but not the highest dose in the series and encountered one animal mortality at 600 mg/kg/d. Subcutaneous administration of BPA (Protocols B, C, D) produced statistically significant increases at lower doses than oral gavage. This is consistent with the available pharmacokinetic data (reviewed 25, 26). One laboratory (Laboratory #20) using Protocol B apparently reversed the doses administered and also recorded two groups with uterine weights statistically less than the vehicle controls (Table 9b). Laboratory #20 vehicle control group mean blotted uterine weight was 54.3 mg, and two treated groups that were statistically lower had weights of 27.4 and 31.6 mg.

Table 9a. Bisphenol A: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol A

mg/kg/d	Lab. #2	Lab. #7	Lab. #12	Lab. #13
60	0.99 (0.83 - 1.17)	0.89 (0.70 - 1.13)	ND	1.00 (0.77 - 1.31)
200	1.13 (0.95 - 1.34)	0.97 (0.76 - 1.22)	1.25 (0.9995[b] - 1.56)	1.16 (0.88 - 1.51)
375	1.26 (1.06 - 1.50)*	1.00 (0.79 - 1.27)	1.36 (1.05 - 1.76)*	1.03 (0.78 - 1.35)
600	1.49 (1.25 - 1.77)*	1.31 (1.03 - 1.66)*	1.63 (1.29 - 2.06)* [1][c]	1.17 (0.79 - 1.72) [1]
1000	1.73 (1.45 - 2.07)*[1][c]	1.40 (1.10 - 1.78)*	ND	1.57 (1.18 - 2.08)*
EE-0.3[a]	ND	1.11 (0.89 - 1.39)	ND	ND
EE-1.0[a]	3.17 (2.52 - 3.99)^*	2.16 (1.73 - 2.69)*	2.85 (2.21 - 3.67)^*	1.44 (1.06 - 1.95)^*
EE-3.0[a]	4.13 (3.27 - 5.22)^*	ND	4.68 (3.63 - 6.02)^*	2.55 (2.06 - 3.17)^*

For footnotes see after Table 9d.

Table 9b. Bisphenol A: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol B

mg/kg/d	Lab. #2	Lab. #6	Lab. #7	Lab. #8
10	1.12 (0.92 - 1.36)	ND	1.01 (0.85 - 1.20)	1.17 (0.92 - 1.50)
100	1.67 (1.37 - 2.02)*	1.18 (0.90 - 1.54)	1.31 (1.10 - 1.56)*	1.47 (1.15 - 1.87)*
300	2.30 (1.88 - 2.81)*	1.37 (1.05 - 1.79)*	1.95 (1.64 - 2.32)*	1.91 (1.50 - 2.43)*
600	3.30 (2.72 - 4.01)*	1.75 (1.31 - 2.33)*	3.47 (2.90 - 4.16)*	2.13 (1.67 - 2.71)*
800	4.00 (3.28 - 4.87)*	ND	3.66 (3.06 - 4.37)*	3.01 (2.36 - 3.84)*
EE-0.3[a]	2.11 (1.70 - 2.62)*	1.59 (1.15 - 2.18)^* [1][c]	1.73 (1.48 - 2.01)*	2.65 (2.37 - 2.97)^*
EE-1.0[a]	4.44 (3.60 - 5.48)*	2.30 (1.71 - 3.10)^*	4.06 (3.49 - 4.72)*	4.96 (4.43 - 5.55)^*
mg/kg/d	**Lab. #12**	**Lab. #13**	**Lab. #15**	**Lab. #18**
10	ND	1.14 (0.71 - 1.82)	0.95 (0.72 - 1.25)	1.28 (1.08 - 1.51)*
100	1.47 (0.99 - 2.19)	1.50 (0.91 - 2.47)	0.98 (0.75 - 1.29)	1.57 (1.34 - 1.83)*
300	1.33 (0.88 - 1.99)	1.72 (1.08 - 2.76)*	1.37 (1.03 - 1.81)*	2.12 (1.81 - 2.50)*
600	2.51 (1.70 - 3.70)*	2.88 (1.78 - 4.64)*	2.54 (1.91 - 3.37)*	3.33 (2.85 - 3.91)*
800	ND	4.15 (2.55 - 6.77)*	3.11 (2.37 - 4.08)*	4.42 (3.78 - 5.17)*
EE-0.3[a]	1.68 (1.11 - 2.53)^*	1.61 (1.08 - 2.42)*	4.45 (3.46 - 5.71)^*	3.81 (3.28 - 4.50)^*
EE-1.0[a]	3.64 (2.43 - 5.45)^*	3.44 (2.25 - 5.27)*	4.95 (3.66 - 6.69)^*	5.62 (4.89 - 6.49)^*
mg/kg/d	**Lab. #20**	**Lab. #21**		
10	1.75 (1.26 - 2.43)*	1.44 (1.18 - 1.74)*[#]		
100	1.57 (1.12 - 2.19)*	1.50 (1.24 - 1.83)*[#]		
300	0.95 (0.69 - 1.32)	1.89 (1.55 - 2.29)*[#]		
600	0.59 (0.42 - 0.81)**	1.97 (1.63 - 2.40)*[#]		
800	0.50 (0.36 - 0.69)**	2.42 (1.99 - 2.93)*[#]		
EE-0.3[a]	1.83 (1.45 - 2.31)^*	3.19[++]		
EE-1.0[a]	2.38 (1.90 - 2.99)^*	1.97[++]		

For footnotes see after Table 9d.

Table 9c. Bisphenol A: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol C

mg/kg/d	Lab. #2	Lab. #6	Lab. #7	Lab. #8
10	1.13 (0.93 - 1.37)	ND	1.03 (0.86 - 1.24)	0.98 (0.80 - 1.20)
100	1.89 (1.55 - 2.30)*	2.05 (1.58 - 2.66)*	1.67 (1.38 - 2.01)*	1.60 (1.31 - 1.96)*
300	2.79 (2.28 - 3.41)*	2.41 (1.79 - 3.23)*	3.44 (2.76 - 4.30)*	2.65 (2.16 - 3.24)*
600	3.08 (2.52 - 3.78)*	3.92 (2.97 - 5.18)*	3.85 (3.16 - 4.70)*	2.85 (2.32 - 3.51)*
800	3.03 (2.45 - 3.75)*	ND	3.67 (3.02 - 4.47)*	2.79 (2.27 - 3.44)*
EE-0.3[a]	2.41 (2.06 - 2.81)*	2.43 (1.55 - 3.82)^*	1.78 (1.47 - 2.16)*	2.16 (1.91 - 2.43)^*
EE-1.0[a]	3.19 (2.69 - 3.78)*	3.89 (2.45 - 6.17)^*	3.29 (2.69 - 4.01)*	2.70 (2.39 - 3.05)^*

mg/kg/d	Lab. #12
10	ND
100	2.03 (1.53 - 2.70)*
300	2.72 (2.05 - 3.61)*
600	3.24 (2.43 - 4.32)*
800	ND
EE-0.3[a]	1.95 (1.52 - 2.49)^*
EE-1.0[a]	3.08 (2.41 - 3.94)^*

For footnotes see after Table 9d.

Table 9d. Bisphenol A: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocols D and E

	Protocol D			Protocol E	
mg/kg/d	Lab. #2	Lab. #7		mg/kg/d	Lab. #12
10	1.14 (0.91 - 1.41)	1.10 (0.97 - 1.26)		60	ND
100	2.53 (1.99 - 3.21)*	2.35 (2.00 - 2.77)*		200	1.16 (0.86 - 1.56)
300	3.74 (2.89 - 4.84)*	3.90 (3.18 - 4.78)*		375	1.27 (0.97 - 1.68)
600	4.69 (3.67 - 5.99)*	4.30 (3.56 - 5.19)*		600	1.29 (0.94 - 1.75)
800	4.31 (3.28 - 5.67)*	4.70 (3.84 - 5.75)*		1000	ND
EE-0.3[a]	3.71 (2.91 - 4.74)*	2.45 (1.97 - 3.05)*			
EE-1.0[a]	4.86 (3.55 - 6.64)*	4.50 (3.53 - 5.73)*			

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[a] EE doses in µg/kg/day.

[b] With the lower 95% confidence limit not > 1.0, the result is not statistically significant.

[c] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; **, significantly decreased at $p<0.05$.

^, shared EE controls.

[#], calculations based on body weight at day 3 of dosing.

[++], body weights at necropsy were not recorded, therefore body weights were not used as a covariable, and confidence intervals were not calculated.

65. <u>*o,p'*-DDT (Tables 10a-d).</u> *o,p'*-DDT produced statistically significant dose-related increases in blotted uterine weight gains in at least one dose group in all protocols in all laboratories, with the exception of Protocol C (Laboratory #12) (Table 9c). This laboratory performed only the three intermediate doses and did not generate data for the highest *o,p'*-DDT dose. In the case of *o,p'*-DDT, oral gavage administration (Protocols A and E – Tables 10a and 10d) produced statistically significant increases at lower doses (10 and 50 mg/kg/d) and much stronger responses with higher relative ratios (2-4 fold increases) than subcutaneous administration (50-200 mg/kg/d and with relative ratios never exceeding

2-fold, respectively, Tables 10b, 10c, and 10d). This was unexpected, but data available to select the doses for and to predict the responses of *o,p'*-DDT were limited.

Table 10a. o,p'-DDT: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol A

mg/kg/d	Lab. #3	Lab. #5	Lab. #11	Lab. #12
10	1.09 (0.84 - 1.40)	1.19 (0.91 - 1.54)	1.21 (1.04 - 1.41)*	ND
50	1.60 (1.24 - 2.05)*	1.53 (1.18 - 1.99)*	2.25 (1.94 - 2.63)*	2.61 (2.01 - 3.40)*
125	2.04 (1.58 - 2.63)*	2.01 (1.54 - 2.63)*	2.60 (2.24 - 3.01)*	3.18 (2.43 - 4.15)*
300	2.67 (1.99 - 3.59)*	2.71 (1.92 - 3.83)* [1] [b]	3.43 (2.96 - 3.98)*	3.45 (2.41 - 4.94)* [3] [b]
600	3.30 (1.80 - 6.04)*[4] [b]	ND	4.33 (3.35 - 5.59)*	ND
1000	ND	3.72 (2.24 - 6.18)* [4] [b]	ND	ND
EE-1.0[a]	2.25 (1.77 - 2.86)*	1.40 (1.04 - 1.90)*	3.04 (2.42 - 3.83)*	2.85 (2.21 - 3.67)^*
EE-3.0[a]	2.55 (2.00 - 3.25)*	1.91 (1.41 - 2.59)*	4.52 (3.54 - 5.78)*	4.68 (3.63 - 6.02)^*

For footnotes see after Table 10d.

Table 10b. o,p'-DDT: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol B

mg/kg/d	Lab. #3	Lab. #5	Lab. #11	Lab. #12
5	0.88 (0.72 - 1.08)	1.15 (0.88 - 1.49)	1.06 (0.85 - 1.31)	ND
25	1.03 (0.85 - 1.26)	1.06 (0.82 - 1.38)	1.06 (0.86 - 1.32)	1.33 (1.00 - 1.76)
50	1.02 (0.84 - 1.25)	0.97 (0.75 - 1.27)	1.04 (0.84 - 1.29)	1.30 (0.98 - 1.72)
100	1.01 (0.83 - 1.23)	1.18 (0.91 - 1.54)	1.08 (0.87 - 1.34)	1.47 (1.11 - 1.94)*
200	1.31 (1.07 - 1.59)*	1.41 (1.09 - 1.84)*	1.36 (1.10 - 1.68)*	ND
EE-0.3[a]	2.00 (1.68 - 2.38)*	ND	3.50 (2.83 - 4.34)*	1.68 (1.11 - 2.53)^*
EE-1.0[a]	3.82 (3.17 - 4.60)*	3.61 (2.91 - 4.46)*	4.58 (3.70 - 5.69)*	3.64 (2.43 - 5.45)^*
EE-3.0[a]	ND	4.78 (3.83 - 5.96)*	ND	ND

For footnotes see after Table 10d.

Table 10c. o,p'-DDT: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol C

mg/kg/d	Lab. #3	Lab. #11	Lab. #12
5	1.13 (0.96 - 1.33)	0.97 (0.76 - 1.23)	ND
25	1.17 (0.99 - 1.37)	1.15 (0.90 - 1.46)	1.07 (0.79 - 1.45)
50	1.30 (1.11 - 1.53)*	1.25 (0.98 - 1.59)	1.10 (0.81 - 1.49)
100	1.43 (1.21 - 1.69)*	1.25 (0.98 - 1.59)	1.31 (0.96 - 1.78)
200	1.86 (1.55 - 2.21)*	1.34 (1.05 - 1.71)*	ND
EE-0.3[a]	2.43 (2.14 - 2.77)*	3.04 (2.63 - 3.51)*	1.95 (1.52 - 2.49)^*
EE-1.0[a]	3.57 (3.06 - 4.18)*	3.97 (3.36 - 4.69)*	3.08 (2.41 - 3.94)^*

For footnotes see after Table 10d.

Table 10d. o,p'-DDT: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocols D and E

	Protocol D			Protocol E	
mg/kg/d	**Lab. #3**	**Lab. #11**		**mg/kg/d**	**Lab. #12**
5	0.94 (0.82 - 1.08)	1.05 (0.84 - 1.32)		10	ND
25	1.01 (0.88 - 1.16)	1.08 (0.87 - 1.35)		50	2.03 (1.53 - 2.71)*
50	1.09 (0.95 - 1.25)	1.34 (1.07 - 1.68)*		125	2.64 (1.92 - 3.61)*
100	1.18 (1.03 - 1.36)*	1.48 (1.17 - 1.87)*		300	2.94 (2.05 - 4.23)*
200	1.54 (1.34 - 1.78)*	1.73 (1.36 - 2.20)*		600	ND
EE-0.3[a]	2.77 (2.44 - 3.14)*	5.16 (3.65 - 7.28)*		EE-1.0[a]	ND
EE-1.0[a]	3.67 (3.13 - 4.29)*	5.85 (4.26 - 8.05)*		EE-3.0[a]	ND

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[a] EE doses in µg/kg/day.

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; ^ shared EE controls.

66. Genistein (Tables 11a-d). GEN produced statistically significant dose-related increases in blotted uterine weight gains in at least one dose group in all laboratories and all protocols. In fact, genistein produced significant increases at the lowest dose in at least one lab in Protocols A and B. In the case of GEN, subcutaneous administration (Protocols B, C, D) produced statistically significant increases at lower doses than oral gavage (Protocols A and E). This is consistent with the available pharmacokinetic data (reviewed in (25)(26)). With GEN, Protocol B appeared to be somewhat more sensitive than Protocol C or D to the oestrogenic effects of GEN in regard to the dose at which statistical significance occurred and in a higher relative ratio response at a given dose, e.g., at or greater than 3-fold at 50 and 80 mg/kg/d for Protocol B versus 2-fold or less for Protocol C. Extended dosing in Protocol D did compensate to some degree.

Table 11a. Genistein: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol A

mg/kg/d	Lab. #1	Lab. #8	Lab. #9	Lab. #12
20	1.42 (1.08 - 1.85)*	1.12 (0.91 - 1.37)	1.36 (1.07 - 1.71)*	ND
60	1.76 (1.35 - 2.30)*	1.61 (1.33 - 1.96)*	2.23 (1.77 - 2.82)*	2.49 (1.97 - 3.15)*
120	1.97 (1.50 - 2.58)*	2.36 (1.94 - 2.87)*	2.63 (2.08 - 3.31)*	3.03 (2.39 - 3.85)*
300	2.22 (1.67 - 2.95)*	2.96 (2.42 - 3.61)*	2.57 (2.03 - 3.25)*	3.47 (2.71 - 4.45)*
500	2.56 (1.93 - 3.41)*	3.16 (2.59 - 3.86)*	3.04 (2.41 - 3.84)*	ND
EE-1.0[a]	1.10 (0.92 - 1.32)	3.09 (2.55 - 3.73)*	2.19 (1.72 - 2.79)^*	2.85 (2.21 - 3.67)^*
EE-3.0[a]	1.50 (1.25 - 1.79)*	4.69 (3.88 - 5.66)*	5.19 (4.10 - 6.58)^*	4.68 (3.63 - 6.02)^*

For footnotes see after Table 11d.

Table 11b. Genistein: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol B

mg/kg/d	Lab. #1	Lab. #8	Lab. #9	Lab. #12
1	1.20 (0.90 - 1.59)	1.18 (0.96 - 1.45)	1.18 (1.00 - 1.38)*	ND
15	1.79 (1.35 - 2.38)*	2.10 (1.71 - 2.58)*	1.91 (1.63 - 2.25)*	1.48 (1.10 - 1.98)*
35	2.33 (1.75 - 3.10)*	2.69 (2.19 - 3.30)*	2.57 (2.19 - 3.02)*	2.28 (1.70 - 3.05)*
50	2.91 (2.19 - 3.86)*	3.08 (2.51 - 3.79)*	3.10 (2.63 - 3.64)*	2.70 (2.02 - 3.61)*
80	3.30 (2.47 - 4.42)*	3.83 (3.12 - 4.71)*	3.50 (2.98 - 4.11)*	ND
EE-0.3[a]	2.17 (1.80 - 2.62)*	2.65 (2.37 - 2.97)^*	4.22 (3.63 - 4.91)^*	1.68 (1.11 - 2.53)^*
EE-1.0[a]	4.19 (3.47 - 5.05)*	4.96 (4.43 - 5.55)^*	4.26 (3.64 - 4.98)^*	3.64 (2.43 - 5.45)^*

For footnotes see after Table 11d.

Table 11c. Genistein: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol C

mg/kg/d	Lab. #1	Lab. #9	Lab. #12
1	0.90 (0.74 - 1.10)	0.99 (0.83 - 1.18)	ND
15	1.53 (1.25 - 1.88)*	1.57 (1.32 - 1.88)*	1.31 (0.92 - 1.87)
35	1.78 (1.46 - 2.18)*	1.87 (1.56 - 2.23)*	1.56 (1.09 - 2.21)*
50	1.68 (1.37 - 2.05)*	2.08 (1.74 - 2.48)*	1.62 (1.14 - 2.32)*
80	1.89 (1.54 - 2.31)*	1.98 (1.66 - 2.37)*	ND
EE-0.3[a]	1.98 (1.70 - 2.30)*	ND	1.95 (1.52 - 2.49)^*
EE-1.0[a]	3.13 (2.66 - 3.67)*	ND	3.08 (2.41 - 3.94)^*

For footnotes see after Table 11d.

Table 11d. Genistein: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocols D and E

	Protocol D			Protocol E	
mg/kg/d	Lab. #1	Lab. #9		mg/kg/d	Lab. #12
1	0.99 (0.80 - 1.23)	1.18 (0.9995[b] - 1.39)		20	ND
15	1.67 (1.35 - 2.07)*	2.06 (1.74 - 2.43)*		60	1.82 (1.34 - 2.48)*
35	2.20 (1.78 - 2.73)*	2.54 (2.15 - 2.99)*		120	1.93 (1.40 - 2.66)*
50	2.31 (1.87 - 2.86)*	2.75 (2.33 - 3.25)*		300	2.16 (1.55 - 3.00)*
80	3.55 (2.87 - 4.40)*	2.81 (2.38 - 3.32)*		500	ND
EE-0.3[a]	2.93 (2.38 - 3.60)*	4.14 (3.34 - 5.13)^*		EE-1.0[a]	ND
EE-1.0[a]	4.40 (3.45 - 5.61)*	4.68 (3.66 - 5.99)^*		EE-3.0[a]	ND

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[a] EE doses in µg/kg/day. [b] With the lower 95% confidence limit not > 1.0, the result is not statistically significant. ND, not done; *, significant increase at $p<0.05$; ^, shared EE controls.

67. Methoxychlor (Tables 12a-d). MC produced statistically significant dose-related increases in blotted uterine weight gains in at least one dose group in all laboratories and all protocols. In fact, MC produced significant increases at the lowest dose in all oral gavage groups. In the case of MC, oral gavage administration (Protocols A and E) produced statistically significant increases at lower doses (20 mg/kg/d) than subcutaneous administration (Protocols B, C, and D), where statistical significance was first achieved at 100 mg/kg/d in all experiments. Given the magnitudes of the responses in Protocols A and E at 20 mg/kg) would have been effective. The equivalent 20 mg/kg/d dose did not produce significant increases in the other protocols. One laboratory (Laboratory #14) using Protocol A had toxicity at the three highest doses and in the vehicle control group (Table 12a).

Table 12a. Methoxychlor: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol A - inter-laboratory comparison

mg/kg/d	Lab. #1	Lab. #3	Lab. #12	Lab. #14[+]
20	1.98 (1.65 - 2.39)*	1.88 (1.52 - 2.31)*	ND	3.14 (2.31 - 4.26)*
50	2.31 (1.91 - 2.79)*	2.37 (1.92 - 2.93)*	3.71 (2.87 - 4.79)*	3.03 (2.27 - 4.05)*
120	2.30 (1.90 - 2.77)*	2.47 (2.00 - 3.05)*	3.79 (2.93 - 4.90)*	3.59 (2.57 - 5.01)* [1][b]
300	2.59 (2.13 - 3.15)*	2.94 (2.34 - 3.69)*	3.98 (3.07 - 5.15)*	3.46 (2.51 - 4.77)* [1][b]
500	2.83 (2.31 - 3.46)*	2.65 (2.11 - 3.35)*	ND	3.19 (2.19 - 4.63)* [3][b]
EE-1.0[a]	0.99 (0.77 - 1.26)	1.53 (1.20 - 1.96)*	2.85 (2.21 - 3.67)^*	3.11 (2.44 - 3.98)*
EE-3.0[a]	1.64 (1.29 - 2.10)*	2.80 (2.20 - 3.58)*	4.68 (3.63 - 6.02)^*	4.69 (3.74 - 5.89)*

For footnotes see after Table 12d.

Table 12b. Methoxychlor: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol B - inter-laboratory comparison

mg/kg/d	Lab. #1	Lab. #3	Lab. #12	Lab. #14
20	1.16 (0.89 - 1.50)	1.21 (0.99 - 1.49)	ND	1.07 (0.79 - 1.45)
100	1.36 (1.05 - 1.76)*	2.21 (1.80 - 2.71)*	2.86 (2.18 - 3.77)*	1.62 (1.20 - 2.19)*
250	1.94 (1.50 - 2.51)*	2.88 (2.34 - 3.55)*	3.53 (2.68 - 4.65)*	2.89 (2.13 - 3.93)*
500	2.47 (1.91 - 3.21)*	2.98 (2.42 - 3.65)*	3.34 (2.53 - 4.40)*	3.76 (2.78 - 5.09)*
800	2.69 (2.08 - 3.49)*	3.52 (2.87 - 4.31)*	ND	3.55 (2.58 - 4.88)* [1][b]
EE-0.3[a]	2.49 (2.12 - 2.93)*	2.31 (1.89 - 2.81)*	1.68 (1.11 - 2.53)^*	2.61 (2.05 - 3.32)*
EE-1.0[a]	4.07 (3.46 - 4.80)*	3.79 (3.11 - 4.63)*	3.64 (2.43 - 5.45)^*	4.55 (3.59 - 5.76)*

For footnotes see after Table 12d.

Table 12c. Methoxychlor: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol C

mg/kg/d	Lab. #1	Lab. #3	Lab. #12
20	0.95 (0.77 - 1.19)	1.08 (0.89 - 1.32)	ND
100	1.28 (1.03 - 1.59)*	1.72 (1.41 - 2.10)*	1.63 (1.21 - 2.19)*
250	1.85 (1.49 - 2.31)*	1.99 (1.63 - 2.43)*	1.79 (1.33 - 2.39)*
500	2.32 (1.86 - 2.89)*	2.42 (1.96 - 2.98)*	1.95 (1.45 - 2.62)*
800	2.42 (1.93 - 3.02)*	2.59 (2.10 - 3.19)*	ND
EE-0.3[a]	2.14 (1.88 - 2.43)*	2.96 (2.36 - 3.71)*	1.95 (1.52 - 2.49)^*
EE-1.0[a]	3.77 (3.27 - 4.35)*	3.61 (2.84 - 4.59)*	3.08 (2.41 - 3.94)^*

For footnotes see after Table 12d.

Table 12d. Methoxychlor: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocols D and E

	Protocol D			Protocol E	
mg/kg/d	**Lab. #1**	**Lab. #3**		**mg/kg/d**	**Lab. #12**
20	0.94 (0.72 - 1.23)	1.08 (0.89 - 1.30)		20	ND
100	1.33 (1.02 - 1.75)*	1.55 (1.27 - 1.90)*		50	2.11 (1.71 - 2.60)*
250	1.75 (1.30 - 2.34)*	2.41 (1.94 - 2.98)*		120	2.36 (1.89 - 2.94)*
500	2.38 (1.76 - 3.22)*	2.46 (1.98 - 3.06)*		300	2.50 (2.03 - 3.08)*
800	2.46 (1.82 - 3.34)*	2.61 (2.08 - 3.26)*		500	ND
EE-0.3[a]	2.75 (2.30 - 3.30)*	2.85 (2.50 - 3.26)*		EE-1.0[a]	ND
EE-1.0[a]	4.11 (3.36 - 5.04)*	4.04 (3.49 - 4.69)*		EE-3.0[a]	ND

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[a] EE doses in µg/kg/day.

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.ND, not done;

*, significant increase at $p<0.05$; ^ shared EE controls.

[+] one animal in the vehicle control group died prior to necropsy.

68. Nonylphenol (Tables 13a-d). NP did not always produce statistically significant dose-related increases in blotted uterine weight gains in all laboratories and all protocols. All four laboratories in Protocol A obtained significant increases at doses of 75 mg/kg and above (Table 13a). In Protocol B, two laboratories (Laboratories #6, 20) did not obtain statistically significant increases (Table 13b). Laboratory #6 tested the three intermediate doses, but not the highest dose in the NP series. Laboratory #20 had a vehicle control group mean blotted uterine weight of 54.3 mg, and all test substance group means were 41.3 mg or less. One of these groups at 15 mg/kg/d was less than vehicle weights by a statistically significant degree. Another laboratory (Laboratory #21) using Protocol B had statistical significance at 5, 35, and 80 mg/kg/d, but not at 15 or 100 mg/kg/d. In Protocol C, Laboratory #6 did not obtain statistically significant increases (Table 13c), but again Laboratory #6 only tested the three intermediate doses, but not the highest dose in the NP series. In Protocol D, both laboratories achieved statistically significant increases.

69. In the case of NP, the oral gavage response (Protocols A and E) was not substantially different from subcutaneous administration (Protocols B, C, D) in the doses first producing statistically significant increases (80 and 75 mg/kg/d, respectively) or in the magnitude of the relative response. Extending the dosing to 7 days did appear to reduce the dose at which statistical significance was achieved and to increase the relative response at a given dose.

Table 13a. Nonylphenol: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol A

mg/kg/d	**Lab. #4**	**Lab. #7[+]**	**Lab. #9**	**Lab. #12**
15	1.07 (0.80 - 1.43)	1.06 (0.87 - 1.30)	1.20 (0.94 - 1.54)	ND
75	1.67 (1.26 - 2.22)*	1.46 (1.19 - 1.80)*	1.42 (1.11 - 1.81)*	1.96 (1.45 - 2.64)*
125	1.91 (1.40 - 2.61)*	1.62 (1.32 - 1.98)*	2.02 (1.59 - 2.58)*	1.85 (1.41 - 2.42)*
250	2.61 (1.69 - 4.04)* [2] [b]	2.17 (1.72 - 2.74)*	2.17 (1.72 - 2.74)*	2.95 (2.02 - 4.32)* [4] [b]
350	All died [6] [b]	2.32 (1.71 - 3.14)*	2.61 (1.61 - 4.23)* [3] [b]	ND
EE-0.3[a]	ND	1.88 (1.55 - 2.29)*	ND	ND
EE-1.0[a]	3.20 (2.36 - 4.35)*	3.15 (2.57 - 3.85)*	2.19 (1.72 - 2.79)^*	2.85 (2.21 - 3.67)^*
EE-3.0[a]	4.04 (2.97 - 5.48)*	ND	5.19 (4.10 - 6.58)^*	4.68 (3.63 - 6.02)^*

For footnotes see after Table 13d.

Table 13b. Nonylphenol: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol B

mg/kg/d	Lab. #4	Lab. #6	Lab. #7	Lab. #8
5	1.04 (0.73 - 1.48)	ND	1.05 (0.85 - 1.30)	0.93 (0.74 - 1.16)
15	0.97 (0.68 - 1.38)	0.84 (0.62 - 1.13)	1.22 (0.99 - 1.50)	1.02 (0.82 - 1.28)
35	1.16 (0.81 - 1.65)	1.03 (0.76 - 1.40)	1.12 (0.91 - 1.39)	1.15 (0.92 - 1.44)
80	2.05 (1.44 - 2.92)*	1.24 (0.91 - 1.68)	1.68 (1.36 - 2.08)*	1.44 (1.15 - 1.80)*
100	1.72 (1.21 - 2.46)*	ND	2.25 (1.83 - 2.78)*	1.54 (1.23 - 1.93)*
EE-0.3[a]	2.75 (1.86 - 4.06)*	1.59 (1.15 - 2.18)^* [1] [b]	1.79 (1.52 - 2.10)*	2.65 (2.37 - 2.97)^*
EE-1.0[a]	4.52 (3.06 - 6.67)*	2.30 (1.71 - 3.10)^*	4.16 (3.53 - 4.90)*	4.96 (4.43 - 5.55)^*
mg/kg/d	**Lab. #9**	**Lab. #12**	**Lab. #15**	**Lab. #18**
5	1.06 (0.83 - 1.34)	ND	0.94 (0.70 - 1.26)	0.88 (0.71 - 1.08)
15	1.11 (0.88 - 1.41)	1.05 (0.76 - 1.44)	0.82 (0.61 - 1.10)	0.90 (0.72 - 1.12)
35	1.33 (1.05 - 1.70)*	1.31 (0.95 - 1.80)	0.87 (0.65 - 1.17)	1.12 (0.89 - 1.40)
80	1.86 (1.47 - 2.36)*	2.02 (1.49 - 2.75)*	1.22 (0.91 - 1.65)	1.93 (1.56 - 2.39)*
100	2.38 (1.87 - 3.03)*	ND	1.52 (1.13 - 2.05)*	2.92 (2.32 - 3.69)*
EE-0.3[a]	4.22 (3.63 - 4.91)^*	1.68 (1.11 - 2.53)^*	4.45 (3.46 - 5.71)^*	3.81 (3.28 - 4.50)^*
EE-1.0[a]	4.26 (3.64 - 4.98)^*	3.64 (2.43 - 5.45)^*	4.95 (3.66 - 6.69)^*	5.62 (4.89 - 6.49)^*
mg/kg/d	**Lab. #20**	**Lab. #21**		
5	0.68 (0.46 - 1.00)	1.39 (1.18 - 1.89)*[#]		
15	0.62 (0.42 - 0.91)	1.34 (0.93 - 1.82)[#]		
35	0.68 (0.46 - 1.01)	1.44 (1.06 - 1.96)*[#]		
80	0.75 (0.51 - 1.11)	1.58 (1.16 - 2.15)*[#]		
100	0.71 (0.48 - 1.05)	1.25 (0.92 - 1.70)[#]		
EE-0.3[a]	1.83 (1.45 - 2.31)^*	3.52[++]		
EE-1.0[a]	2.38 (1.90 - 2.99)^*	1.78[++]		

For footnotes see after Table 13d.

Table 13c. Nonylphenol: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocol C

mg/kg/d	Lab. #6	Lab. #7	Lab. #8	Lab. #9
5	ND	1.21 (0.97 - 1.50)	0.97 (0.81 - 1.15)	1.00 (0.81 - 1.24)
15	1.02 (0.79 - 1.30)	1.12 (0.90 - 1.39)	0.98 (0.83 - 1.17)	0.99 (0.80 - 1.23)
35	1.14 (0.89 - 1.46)	1.27 (1.02 - 1.58)*	1.15 (0.96 - 1.36)	1.08 (0.88 - 1.34)
80	1.16 (0.90 - 1.48)	1.64 (1.32 - 2.03)*	1.17 (0.98 - 1.39)	1.23 (0.99 - 1.52)
100	ND	1.52 (1.22 - 1.89)*	1.42 (1.19 - 1.69)*	1.61 (1.30 - 1.99)*
EE-0.3[a]	2.43 (1.55 - 3.82)^*	2.71 (2.27 - 3.24)*	2.16 (1.91 - 2.43)^*	ND
EE-1.0[a]	3.89 (2.45 - 6.17)^*	4.32 (3.55 - 5.25)*	2.70 (2.39 - 3.05)^*	ND
mg/kg/d	**Lab. #12**			
5	ND			
15	1.09 (0.83 - 1.41)			
35	1.08 (0.83 - 1.41)			
80	1.33 (1.02 - 1.73)*			
100	ND			
EE-0.3[a]	1.95 (1.52 - 2.49)^*			
EE-1.0[a]	3.08 (2.41 - 3.94)^*			

For footnotes see after Table 13d.

Table 13d. Nonylphenol: Inter-laboratory dose-response comparison of blotted uterine weight increase using Protocols D and E

	Protocol D			Protocol E	
mg/kg/d	**Lab. #7**	**Lab. #9**		**mg/kg/d**	**Lab. #12**
5	1.02 (0.84 - 1.25)	1.04 (0.87 - 1.26)		15	ND
15	1.08 (0.88 - 1.31)	1.15 (0.95 - 1.38)		75	1.60 (1.23 - 2.09)*
35	1.31 (1.08 - 1.60)*	1.38 (1.14 - 1.66)*		125	1.74 (1.34 - 2.27)*
80	2.11 (1.73 - 2.58)*	1.83 (1.51 - 2.21)*		250	1.76 (1.34 - 2.32)*
100	1.96 (1.60 - 2.40)*	2.08 (1.71 - 2.51)*		350	
EE-0.3[a]	3.28 (2.48 - 4.35)*	4.14 (3.34 - 5.13)^*		EE-1.0[a]	ND
EE-1.0[a]	5.67 (4.15 - 7.74)*	4.68 (3.66 - 5.99)^*		EE-3.0[a]	ND

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval)

[a] EE doses in µg/kg/day

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; ^ shared EE controls

+ one vehicle control animal died prior to necropsy

[++], body weights at necropsy were not recorded, therefore body weights were not used as a covariable, and confidence intervals were not calculated.

[#], calculations based on body weight at day 3 of dosing

Data for the Reference Agonist, 17α-Ethinyl oestradiol

70. 17α-Ethinyl oestradiol (EE) (Tables 14a, 14b). The selected doses of EE produced statistically significant dose-related increases in blotted uterine weights gains in all laboratories and all protocols with the following exceptions:

- Laboratory #9 (Protocol C) did not run concurrent EE controls.
- Laboratory #7 (Protocol A) used 0.3 and 1 µg EE/kg, instead of 1 and 3 mg/kg. 0.3 µg/kg EE was not statistically in the GEN experiment, but was in the NP experiment. This is consistent with the MED results from Phase-1.
- Laboratory #1 (Protocol A) did not achieve statistical significance with 1 µg/kg EE in two experiments, but did with 3 µg/kg EE in both.
- No concurrent EE controls were run in Protocol E (Laboratory #12).

71. The following tables report the ratio of blotted uterine weight mean to vehicle control uterine weight mean after log transformation of the data and with body weights at necropsy as a covariable. The lower and the upper 95% confidence intervals are reported in parentheses.

Table 14a. Blotted uterine weight increases in response to the low dose of ethinyl oestradiol used as the positive reference dose in the dose-response studies

Dose	1.0 µg EE/kg/day by oral gavage	0.3 µg EE/kg/day by subcutaneous injection		
Lab.	Protocol A	Protocol B	Protocol C	Protocol D
1 [a]	0.99 (0.77 - 1.26)[b]	2.49 (2.12 - 2.93)*	2.14 (1.88 - 2.43)*	2.75 (2.30 - 3.30)*
1	1.10 (0.92 - 1.32)	2.17 (1.80 - 2.62)*	1.98 (1.70 - 2.30)*	2.93 (2.38 - 3.60)*
2	3.17 (2.52 - 3.99)*	2.11 (1.70 - 2.62)*	2.41 (2.06 - 2.81)*	3.71 (2.91 - 4.74)*
3	2.25 (1.77 - 2.86)*	2.00 (1.68 - 2.38)*	2.43 (2.14 - 2.77)*	2.77 (2.44 - 3.14)*
3	1.53 (1.20 - 1.96)*	2.31 (1.89 - 2.81)*	2.96 (2.36 - 3.71)*	2.85 (2.50 - 3.26)*
4	3.20 (2.36 - 4.35)*	2.75 (1.86 - 4.06)*		
5	1.40 (1.04 - 1.90)*	++		
6		1.59 (1.15 - 2.18)*	2.43 (1.55 - 3.92)*	
7	2.16 (1.73 - 2.69)*	1.73 (1.48 - 2.01)*	1.78 (1.47 - 2.16)*	2.45 (1.97 - 3.05)*
7	3.15 (2.57 - 3.85)*	1.79 (1.52 - 2.10)*	2.71 (2.27 - 3.24)*	3.28 (2.48 - 4.35)*
8	3.09 (2.55 - 3.73)*	2.65 (2.37 - 2.97)*	2.16 (1.91 - 2.43)*	
9	2.19 (1.72 - 2.79)*	4.22 (3.63 - 4.91)*		4.14 (3.34 - 5.13)*
11	3.04 (2.42 - 3.83)*	3.50 (2.83 - 4.34)*	3.04 (2.63 - 3.51)*	5.16 (3.65 - 7.28)*
12	2.85 (2.21 - 3.67)*	1.68 (1.11 - 2.53)*	1.95 (1.52 - 2.49)*	
13	1.44 (1.06 - 1.95)*	1.61 (1.08 - 2.42)*		
14	3.11 (2.44 - 3.98)*	2.61 (2.05 - 3.32)*		
15		4.45 (3.46 - 5.71)*		
18		3.81 (3.28 - 4.50)*		
20		1.83 (1.45 - 2.31)*		
21		3.19*		
21		3.52*		

For footnotes, see after Table 14b.

Table 14b. Blotted uterine weight increase in response to the high dose of ethinyl oestradiol used as the positive reference dose in the dose-response studies

Dose	3.0 µg EE/kg/d by oral gavage	1.0 µg EE/kg/day by subcutaneous injection		
Lab.	Protocol A	Protocol B	Protocol C	Protocol D
1 [a]	1.50 (1.25 - 1.79)* [b]	4.19 (3.47 - 5.05)*	3.13 (2.66 - 3.67)*	4.40 (3.45 - 5.61)*
1	1.64 (1.29 - 2.10)*	4.07 (3.46 - 4.80)*	3.77 (3.27 - 4.35)*	4.11 (3.36 - 5.04)*
2	4.13 (3.27 - 5.22)*	4.44 (3.60 - 5.48)*	3.19 (2.69 - 3.78)*	4.86 (3.55 - 6.64)*
3	2.55 (2.00 - 3.25)*	3.82 (3.17 - 4.60)*	3.57 (3.06 - 4.18)*	3.67 (3.13 - 4.29)*
3	2.80 (2.20 - 3.58)*	3.79 (3.11 - 4.63)*	3.61 (2.84 - 4.59)*	4.04 (3.49 - 4.69)*
4	4.04 (2.97 - 5.48)*	4.52 (3.06 - 6.67)*		
5	1.91 (1.41 - 2.59)*	3.61 (2.91 - 4.46)*		
6		2.30 (1.71 - 3.10)*	3.89 (2.45 - 6.17)*	
7		4.06 (3.49 - 4.72)*	3.29 (2.69 - 4.01)*	4.50 (3.53 - 5.73)*
7		4.16 (3.53 - 4.90)*	4.32 (3.55 - 5.25)*	5.67 (4.15 - 7.74)*
8	4.69 (3.88 - 5.66)*	4.96 (4.43 - 5.55)*	2.70 (2.39 - 3.05)*	
9	5.19 (4.10 - 6.58)*	4.26 (3.64 - 4.98)*		4.68 (3.66 - 5.99)*
11	4.52 (3.54 - 5.78)*	4.58 (3.70 - 5.69)*	3.97 (3.36 - 4.69)*	5.85 (4.26 - 8.05)*
12	4.68 (3.63 - 6.02)*	3.64 (2.43 - 5.45)*	3.08 (2.41 - 3.94)*	
13	2.55* (2.06 - 3.17)*	3.44 (2.25 - 5.27)*		
14	4.69 (3.74 - 5.89)*	4.55 (3.59 - 5.76)*		
15		4.95 (3.66 - 6.69)*		
18		5.62 (4.89 - 6.49)*		
20		2.38 (1.90 - 2.99)*		
21		1.97*		
21		1.78*		

[a] When a laboratory ran more than one reference control, the experiments are recorded separately.
[b] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval)
Superscript numbers indicate the numbers of animals that died prior to necropsy or were not weighed
*, significant increase at $p<0.05$
[++], body weights at necropsy were not recorded, therefore body weights were not used as a covariable, and confidence intervals were not calculated.

Minimal Effective Dose

72. The minimal effective dose (MED) for each chemical in all laboratories and Protocols A, B, C, and D are shown in Tables 15a-15e. The MED is defined as the lowest dose concentration in a series that achieved statistical significance. In addition, after calculation of the MED, the data for each protocol were pooled and a mixed effects linear model was used in order to calculate an overall protocol mean for the relative increase in uterine weight, an overall protocol lower and upper 95% confidence levels, and an overall protocol minimal effective dose. The mixed effects linear models are referred to as the "Global Analysis" and the results are placed at the bottom of each table.

73. For BPA, the MED for Protocols C and D is 100 mg/kg/d across all laboratories; the MED for Protocol A are within a 0.5 log range (375-1000 mg/kg/d); and the MEDs for Protocol B have the widest range from 10 to 600 mg/kg/d. Thus, Protocol B would be judged to be slightly less sensitive and more variable than Protocols C and D.

Table 15a. Minimal effective doses (mg/kg/d) and the corresponding uterine weight increase for Bisphenol A in the dose response studies

Lab	Protocol[a]			
	A	**B**	**C**	**D**
2	375 1.26 (1.06 - 1.50)	100 1.67 (1.37 - 2.02)	100 1.89 (1.55 - 2.30)	100 2.53 (1.99 - 3.21)
6	- - - -	300 1.37 (1.05 - 1.79)	100 2.05 (1.58 - 2.66)	- - - -
7	600 1.31 (1.03 - 1.66)	100 1.31 (1.10 - 1.56)	100 1.67 (1.38 - 2.01)	100 2.35 (2.00 - 2.77)
8	- - - -	100 1.47 (1.15,1.87)	100 1.60 (1.31,1.96)	- - - -
12	375 1.36 (1.05 - 1.76)	600 2.51 (1.70 - 3.70)	100 2.03 (1.53 - 2.70)	- - - -
13	1000 1.57 (1.18 - 2.08)	300 1.72 (1.08 - 2.76)	- - - -	- - - -
15	- - - -	300 1.37 (1.03 - 1.81)	- - - -	- - - -
18	- - - -	10 1.28 (1.08,1.51)	- - - -	- - - -
20	- - - -	10[b] 1.75 (1.26 - 2.43)	- - - -	- - - -
Global analysis	600 1.41 (1.07 - 1.85)	300 1.61 (1.00 - 2.58)	100 1.83 (1.39 - 2.42)	100 2.42 (1.95 - 3.00)

[a] Protocol E was run in a single lab p.o. (like Protocol A), there was no MED up to 600 mg/kg/d and the highest dose of 1000 mg/kg/d was not administered.
[b]See Table 9b, if the doses were indeed reversed in Laboratory #20, then the MED would be 600 mg/kg/d.

74. For *o,p'*-DDT, the MED for Protocols A was the lowest (10-50 mg/kg/d), Protocols C and D were similar (50-200 mg/kg/d), and Protocol B had somewhat higher MEDs (100-200 mg/kg/d). Absent the testing of the highest dose in Laboratory #12, similar performance in Protocol C cannot be excluded. In the global analysis, Protocol B was again slightly less sensitive compared to Protocols C and D. More significantly, the oral route of administration was more sensitive than subcutaneous injection for *o,p'*-DDT.

Table 15b. Minimal effective doses (mg/kg/d) and the corresponding uterine weight increase for *o,p'*-DDT in the dose response studies

Lab	Protocol[a]			
	A	**B**	**C**	**D**
3	50 1.60 (1.24 - 2.05)	200 1.31 (1.07,1.59)	50 1.30 (1.11 - 1.53)	100 1.18 (1.03 - 1.36)
5	50 1.53 (1.18 - 1.99)	200 1.41 (1.09 - 1.84)	- - - -	- - - -
11	10 1.21 (1.04,1.41)	200 1.36 (1.10,1.68)	200 1.34 (1.05 - 1.71)	50 1.34 (1.07 ,1.68)
12	50 2.61 (2.01 - 3.4)	100 1.47 (1.11 - 1.94)	No dose was Significant[b]	- - - -
Global analysis	50 1.95 (1.49 - 2.54)	200 1.38 (1.10 - 1.74)	100 1.33 (1.04 - 1.69)	100 1.31 (1.08 - 1.59)

[a] Protocol E was run in a single lab p.o. (like Protocol A), the MED was 50 mg/kg/d and the lowest dose of 10 mg/kg/d was not administered.
[b] Did not administer the highest dose, only the three intermediate doses were used.

For genistein, the MEDs for Protocols B, C and D are 1-35 mg/kg/d and 20-60 mg/kg/d for Protocol A. Subcutaneous administration was somewhat more sensitive than oral gavage.

Table 15c. Minimal effective doses (mg/kg/d) and the corresponding uterine weight increase for genistein in the dose response studies

Lab	Protocol[a]			
	A	**B**	**C**	**D**
1	20 1.42 (1.08 - 1.85)	15 1.79 (1.35 - 2.38)	15 1.53 (1.25 - 1.88)	15 1.67 (1.35 - 2.07)
8	60 1.61 (1.33 - 1.96)	15 2.1 (1.71 - 2.58)	- - - -	- - - -
9	20 1.36 (1.07 - 1.71)	1 1.18 (1.00 - 1.38)	15 1.57 (1.32 - 1.88)	15 2.06 (1.74 - 2.43)
12	60[b] 2.49 (1.97 - 3.15)	15 1.48 (1.10 - 1.98)	35 1.56 (1.09 - 2.21)	- - - -
Global analysis	60 1.99 (1.44 - 2.75)	15 1.83 (1.34 - 2.49)	15 1.48 (1.24 - 1.77)	15 1.86 (1.27 - 2.72)

[a] Protocol E was run in a single lab p.o. (like Protocol A), the MED was 60 mg/kg/d and the lowest dose of 20 mg/kg/d was not administered.
[b] Did not administer the lowest dose of 20 mg/kg/d, only the three intermediate doses were used.

75. For methoxychlor, the actual MEDs for Protocol A are less than 20 mg/kg/d based on the high relative responses and the lack of testing in Laboratory #12 of the lower 20 mg/kg/d dose. While acknowledging the results of the Global Analysis, Protocols B, C, and D would appear to be similar in sensitivity.

Table 15d. Minimal effective doses (mg/kg/d) and the corresponding uterine weight increase for methoxychlor in the dose response studies

Lab	Protocol[a]			
	A	**B**	**C**	**D**
1	20 1.98 (1.65 - 2.39)	100 1.36 (1.05 - 1.76)	100 1.28 (1.03 - 1.59)	100 1.33 (1.02 - 1.75)
3	20 1.88 (1.52 - 2.31)	100 2.21 (1.80 - 2.71)	100 1.72 (1.41 - 2.10)	100 1.55 (1.27 - 1.90)
12	50[b] 3.71 (2.87 - 4.79)	100 2.86 (2.18 - 3.77)	100 1.63 (1.21 - 2.19)	- - - -
14	20 3.14 (2.31 - 4.26)	100 1.62 (1.20 - 2.19)	- - - -	- - - -
Global analysis	20 2.23 (1.42 - 3.49)	100 1.94 (1.06 - 3.57)	100 1.54 (1.23 - 1.93)	250 2.06 (1.31 - 3.23)

[a] Protocol E was run in a single lab p.o. (like Protocol A), the MED was 50 mg/kg/d and the lowest dose of 20 mg/kg/d was not administered.
[b] Did not administer the lowest dose of 20 mg/kg/d, only the three intermediate doses were used.

76. For nonylphenol, the MEDs for Protocols B and C were similar; the MED for Protocol D was lower suggesting that extending dosing was beneficial in this case; and the MEDs for oral gavage in Protocol A were not substantially different from subcutaneous administration. Where differences in the MED existed in Protocols B and C, these were within 3- to 4-fold.

Table 15e. Minimal effective doses (mg/kg/d) and the corresponding uterine weight increase for nonylphenol in the dose response studies

Lab	Protocol[a]			
	A	**B**	**C**	**D**
4	75 1.67 (1.26 - 2.22)	80 2.05 (1.44 - 2.92)	- - - -	- - - -
6	 - -	No dose was Significant[b]	No dose was Significant[b]	- - - -
7	75 1.46 (1.19 - 1.80)	80 1.68 (1.36 - 2.08)	35 1.27 (1.02 - 1.58)	35 1.31 (1.08 - 1.60)
8	- - - -	80 1.44 (1.15 - 1.80)	100 1.42 (1.19 - 1.69)	- - - -
9	75 1.42 (1.11 - 1.81)	35 1.33 (1.05,1.70)	100 1.61 (1.30 - 1.99)	35 1.38 (1.14,1.66)
12	75 1.96 (1.45 - 2.64)	80 2.02 (1.49 - 2.75)	80 1.33 (1.02 - 1.73)	- - - -
15	- - - -	100 1.52 (1.13 - 2.05)	- - - -	- - - -
18	- - - -	80 1.93 (1.56 - 2.39)	- - - -	- - - -
20	- - - -	No dose was significant	- - - -	- - - -
Global analysis	75 1.64 (1.32 - 2.05)	80 1.57 (1.10 - 2.26)	80 1.29 (1.06 - 1.58)	35 1.34 (1.09 - 1.66)

[a] Protocol E was run in a single lab p.o. (like Protocol A), the MED was 75 mg/kg/d and the lowest dose of 15 mg/kg/d was not administered.

[b] Did not administer the highest dose, only the three intermediate doses were used.

Summary of the dose-response studies

77. A total of 86 chemical/laboratory/protocol dose-response combinations were performed (Tables 9-13). There was good agreement among laboratories, and across protocols, with regard to the results obtained. All laboratories reported statistically significant results in at least one dose for all chemicals in all protocols, with the five following exceptions: Laboratory #12, Protocol E (BPA); Laboratory #12, Protocol C (DDT); Laboratory #6, Protocols B and C (NP); and Laboratory #20, Protocol B (NP).

78. It should be noted that Laboratories #6 and 12 tested the three intermediate of the five designated doses, and did not test the highest dose. The lower 95% confidence level had to equal or exceed a value of 1 to achieve statistical significance, and this value at the next to highest dose was 0.90, 0.91, 0.94, and 0.96 in these four cases. The coefficient of variation values for the vehicle controls in these cases was somewhat higher than average: 17.3, 24.1, 17.7, and 22.4%, which would have reduced the power. No animal mortalities occurred in these groups to reduce the power.

79. In the case of NP Laboratory #20, all relative responses with the test substance were ≤ 0.75 and one response was significantly less than the control (see Table 13b)! An examination of the data showed a control blotted mean weight of 54.3 mg and individual values of 40.8, 44.6, 49.2, 54.9, 65.6 and 70.5 mg, substantially above the means for most other vehicle controls (the group was number 58 out of 60 when ranked in ascending order). As a result, this control group and the results calculated from it are considered an anomaly.

80. An analysis of the MED or the lowest dose of a test substance producing a statistically significant effect reinforces the conclusion of agreement and reproducibility among the laboratories. In several protocols, there was no difference observed or the doses were within a 3- to 4-fold range despite differences in rat strains, diets, and other variables.

81. An additional observation from these MED data is that a blotted uterine weight increase of about 30% or more relative to controls results in statistical significance with a group size of six in a number of cases.

82. The MED calculated for the five weak agonists were substantially higher than for the potent reference EE. In Phase-1, the MED for EE in Protocol A was 0.3-1.0 μg/kg/d and in Protocols B, C, and D was 0.1-0.3 μg/kg/d. In Phase-2, the MEDs for the weak agonists in Protocol A ranged from < 20 mg/kg/d for MC to 600 mg/kg/d for BPA; the latter MED being approximately 600,000-fold higher than for EE by the same route of exposure. This indicates the uterotrophic bioassay is capable of operating over an extensive range and can address substances up to a limit dose of 1000 mg/kg/d or the MTD, whichever comes first.

83. No animal model (i.e., immature or OVX), protocol or route of administration was clearly superior. The MEDS achieved by oral administration in Protocols A (immature) and E (OVX) were similar with all compounds, and the MEDS achieved by SC administration in Protocols B (immature) and C and D (OVX) were similar. The specific characteristics of the individual chemical were equally or more important. For BPA, Protocol C appeared to be somewhat more sensitive than B. However, with other chemicals, such as GEN and MC, Protocol B seemed somewhat more sensitive. Similarly, oral administration in Protocol A appeared to achieve higher and earlier responses with MC and DDT, subcutaneous administration achieved higher and earlier responses with GEN and BPA, and subcutaneous administration was only modestly better with NP. Extending administration to seven consecutive days (Protocol D) showed no significant advantage over the 3-day treatment. While increasing the relative response slightly with BPA, no advantage of extending dosing in the magnitude of the response or the MED was obvious with the other weak agonists. In addition, the seven-day treatment protocol would have a modest disadvantage of higher costs as a result of the additional dosing and additional animal maintenance time. The seven-day treatment protocol cannot be recommended for routine use, but may be useful for chemicals that require longer dosing times to reach effective body burden concentrations or to induce specific metabolic enzymes.

Coded single-dose studies

84. The laboratories participating in the coded single-dose studies and the protocols they used were described in Table 2, and the absolute mean wet and blotted uterine weights and the body weights are in Annex 4. The 16 laboratories were to test all five weak agonists (BP, DDT, GEN, MC, NP) and the negative chemical (DBP) under code, and two doses of EE. The identity of EE was known because the laboratories were required to make two dilutions to obtain the high and low doses.

85. Specific instructions as to dosage formulation, preparation, and dilution accompanied the test samples to laboratories because this segment of the work was being carried out under code. However, two laboratories reported dose preparation difficulties, where the substances were difficult to solubilise in their vehicles. The laboratories chose not to administer the resulting suspensions. Thus, Laboratory #19 did not test BP and GEN in Protocol B and GEN and MC in Protocol C, and Laboratory #17 did not test GEN in Protocol B.

86. The summary single-dose (CSD) data for all laboratories and coded chemicals are in Tables 16 - 21. As with the dose-response data, the results are reported as the ratio of the blotted uterine weight mean

for the test substance group relative to the mean uterus weight of the vehicle group adjusted for body weight as a covariable. The 95% upper and lower confidence levels for this ratio are reported, respectively, in parentheses. If statistical significance was achieved, then the data are marked with an asterisk. Other considerations such as animal mortality are noted. The absolute wet and blotted uterine weights and the body weights are reported in Annex 4.

87. As noted previously, the doses were selected based on the judgement that they would correspond to an ED_{50} or an ED_{80} dose. This was not always the case. For each chemical and protocol in the following sections, the selected dose will be compared to the MED seen in the dose response study. This will provide a necessary perspective on whether some, most or all laboratories should have achieved statistical significance because the proportion achieving statistical significance should increase as the administered dose rises up the dose response curve from the MED.

Results for each individual test substance in the coded single-dose studies

88. Bisphenol A (Table 16). The administered dose for BPA in Protocol A was 600 mg/kg/d, and the MED based on the mixed effect linear models of the pooled data, referred to as the global analysis, was also 600 mg/kg/d. Four laboratories out of ten failed to achieve statistical significance. In three of these laboratories, the lower 95% confidence level was 0.90 or greater, and the group size was reduced by 2 treatment related mortalities in Laboratory #14 and 3 in Laboratory #12. The administered doses for Protocols B, C, and D were 300 mg/kg/d, and the MEDs were 300, 100, and 100 mg/kg/d, respectively. In Protocol B, only Laboratory #20 failed to get a statistically significant response and there were no mortalities. All laboratories using Protocols C and D had statistically significant responses. The mean relative ratio for Protocol A was only 1.37, and among the subcutaneous injection the protocols the means were nearly a full unit apart with B < C <D.

Table 16. Bisphenol A: Summary responses of mean blotted uterine weight increases in the coded single-dose study

Lab. #	Protocol (dose)			
	A (600 mg/kg)	**B (300 mg/kg)**	**C (300 mg/kg)**	**D (300 mg/kg)**
1	1.11 (0.90 - 1.37) [a]	1.58 (1.21 - 2.08)*	2.61 (2.23 - 3.06)*	3.65 (2.84 - 4.68)*
2	1.45 (1.14 - 1.84)*	1.77 (1.40 - 2.24)*	2.61 (1.99 - 3.42)*	3.91 (3.21 - 4.76)*
3	1.40 (1.14 - 1.73)*	2.00 (1.62 - 2.48)*	2.89 (2.24 - 3.72)*	3.26 (2.51 - 4.24)*
4	1.36 (1.05 - 1.74)*	1.45 (1.08 - 1.94)*	ND	ND
5	1.23 (0.95 - 1.57)	2.02 (1.64 - 2.49)*	ND	ND
8	1.91 (1.58 - 2.31)*	1.91 (1.53 - 2.39)*	2.89 (2.16 - 3.88)*	ND
11	1.41 (1.11 - 1.80)*	1.82 (1.36 - 2.44)*	3.39 (2.85 - 4.05)*	4.05 (3.08 - 5.33)*
12	1.08 (0.43 - 2.71) [3] [b]	1.60 (1.12 - 2.31)*	2.30 (1.67 - 3.18)*	ND
13	1.25 (1.01 - 1.56)* [1] [b]	1.52 (1.11 - 2.08)*	ND	ND
14	1.50 (0.95 - 2.37) [2] [b]	2.82 (2.05 - 3.87)*	ND	ND
16	ND	2.11 (1.37 - 3.22)*	ND	ND
17	ND	2.35 (1.64 - 3.37)*	ND	ND
18	ND	2.32 (1.88 - 2.86)*	ND	ND
19	ND	ND	2.42 (1.99 - 2.95)*	ND
20	ND	1.11 (0.86 - 1.44)	ND	ND
21	ND	1.67[++]	ND	ND
mean[+]	**1.37 ± 0.22**	**1.88 ± 0.41**	**2.73 ± 0.34**	**3.72 ± 0.30**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; [+] mean of uterine weight ratios ± standard deviation

[++], body weights at necropsy were not recorded, therefore body weights were not used as a covariable, and confidence intervals were not calculated.

89. Dibutyl phthalate (Table 17). As DBP was not tested in the dose response and was the negative chemical, there was no MED. For DBP, the overall relative ratio mean was approximately one in all protocols. Protocols A and D were uniformly negative with no instances of statistical significance. Laboratory #12 had increased upper and lower 95% confidence levels in Protocol A apparently due to a decreased group size from 3 mortalities. In Protocols B and C, five laboratories had statistically significant changes from the vehicle control group. In Protocol B, Laboratories #11 and 14, and, in Protocol C, Laboratory #1 had statistically significant increases with DBP. The ratios were 1.39, 1.38, and 1.37 and the lower 95% confidence levels were 1.06, 1.01, and 1.17, respectively. In Protocol B, Laboratories #18 and 20 had statistically significant decreases. The ratios were 0.74 and 0.74 and the upper 95% confidence levels were 0.91 and 0.96, respectively. While these instances of statistical significance are marginal, they do indicate that the uterotrophic bioassay is likely to encounter both false positive and false negative events.

Table 17. Dibutyl phthalate: Summary responses of mean blotted rat uterine weight increase in the coded single-dose study

Lab. #	Protocol (dose)			
	A (1000 mg/kg)	**B (500 mg/kg)**	**C (500 mg/kg)**	**D (500 mg/kg)**
1	0.91 (0.74 - 1.13) [a]	0.97 (0.74 - 1.28)	1.37 (1.17 - 1.61)*	0.91 (0.73 - 1.15)
2	0.99 (0.78 - 1.26)	1.05 (0.83 - 1.31)	0.99 (0.76 - 1.28)	1.03 (0.86 - 1.24)
3	1.00 (0.81 - 1.23)	1.01 (0.81 - 1.25)	0.90 (0.70 - 1.15)	1.01 (0.81 - 1.26)
4	0.99 (0.77 - 1.28)	0.85 (0.64 - 1.14)	ND	ND
5	1.03 (0.81 - 1.32)	1.06 (0.86 - 1.31)	ND	ND
8	0.98 (0.81 - 1.18)	1.00 (0.80 - 1.18)	1.24 (0.92 - 1.65)	ND
11	0.95 (0.75 - 1.22)	1.39 (1.06 - 1.82)*	0.99 (0.83 - 1.16)	1.00 (0.78 - 1.28)
12	0.91 (0.38 - 2.20) [3] [b]	0.97 (0.67 - 1.40)	0.93 (0.68 - 1.26)	ND
13	0.86 (0.69 - 1.07)	0.98 (0.72 - 1.34)	ND	ND
14	0.91 (0.60 - 1.38)	1.38 (1.01 - 1.89)*	ND	ND
17	ND	0.88 (0.62 - 1.26)	ND	ND
16	ND	0.90 (0.59 - 1.38)	ND	ND
18	ND	0.74 (0.60 - 0.91)#	ND	ND
19	ND	0.75 (0.47 - 1.20)	0.84 (0.69 - 1.02)	ND
20	ND	0.74 (0.56 - 0.96)#	ND	ND
21	ND	1.23[++]	ND	ND
mean[+]	**0.95 ± 0.05**	**0.97 ± 0.20**	**1.04 ± 0.18**	**0.99 ± 0.05**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval)

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; [+] mean of uterine weight ratios ± standard deviation #, significantly decreased at $p<0.05$

90. *o,p'*-DDT (Table 18). The administered dose for DDT in Protocol A was 300 mg/kg/d, and the MED based on the global analysis was 50 mg/kg/d. All laboratories achieved statistical significance even though three of six animals died in Laboratory #14. The administered doses for Protocols B, C, and D were 100 mg/kg/d, and the dose response MEDs were 200, 100, and 100 mg/kg/d, respectively. In Protocol B, 10 of 14 laboratories failed to get a statistically significant response, and there were no mortalities. In Protocols C and D, 2 of 6 and 1 of 4 laboratories failed to achieve statistical significance,

respectively. The mean relative ratio for Protocol A was 3.55, and among the subcutaneous injection the protocols the means were 1.27, 1.26, and 1.18 for B, C, and D, respectively.

Table 18. o,p'-DDT: Summary responses of mean blotted uterine weight increase in the coded single-dose study

Lab. #	Protocol (dose)			
	A (300 mg/kg)	**B (100 mg/kg)**	**C (100 mg/kg)**	**D (100 mg/kg)**
1	2.70 (2.15 - 3.39)* [a]	2.05 (1.57 - 2.70)*	1.65 (1.41 - 1.93)*	1.05 (0.83 - 1.31)
2	3.68 (2.88 - 4.71)*	1.13 (0.90 - 1.42)	1.43 (1.09 - 1.86)*	1.21 (1.01 - 1.46)*
3	3.05 (2.45 - 3.81)*	1.18 (0.95 - 1.46)	1.29 (1.00 - 1.65)*	1.14 (0.92 - 1.43)
4	3.76 (2.91 - 4.87)*	1.57 (1.17 - 2.10)*	ND	ND
5	2.92 (2.23 - 3.83)*	0.95 (0.78 - 1.18)	ND	ND
8	3.87 (3.18 - 4.71)*	1.06 (0.85 - 1.32)	1.17 (0.87 - 1.57)	ND
11	3.58 (2.79 - 4.60)*	1.03 (0.78 - 1.37)	1.24 (1.05 - 1.47)*	1.31 (1.03 - 1.68)*
12	All died [6] [b]	1.50 (1.04 - 2.16)*	0.96 (0.71 - 1.31)	ND
13	4.12 (3.32 - 5.12)*	1.79 (1.31 - 2.45)*	ND	ND
14	4.26 (2.65 - 6.83)* [3] [b]	1.17 (0.85 - 1.60)	ND	ND
16	ND	1.29 (0.84 - 1.97)	ND	ND
17	ND	1.46 (1.02 - 2.08)*	ND	ND
18	ND	0.98 (0.80 - 1.21)	ND	ND
19	ND	1.07 (0.67 - 1.69)	1.06 (0.87 - 1.29)	ND
20	ND	0.79 (0.61 - 1.02)	ND	ND
21	ND	1.49 [++]	ND	ND
mean [+]	**3.55 ± 0.51**	**1.27 ± 0.34**	**1.26 ± 0.21**	**1.18 ± 0.10**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval)

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; [+] mean of uterine weight ratios ± standard deviation

91. Genistein (Table 19). The administered dose for GEN in Protocol A was 300 mg/kg/d, and the MED based on the global analysis was 60 mg/kg/d. The administered doses for Protocols B, C, and D were 35 mg/kg/d, and the dose response MEDs were 15 mg/kg/d in all cases. All laboratories in all protocols achieved statistical significance. The mean relative ratio for Protocol A was 2.72, and among the subcutaneous injection the protocols the means were 2.35, 1.78, and 2.19 for B, C, and D, respectively.

Table 19. Genistein: Summary responses of mean blotted uterine weight increase in the coded single-dose study

Lab. #	Protocol (dose)			
	A (300 mg/kg)	**B (35 mg/kg)**	**C (35 mg/kg)**	**D (35 mg/kg)**
1	2.39 (1.94 - 2.95)* [a]	2.16 (1.64 - 2.84)*	1.67 (1.42 - 1.97)*	2.11 (1.68 - 2.65)*
2	2.47 (1.93 - 3.17)* [1] [b]	2.95 (2.35 - 3.70)*	2.07 (1.59 - 2.70)*	2.33 (1.93 - 2.80)*
3	2.73 (2.21 - 3.36)*	2.69 (2.17 - 3.33)*	1.66 (1.30 - 2.13)*	1.85 (1.48 - 2.32)*
4	2.58 (2.01 - 3.31)*	2.26 (1.69 - 3.03)*	ND	ND
5	1.60 (1.25 - 2.05)*	2.31 (1.88 - 2.85)*	ND	ND
8	3.21 (2.65 - 3.88)*	2.53 (2.03 - 3.15)*	1.95 (1.46 - 2.61)*	ND
11	2.86 (2.25 - 3.65)*	2.38 (1.78 - 3.17)*	1.73 (1.47 - 2.05)*	2.46 (1.93 - 3.14)*
12	3.74 (1.83 - 7.62)*	2.20 (1.53 - 3.17)*	1.57 (1.12 - 2.20)*	ND
13	2.64 (2.17 - 3.22)*	2.25 (1.64 - 3.07)*	ND	ND
14	2.98 (1.93 - 4.61)*	3.44 (2.50 - 4.72)*	ND	ND
16	ND	3.21 (2.10 - 4.89)*	ND	ND
18	ND	2.53 (2.06 - 3.11)*	ND	ND
21	ND	1.78[++]	ND	ND
20	ND	1.32 (1.02 - 1.70)*	ND	ND
mean[+]	**2.72 ± 0.53**	**2.35 ± 0.52**	**1.78 ± 0.18**	**2.19 ± 0.23**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done. *, significant increase at $p<0.05$. [+] mean of uterine weight ratios ± standard deviation.

92. Methoxychlor (Table 20). The administered dose for MC in Protocol A was 300 mg/kg/d, and the MED based on the global analysis was <20 mg/kg/d. The administered doses for Protocols B, C, and D were 500 mg/kg/d, and the dose response MEDs were 100, 100, and 250 mg/kg/d, respectively. All laboratories in all protocols achieved statistical significance. The mean relative ratio for Protocol A was 3.16, and among the subcutaneous injection the protocols the means were 2.84, 2.15, and 2.74 for B, C, and D, respectively.

Table 20. Methoxychlor: Summary responses of mean blotted uterine weight increase in the coded single-dose study

Lab. #	Protocol (dose)			
	A (300 mg/kg)	**B (500 mg/kg)**	**C (500 mg/kg)**	**D (500 mg/kg)**
1	2.97 (2.39 - 3.71)* [a]	2.73 (2.08 - 3.58)*	1.96 (1.67 - 2.30)*	2.23 (1.77 - 2.80)*
2	3.14 (2.41 - 4.00)*	3.01 (2.39 - 3.80)*	2.08 (1.58 - 2.73)*	2.71 (2.21 - 3.31)*
3	2.77 (2.24 - 3.41)*	2.66 (2.15 - 3.29)*	2.11 (1.64 - 2.71)*	2.67 (2.03 - 3.51)*
4	3.01 (2.34 - 3.86)*	3.33 (2.49 - 4.45)*	ND	ND
5	3.10 (2.41 - 3.99)*	3.61 (2.93 - 4.45)*	ND	ND
8	3.71 (3.07 - 4.49)*	2.91 (2.33 - 3.63)*	2.08 (1.54 - 2.80)*	ND
11	3.46 (2.67 - 4.48)*	2.39 (1.81 - 3.16)*	3.14 (2.63 - 3.75)*	3.34 (2.55 - 4.36)*
12	3.20 (1.34 - 7.61)* [3] [b]	3.14 (2.18 - 4.51)*	1.50 (1.09 - 2.03)*	ND
13	3.31 (2.72 - 4.02)*	2.89 (2.11 - 3.96)*	ND	ND
14	2.95 (1.94 - 4.48)*	4.07 (2.97 - 5.56)*	ND	ND
16	ND	4.29 (2.81 - 6.55)*	ND	ND
17	ND	3.25 (2.28 - 4.63)*	ND	ND
18	ND	3.18 (2.59 - 3.90)*	ND	ND
20	ND	1.76 (1.37 - 2.28)*	ND	ND
21	ND	1.96[++]	ND	ND
mean[+]	**3.16 ± 0.26**	**2.84 ± 0.69**	**2.15 ± 0.49**	**2.74 ± 0.40**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; [+] mean of uterine weight ratios ± standard deviation.

93. Nonylphenol (Table 21). The administered dose for NP in Protocol A was 250 mg/kg/d, and the MED based on the global analysis was 75 mg/kg/d. The administered doses for Protocols B, C, and D were 80 mg/kg/d, and the dose response MEDs were 80, 80, and 35 mg/kg/d, respectively. One of 10 laboratories in Protocol A failed to achieved statistical significance. However, seven laboratories had mortalities. In Laboratory #12, which was the lab that did not achieve statistical significance, there were four exposure-related mortalities among six animals sharply reducing the statistical power. Four of 16 laboratories in Protocol B, 1 of 7 laboratories in Protocol C, and 0 of 4 laboratories in Protocol D failed to achieve statistical significance. There were no mortalities to reduce group sizes. The mean relative ratio for Protocol A was 2.07, and among the subcutaneous injection the protocols the means were 1.66, 1.40, and 1.79 for B, C, and D, respectively.

Table 21. Nonylphenol: Summary responses of mean blotted uterine weight increase in the coded single-dose study

Lab. #	Protocol (dose)			
	A (250 mg/kg)	**B (80 mg/kg)**	**C (80 mg/kg)**	**D (80 mg/kg)**
1	1.71 (1.37 - 2.14)* [a]	1.65 (1.26 - 2.17)*	1.43 (1.22 - 1.68)*	1.54 (1.23 - 1.93)*
2	2.03 (1.48 - 2.77)*	1.34 (1.06 - 1.68)*	1.24 (0.95 - 1.62)	1.86 (1.55 - 2.24)*
3	1.80 (1.43 - 2.27)*	1.81 (1.46 - 2.24)*	1.37 (1.07 - 1.76)*	1.73 (1.37 - 2.18)*
4	1.89 (1.24 - 2.88)* [4] [b]	1.45 (1.08 - 1.94)*	ND	ND
5	1.74 (1.28 - 2.35)* [2] [b]	1.64 (1.30 - 2.07)*	ND	ND
8	2.89 (2.33 - 3.57)* [1] [b]	1.32 (1.06 - 1.69)*	1.59 (1.19 - 2.13)*	ND
11	2.33 (1.65 - 3.28)* [2] [b]	2.05 (1.54 - 2.73)*	1.38 (1.16 - 1.63)*	2.02 (1.57 - 2.60)*
12	1.97 (0.73 - 5.33) [4] [b]	1.71 (1.19 - 2.47)*	1.38 (1.01 - 1.90)*	ND
13	2.24 (1.81 - 2.78)* [1] [b]	1.08 (0.79 - 1.48)	ND	ND
14	2.05 (1.17 - 3.59)* [2] [b]	1.72 (1.26 - 2.35)*	ND	ND
16	ND	1.30 (0.85 - 1.99)	ND	ND
17	ND	2.50 (1.74 - 3.55)*	ND	ND
18	ND	1.73 (1.40 - 2.14)*	ND	ND
19	ND	1.22 (0.76 - 1.96)	1.40 (1.15 - 1.70)*	ND
\20	ND	1.07 (0.83 - 1.38)	ND	ND
21	ND	1.04 [++]	ND	ND
mean [+]	**2.07 ± 0.34**	**1.66 ± 0.44**	**1.40 ± 0.10**	**1.79 ± 0.18**

[a] Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval).

[b] Numbers in brackets are the number of animal deaths where uteri were not weighed.

ND, not done; *, significant increase at $p<0.05$; [+] mean of uterine weight ratios ± standard deviation.

Summary of the coded single-dose studies

94. The coded single-doses were selected based on positions presumed to be near the mid-point or in the upper half of a dose-response curve (ED_{50}-ED_{80}). As judgement was used in the selection based on literature values, it is anticipated that there might be some differences in the magnitude of responses among the laboratories when the actual experiments were performed. However, in several cases, the selected doses were at or near the MED, which might have been then in an ED_{10}-ED_{20} range rather than the intended ED_{50}-ED_{80} range. Examples are Protocol A with BPA, Protocols B, C, and D with DDT, and Protocols B and C with NP. As this would place the dose in the low region of the dose response curve, it would be anticipated that some laboratories would not achieve statistical significance. This was, in fact, the result. Despite this, the agreement of laboratories and their repeatability with the same dose in the dose response studies can still be evaluated.

95. There was good agreement and consistency among laboratories within a given chemical and protocol with respect to the relative uterine weight increases. When the coefficients of variations (CV) are calculated and used to assess the agreement within a protocol, Protocols A and D had the lowest CVs, and Protocol B had the highest (Table 22). Protocol B was performed by the largest number of laboratories (17), however, only 9 of these also performed Protocol C. This included the two labs that did not participate in Phase-1 of the validation study. A review of Table 22 does not indicate that any specific chemical had a lower or higher CV than the others. DBP was the negative chemical with a corresponding minimal relative response. The relative response was high with GEN and MC, modest to low with NP, and varied depending upon route of administration with BPA and DDT. There is no evidence in this analysis that the CV of the blotted weight would vary strongly by position on the dose response curve. This needs to be assessed further in a detailed statistical analysis.

Table 22. Summary of the relative uterus weight increase expressed as means ± standard deviations [coefficients of variance] of the coded single-dose responses

	Protocol			
Chemical	**A**	**B**	**C**	**D**
BPA	1.37 ± 0.22 [16]	1.88 ± 0.41 [20]	2.73 ± 0.34 [12]	3.72 ± 0.30 [8]
DBP	0.95 ± 0.05 [5]	0.97 ± 0.20 [21]	1.04 ± 0.18 [17]	0.99 ± 0.05 [5]
DDT	3.55 ± 0.51 [14]	1.27 ± 0.34 [27]	1.26 ± 0.21 [17]	1.18 ± 0.10 [8]
GEN	2.72 ± 0.53 [19]	2.35 ± 0.52 [22]	1.78 ± 0.18 [10]	2.19 ± 0.23 [11]
MC	3.16 ± 0.26 [8]	2.84 ± 0.69 [24]	2.15 ± 0.49 [23]	2.74 ± 0.40 [15]
NP	2.07 ± 0.34 [16]	1.66 ± 0.44 [27]	1.40 ± 0.10 [7]	1.79 ± 0.18 [10]

96. The pattern from the dose response experiments where no protocol or route of administration was clearly superior was repeated in the coded dose experiments. Again, the specific characteristics of the individual chemical were equally or more important. Using the overall means of uterus weight, the relative increase in Protocol C was better than B and extending the time of dosing in Protocol D saw a further increase in the relative ratio. However, with other chemicals, such as GEN and MC, the relative increase with Protocol B seemed somewhat higher than C and no benefit was clearly evident for extending the time of dosing. Similarly, oral administration in Protocol A appeared to achieve higher responses with MC and DDT, subcutaneous administration achieved higher responses with GEN and BPA, and subcutaneous administration was only modestly better with NP considering the differences in doses administered. More detailed comparisons are conducted in the next section.

INTRA- AND INTER-LABORATORY COMPARISONS

97. This section addresses the crucial validation issue of intra- and inter-laboratory reproducibility (reliability). There are both general and specific ways to assess reproducibility. The most relevant approach to address the issue is the reproducibility of responses (positive or negative) where the comparisons can specifically be made and not inferred. This would be for the same protocol and, for more detail, for each specific chemical using the same protocol as first compiled in Tables 9 - 13. In addition, the place of a particular dose on the dose response curve for a chemical should be considered, as there will be increasing uncertainty in the lower portions of the dose response curve.

98. This section assesses reproducibility from the dose response and the coded single-dose experiments in four ways. The first way is the proportion of assays achieving statistical significance when considering the position of the selected dose on the dose response curve (Table 23). The second way is a general approach of pooling data across all laboratories for a given chemical and protocol (Table 24). This would mean including a laboratory that performed only one portion of the overall experiment such as only the coded single-dose experiments for Protocol B. The third way is a specific approach that restricts consideration only to comparable data for example in comparisons of Protocols B and C to only those laboratories performing experiments on a chemical in both Protocols B and C (Tables 25a-e). The fourth way is to examine those instances where the results from the dose response and coded single-dose experiments are statistically significantly different from one another.

99. This section also recognises that the EE results at equivalent doses in the dose-response and coded single-dose studies provides another opportunity to compare intra-laboratory test results and reproducibility. Because EE was also tested approximately one year earlier in the Phase-1 studies, the data obtained from similar doses can be compared to the responses in the Phase-2 tests to assess reproducibility over time (Tables 27a-d).

Inter-laboratory reproducibility of the uterotrophic bioassay results

Proportions of studies achieving statistically significant responses

100. As noted previously, the first assumption is that the proportion of laboratories achieving statistical significance will decrease as the selected dose moves into the lower portion of the dose response curve and approaches the no effect level. The second assumption is the proportion of laboratories achieving statistical significance at the same dose will be similar between the dose response and the coded single-dose experiments. The proportion of studies achieving statistically significant response was indeed consistent between the coded single-dose and the dose response experiments, indicating good inter-laboratory reproducibility. The results for statistical significance in the dose response and the coded single-dose studies are compiled in Table 23. The comparisons are made on the basis of each weak agonist and each protocol.

101. The relative increase in uterine weight with GEN and MC was high and the selected doses well above the observed MEDs in all protocols. Consistent with assumptions, statistical significance was achieved in all laboratories with these chemicals. The relative increase in uterine weight was high and the selected doses were well above the MEDs in other instances (DDT Protocol A, BPA Protocols C and D, NP Protocols A and D). Again, statistical significance was achieved in all laboratories, with the exception of one laboratory in Protocol A with NP where four of the six animals died, reducing the group size to two.

102. In the other experiments, as the selected doses approached the MED, a decreasing proportion of the laboratories achieved statistical significance and many of those achieving statistical significance did so marginally with 95% lower confidence levels equal to or greater than 1.00 and less than 1.10 or conversely failed to achieve statistical significance with the 95% lower confidence levels greater than 0.90 and less than 1.00 (BPA Protocols A and B, NP Protocols B and C, DDT Protocol D). In Protocol C with DDT where the selected dose was at the MED, 4 of 10 laboratories achieved statistical significance. In Protocol B with DDT, where the selected dose was actually just below the MED, only 6 of 18 studies achieved statistical significance. In conclusion, the proportion of studies achieving statistically significant response were consistent between the coded single-dose and the dose response experiments considering the relation of the selected dose and the MED, indicating good inter-laboratory reproducibility of the uterotrophic bioassay.

Table 23. Proportions of laboratories achieving statistically significant responses for each chemical in the different protocols at the same dose

Chemical	Protocol A		Protocol B		Protocol C		Protocol D	
	DR	CSD	DR	CSD	DR	CSD	DR	CSD
BPA	3/4	6/10	7/9 [b]	13/14	5/5	7/7	2/2	4/4
DDT	4/4	9/9[a]	1/4	5/15	1/3	4/7	2/2	2/4
GEN	4/4	10/10	4/4	13/13	3/3	6/6	2/2	4/4
MC	4/4	10/10	4/4	14/14	3/3	6/6	2/2	4/4
NP	4/4	9/10*	6/9 [b]	11/15	2/5	6/7	2/2	4/4

DR, dose-response studies; CSD, coded single-dose studies

[a] Laboratory #12 not included – all animals died

[b] Laboratory #21 not included; the response was low and 95% confidence limits are not available.

* 4 animals died in the laboratory that did not achieve statistical equivalence, leaving a group size of 2.

Global Analyses

103. In the global analyses using mixed effect linear models on the pooled data for a protocol, the data were separately pooled for the coded single-dose and the dose response studies for the common dose of each chemical. The data were analysed to calculate the relative ratio of the blotted uterine weight of the test substance group to the vehicle control group with both lower and upper 95% confidence levels. These calculations are shown in Table 24.

104. In some 20 comparisons, 11 pairs of means are different by only ≤ 0.10, another 6 by ≤ 0.2, the Protocol B BPA by 0.24, the Protocol A NP by 0.28, and the Protocol A DDT by 0.47. All means were within the upper and lower 95% confidence levels of the other, indicating that no statistical difference occurred between any of the means. In conclusion, the global analysis means were consistent between the coded single-dose and the dose response experiments supporting good inter-laboratory reproducibility of the uterotrophic bioassay.

Table 24. Global Analysis of coded single-dose and dose response studies: Ratio of blotted uterine weight of test groups to vehicle control group based on pooled data

	Protocol			
Substance -dose	**A**	**B**	**C**	**D**
BPA mg/kg/d	600	300	300	300
DR	1.41 (1.07 - 1.85) [a]	1.61 (1.00 - 2.58)	2.73 (2.07 - 3.61)	3.78 (2.98 - 4.79)
CSD	1.34 (1.09 - 1.66)	1.85 (1.58 - 2.16)	2.68 (2.36 - 3.04)	3.84 (3.39 - 4.35)
DDT mg/kg/d	300	100	100	100
DR	3.13 (2.38 - 4.12)	1.16 (0.94 - 1.44)	1.33 (1.04 - 1.69)	1.31 (1.08 - 1.59)
CSD	3.60 (2.94 - 4.41)	1.23 (0.97 - 1.58)	1.24 (1.00 - 1.52)	1.17 (1.06 - 1.30)
GN mg/kg/d	300	35	35	35
DR	2.75 (1.98 - 3.80)	2.47 (1.82 - 3.37)	1.73 (1.45 - 2.07)	2.36 (1.61 - 3.46)
CSD	2.65 (2.21 - 3.18)	2.42 (2.05 - 2.86)	1.77 (1.58 - 2.00)	2.18 (1.91 - 2.49)
MX mg/kg/d	300	500	500	500
DR	3.16 (2.09 - 4.79)	3.13 (1.70 - 5.75)	2.25 (1.79 - 2.83)	2.43 (1.55 - 3.83)
CSD	3.21 (2.58 - 3.99)	3.03 (2.54 - 3.62)	2.07 (1.72 - 2.48)	2.62 (2.28 - 3.00)
NP mg/kg/d	250	80	80	80
DR	2.40 (1.90 - 3.04)	1.51 (1.05 - 2.16)	1.29 (1.06 - 1.58)	1.96 (1.59 - 2.42)
CSD	2.12 (1.72 - 2.61)	1.53 (1.26 - 1.88)	1.40 (1.24 - 1.57)	1.77 (1.58 - 1.98)
DBP mg/kg/d	1,000	500	500	500
CSD	0.95 (0.77 - 1.18)	0.97 (0.80 - 1.17)	1.02 (0.84 - 1.24)	0.99 (0.91 - 1.07)

[a] Ratio of geometric means of treated blotted uterine weights to the vehicle control blotted uterine weights after adjusting for the body weights at necropsy as a covariable (lower 95% confidence limit, upper 95% confidence limit).

Specific comparisons in the same laboratory across dose-response and coded single-dose procedures

105. The third approach avoids global data pooling and only considers directly comparable data, e.g., only those laboratories performing both studies on a chemical in the same Protocol. These comparisons are in Tables 25a-d. This is followed by an examination of the subset of data where there was not a consistent achievement of statistical significance. This examination is summarised in Table 26.

106. Protocol A has 16 possible intra-laboratory comparisons covering all 5 weak agonists, but the deaths of all animals in Laboratory #12 with DDT reduces this to 15 comparisons. In 13 instances, the results are in full agreement with the means of each study being within the 95% confidence levels of the other. The two exceptions are from Laboratory #12 with BPA and NP. With BPA, there was one

mortality in the dose response and three in the coded single-dose. With NP, there were four mortalities in both studies with large standard deviations and large 95% confidence intervals in the body weight adjusted analyses.

107. Protocol B has 24 possible intra-laboratory comparisons covering all 5 weak agonists. Because Laboratory #21 failed to record body weights at necropsy, these data sets were statistically analysed using the absolute uterine weights. In 21 instances, the results are in full agreement with the means of each study being within the 95% confidence levels of the other. The three exceptions are Laboratory #2 with BPA, Laboratory #5 with DDT, and Laboratory #4 with NP. In the case of Laboratory #2, the primary reason for the difference in the ratio was a nearly 6 mg difference in the means of the immature vehicle controls (see Table 25b). In the case of Laboratory #5, the test substance group in the coded single-dose studies was slightly less than the vehicle controls so that the upper 95% confidence level was equal to the mean in the dose response studies (see Table 25b).

108. Protocol C has 12 possible intra-laboratory comparisons covering all 5 weak agonists. In 9 instances, the results are in full agreement with the means of each study being within the 95% confidence levels of the other. The three exceptions are Laboratory #12 with DDT, Laboratory #1 with MC, and Laboratory #8 with NP. Laboratory #12 had a high individual value of 197.4 mg in the dose response and a low individual value of 78.7 mg in the coded single-dose, which strongly influenced the means in both cases. In Laboratory #1, the excursion was slight with an upper 95% confidence level of 2.30 in the coded single-dose and a mean of 2.32 in the dose response. The primary factor was a difference of over 18 mg in the OVX vehicle control means (see Table 25c). In Laboratory #8, the response in the dose response studies was limited in all individuals and in the coded single-dose studies, while the mean was influenced by two high individuals (158.8 and 144.9 mg), the other four individuals were all easily greater than the highest individual value in the vehicle control group.

109. Protocol D has 6 possible intra-laboratory comparisons, but no data for a comparison based on NP exists as no lab performed both the dose response and the coded single-dose studies with NP. The results are in full agreement in all 6 instances with the means of each study being within the 95% confidence levels of the other.

110. In conclusion, an intra-laboratory comparison shows that the uterotrophic bioassay is reproducible. Out of 58 possible comparisons, one is eliminated due to animal mortalities (Protocol A, Laboratory #12, BPA), and, in 49 of the 57 remaining cases, the results are in agreement (the means are within the 95% confidence level of the corresponding experiment). Animal mortalities, variability in the vehicle control weights, and high or low values of particular individual appear to be the primary source of the difference in most cases. The latter group is marked by high standard deviations and coefficients of variation. The one clear instance of the lack of reproducibility was Laboratory #8 in Protocol C with NP.

Table 25a. Protocol A: Within-laboratory comparison of dose-response and coded single results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Bisphenol A at 600 mg/kg/d									
2	Blotted (mg)	25.4 ± 4.19	35.8 ± 5.68	1.41[1]	1.49* (1.26 - 1.77)	29.2 ± 2.73	42.5 ± 7.10	1.45	1.49* (1.14 - 1.84)
	Body Wt (g)	46.6 ± 7.14	44.3 ± 4.00			52.0 ± 3.62	51.7 ± 3.23		
12	Blotted (mg)	20.6 ± 1.81	33.8 ± 6.04	1.64[1]	1.63* (1.30 - 2.06)	21.0 ± 8.75	35.5 ± 4.27	1.69[3]	1.08 (0.431 - 2.71)
	Body Wt (g)	39.7 ± 3.10	39.5 ± 6.00			36.5 ± 3.62	30.4 ± 3.39		
13	Blotted (mg)	31.8 ± 3.66	34.4 ± 2.70	1.08[1]	1.17 (0.79 - 1.72)	30.0 ± 6.66	32.6 ± 5.50	1.09[1]	1.25* (1.01 - 1.56)
	Body Wt (g)	41.5 ± 2.74	31.4 ± 3.36			34.5 ± 4.85	27.8 ± 2.17		
Comparison of *o,p'*-DDT at 300 mg/kg/d									
3	Blotted (mg)	32.6 ± 4.23	97.3 ± 11.28	2.98	2.67* (1.99 - 3.59)	35.2 ± 5.74	99.2 ± 14.89	2.82	3.05* (2.45 - 3.81)
	Body Wt (g)	63.2 ± 3.07	54.3 ± 9.56			66.0 ± 3.83	58.3 ± 3.69		
5	Blotted (mg)	43.3 ± 7.91	100.3 ± 20.64	2.32[1]	2.71* (1.92 - 3.83)	40.0 ± 7.05	100.0 ± 13.23	2.50	2.92* (2.23 - 3.83)
	Body Wt (g)	58.6 ± 5.07	44.3 ± 11.26			58.0 ± 4.25	48.8 ± 3.23		
11	Blotted (mg)	25.3 ± 3.75	87.0 ± 3.90	3.44	3.43* (2.96 - 3.98)	27.3 ± 3.44	94.8 ± 13.57	3.47	3.58* (2.79 - 4.60)
	Body Wt (g)	38.6 ± 3.91	39.5 ± 3.56			41.6 ± 3.79	37.9 ± 2.85		
12	Blotted (mg)	20.6 ± 1.81	67.4 ± 16.63	3.02[3]	3.45* (2.41 - 4.90)	21.0 ± 8.75	All 6 died		
	Body Wt (g)	39.7 ± 3.10	33.0 ± 8.16			36.5 ± 3.62	All 6 died		
Comparison of Genistein at 300 mg/kg/d									
1	Blotted (mg)	39.1 ± 4.10	81.4 ± 7.85	2.08	2.22* (1.67 - 2.95)	32.8 ± 1.93	78.5 ± 5.21	2.39	2.39* (1.94 - 2.95)
	Body Wt (g)	67.3 ± 2.62	63.6 ± 2.53			62.4 ± 4.95	62.6 ± 3.81		
8	Blotted (mg)	21.4 ± 2.56	61.7 ± 8.50	2.88	2.96* (2.42 - 3.61)	20.0 ± 2.65	62.8 ± 6.89	3.14	3.21* (2.65 - 3.88)
	Body Wt (g)	46.5 ± 5.61	42.9 ± 3.98			44.7 ± 2.54	41.6 ± 5.29		
12	Blotted (mg)	20.6 ± 1.81	74.6 ± 10.43	3.62	3.47* (2.71 - 4.45)	21.0 ± 8.75	67.3 ± 10.63	3.20	3.74* (1.83 - 7.62)
	Body Wt (g)	39.7 ± 3.10	43.4 ± 4.48			36.5 ± 3.62	34.4 ± 6.05		

Table 25a (continued). Protocol A: Within-laboratory comparison of dose-response and coded single results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Methoxychlor 300 mg/kg/d									
1	Blotted (mg)	38.4 ± 6.50	93.1 ± 7.50	2.43	2.59* (2.13 - 3.15)	32.8 ± 1.93	97.2 ± 10.94	2.96	2.98* (2.39 - 3.71)
	Body Wt (g)	61.6 ± 2.49	59.2 ± 2.21			62.4 ± 4.95	58.5 ± 3.40		
3	Blotted (mg)	39.6 ± 9.12	105.0 ± 9.75	2.65	2.94* (2.34 - 3.69)	35.2 ± 5.74	93.6 ± 7.70	2.66	2.77* (2.24 - 3.41)
	Body Wt (g)	62.9 ± 1.64	58.4 ± 3.31			66.0 ± 3.83	62.9 ± 3.33		
12	Blotted (mg)	20.6 ± 1.81	78.6 ± 10.02	3.82	3.98* (3.07 - 5.15)	21.0 ± 8.75	58.4 ± 12.54	2.78[3]	3.20* (1.34 - 7.61)
	Body Wt (g)	39.7 ± 3.10	38.3 ± 3.30			36.5 ± 3.62	34.7 ± 5.44		
14	Blotted (mg)	14.8 ± 2.28	55.6 ± 8.20	3.76[1]	3.46* (2.51 - 4.77)	19.0 ± 5.66	44.0 ± 9.93	2.32	2.95* (1.94 - 4.48)
	Body Wt (g)	40.3 ± 6.83	46.3 ± 5.47			46.2 ± 8.80	44.1 ± 0.66		
Comparison of Nonylphenol at 250 mg/kg/d									
4	Blotted (mg)	29.3 ± 10.91	60.3 ± 6.99	2.05[2]	2.61* (1.69 - 4.04)	29.8 ± 5.49	49.5 ± 0.71	1.66[4]	1.89* (1.24 - 2.88)
	Body Wt (g)	42.7 ± 2.91	28.5 ± 1.55			40.8 ± 4.67	26.0 ± 1.27		
12	Blotted (mg)	20.6 ± 1.81	62.2 ± 18.95	3.02[4]	2.95* (2.02 - 4.32)	21.0 ± 8.75	38.5 ± 26.90	1.83[4]	1.97 (0.73 - 5.33)
	Body Wt (g)	39.7 ± 3.10	39.9 ± 2.05			36.5 ± 3.62	36.7 ± 1.98		

Table 25b. Protocol B: Within-laboratory comparison of dose-response and coded single-dose results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Bisphenol A at 300 mg/kg/d									
2	Blotted (mg)	26.5 ± 1.80	59.8 ± 5.72	2.25	2.30* (1.88 - 2.81)	32.1 ± 6.43	55.6 ± 12.65	1.73	1.77* (1.40 - 2.24)
	Body Wt (g)	51.5 ± 2.45	48.8 ± 3.53			54.8 ± 2.52	51.7 ± 2.40		
8	Blotted (mg)	23.5 ± 2.33	45.5 ± 7.26	1.93	1.91* (1.50 - 2.43)	25.3 ± 4.77	47.3 ± 10.81	1.87	1.91* (1.53 - 2.39)
	Body Wt (g)	51.9 ± 6.75	52.6 ± 3.59			54.1 ± 3.45	51.4 ± 2.77		
12	Blotted (mg)	22.4 ± 6.47	28.2 ± 6.64	1.26	1.33 (0.89 - 1.99)	31.6 ± 6.10	50.4 ± 10.25	1.59	1.61* (1.12 - 2.31)
	Body Wt (g)	40.4 ± 3.38	36.8 ± 5.79			44.5 ± 2.85	44.2 ± 4.48		
13	Blotted (mg)	28.0 ± 3.46	51.3 ± 15.60	1.83	1.72* (1.08 - 2.76)	29.5 ± 3.21	46.3 ± 12.50	1.57	1.52* (1.11 - 2.08)
	Body Wt (g)	45.2 ± 2.32	45.8 ± 3.06			36.0 ± 2.97	36.5 ± 2.81		
18	Blotted (mg)	21.3 ± 1.50	46.6 ± 5.28	2.19	2.12* (1.81 - 2.50)	27.2 ± 2.22	62.0 ± 10.17	2.28	2.32* (1.88 - 2.87)
	Body Wt (g)	52.1 ± 3.70	55.1 ± 3.76			55.4 ± 3.00	52.4 ± 4.19		
20	Blotted (mg)	54.3 ± 11.77	50.8 ± 9.08	0.94	0.95 (0.69 - 1.32)	54.4 ± 7.73	60.0 ± 11.48	1.10	1.11 (0.86 - 1.44)
	Body Wt (g)	50.7 ± 4.01	51.4 ± 1.87			54.4 ± 7.73	49.8 ± 4.03		
21	Blotted (mg)	47.3 ± 6.92	89.0 ± 11.37	1.88**		53.6 ± 4.26	89.4 ± 12.51	1.67**	
	Body Wt (g)	NA	NA			NA	NA		
Comparison of *o,p'*-DDT at 100 mg/kg/d									
3	Blotted (mg)	31.5 ± 3.97	37.8 ± 3.49	1.20	1.01 (0.83 - 1.23)	33.2 ± 5.06	40.1 ± 6.74	1.21	1.18 (0.95 - 1.46)
	Body Wt (g)	65.4 ± 3.13	64.6 ± 1.70			62.9 ± 1.87	64.0 ± 3.15		
5	Blotted (mg)	36.1 ± 10.07	39.5 ± 8.87	1.09	1.18 (0.94 - 1.54)	32.8 ± 4.11	31.2 ± 3.78	0.95	0.96 (0.78 - 1.18)
	Body Wt (g)	57.5 ± 5.69	55.9 ± 6.36			59.5 ± 4.21	58.6 ± 3.30		
11	Blotted (mg)	23.6 ± 3.87	25.1 ± 2.79	1.06	1.08 (0.87 - 1.34)	26.6 ± 3.75	26.0 ± 4.46	0.97	1.03 (0.78 - 1.37)
	Body Wt (g)	38.6 ± 4.11	38.3 ± 2.50			42.0 ± 3.21	37.7 ± 2.78		
12	Blotted (mg)	22.4 ± 6.47	33.9 ± 10.49	1.51	1.47* (1.11 - 1.94)	31.6 ± 6.10	47.3 ± 10.59	1.50	1.50* (1.04 - 2.16)
	Body Wt (g)	40.4 ± 3.38	41.0 ± 5.01			44.5 ± 2.85	44.0 ± 3.40		

Table 25b (continued). Protocol B: Within-laboratory comparison of dose-response and coded single-dose results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Genistein at 35 mg/kg/d									
1	Blotted (mg)	33.4 ± 9.32	75.4 ± 11.61	2.26	2.33* (1.75 - 3.10)	38.6 ± 10.87	79.9 ± 6.25	2.07	2.16* (1.65 - 2.84)
	Body Wt (g)	63.1 ± 4.45	62.0 ± 3.18			59.5 ± 3.18	58.6 ± 5.09		
8	Blotted (mg)	24.2 ± 2.77	56.4 ± 10.91	2.33	2.53* (2.04 - 3.15)	25.3 ± 4.77	63.2 ± 4.61	2.50	2.69* (2.19 - 3.30)
	Body Wt (g)	52.9 ± 6.02	51.1 ± 5.10			54.1 ± 3.45	53.8 ± 2.41		
12	Blotted (mg)	22.4 ± 6.47	50.6 ± 10.96	2.26	2.28* (1.70 - 3.05)	31.6 ± 6.10	70.2 ± 12.08	2.22	2.20* (1.53 - 3.17)
	Body Wt (g)	40.4 ± 3.38	40.6 ± 4.57			44.5 ± 2.85	45.0 ± 3.53		
Comparison of Methoxychlor at 500 mg/kg/d									
1	Blotted (mg)	35.2 ± 6.34	86.3 ± 10.15	2.45	2.47* (1.91 - 3.21)	38.6 ± 10.87	101.7 ± 8.11	2.63	2.73* (2.08 - 3.58)
	Body Wt (g)	66.6 ± 4.48	64.7 ± 2.92			59.5 ± 3.18	59.2 ± 2.46		
3	Blotted (mg)	37.5 ± 5.61	107.4 ± 17.90	2.86	2.97* (2.42 - 3.65)	33.2 ± 5.06	88.9 ± 14.81	2.68	2.66* (2.15 - 3.29)
	Body Wt (g)	63.5 ± 3.92	62.3 ± 2.59			62.9 ± 1.87	63.2 ± 3.18		
12	Blotted (mg)	22.4 ± 6.47	72.0 ± 9.42	3.21	3.34* (2.53 - 4.40)	31.6 ± 6.10	96.7 ± 12.05	3.06	3.14* (2.18 - 4.51)
	Body Wt (g)	40.4 ± 3.38	38.7 ± 5.27			44.5 ± 2.85	43.9 ± 2.87		
14	Blotted (mg)	16.3 ± 3.67	61.8 ± 13.12	3.79	3.76* (2.79 - 5.09)	16.3 ± 3.78	63.8 ± 8.61	3.91	4.07* (2.97 - 5.56)
	Body Wt (g)	44.2 ± 5.25	44.7 ± 5.00			49.2 ± 4.30	47.8 ± 5.44		
Comparison of Nonylphenol at 80 mg/kg/d									
4	Blotted (mg)	30.8 ± 10.46	61.5 ± 17.41	1.99	2.05* (1.44 - 2.92)	31.2 ± 4.92	45.2 ± 8.33	1.45	1.45* (1.08 - 1.94)
	Body Wt (g)	45.6 ± 3.93	44.4 ± 3.26			41.2 ± 4.42	41.8 ± 4.40		
8	Blotted (mg)	24.2 ± 2.77	35.2 ± 9.30	1.45	1.44* (1.15 - 1. 80)	25.3 ± 4.77	32.4 ± 3.38	1.28	1.32* (1.06 - 1.65)
	Body Wt (g)	52.9 ± 6.02	50.3 ± 4.84			54.1 ± 3.45	52.3 ± 3.89		
12	Blotted (mg)	22.4 ± 6.47	42.5 ± 5.94	1.90	2.02* (1.49 - 2.75)	31.6 ± 6.10	52.8 ± 12.44	1.67	1.71* (1.19 - 2.47)
	Body Wt (g)	40.4 ± 3.38	40.2 ± 2.46			44.5 ± 2.85	43.1 ± 3.96		
18	Blotted (mg)	21.3 ± 1.50	41.9 ± 4.00	1.97	1.93* (1.56 - 2.39)	27.2 ± 2.22	45.8 ± 3.84	1.69	1.73* (1.40 - 2.14)
	Body Wt (g)	52.1 ± 3.70	55.2 ± 3.20			55.4 ± 3.00	52.4 ± 2.82		
20	Blotted (mg)	54.3 ± 11.77	41.3 ± 10.96	0.76	0.75 (0.51 - 1.11)	54.4 ± 7.73	58.8 ± 11.55	1.08	1.07 (0.83 - 1.38)
	Body Wt (g)	50.7 ± 4.01	51.2 ± 3.16			54.4 ± 7.73	52.8 ± 3.13		
21	Blotted (mg)	47.3 ± 6.92	75.2 ± 15.77	1.59**		53.6 ± 4.26	55.6 ± 6.18	1.04	
	Body Wt (g)	NA	NA			NA	NA		

Table 25c. Protocol C: Within-laboratory comparison of dose-response and coded single-dose results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Bisphenol A at 300 mg/kg/d									
2	Blotted (mg)	99.8 ± 10.76	278.6 ± 35.86	2.79	2.79* (2.28 - 3.41)	85.6 ± 9.76	223.3 ± 28.92	2.61	2.61* (1.99 - 3.42)
	Body Wt (g)	250.9 ± 13.24	238.0 ± 13.90			242.3 ± 14.13	231.5 ± 9.17		
8	Blotted (mg)	88.0 ± 9.76	229.2 ± 35.16	2.60	2.65* (2.16 - 3.24)	77.8 ± 7.41	223.6 ± 30.14	2.87	2.89* (2.16 - 3.88)
	Body Wt (g)	291.0 ± 17.09	281.2 ± 14.29			283.5 ± 9.40	273.7 ± 18.42		
12	Blotted (mg)	98.6 ± 22.04	266.3 ± 44.60	2.70	2.72* (2.05 - 3.61)	104.1 ± 17.88	240.6 ± 56.38	2.31	2.30* (1.67 - 3.18)
	Body Wt (g)	297.2 ± 14.54	299.9 ± 10.99			295.1 ± 11.42	282.1 ± 9.52		
Comparison of *o,p'*-DDT at100 mg/kg/d									
3	Blotted (mg)	86.6 ± 4.14	122.2 ± 10.32	1.41	1.43* (1.21 - 1.69)	91.5 ± 7.63	120.4 ± 31.06	1.32	1.29* (1.00 - 1.65)
	Body Wt (g)	268.9 ± 8.50	264.3 ± 10.76			267.4 ± 11.90	269.1 ± 11.45		
11	Blotted (mg)	78.5 ± 8.38	99.6 ± 28.59	1.27	1.25 (0.98 - 1.59)	83.0 ± 4.91	102.8 ± 13.47	1.24	1.24* (1.05 - 1.47)
	Body Wt (g)	217.0 ± 5.55	214.6 ± 9.22			218.1 ± 7.03	215.2 ± 6.93		
12	Blotted (mg)	98.6 ± 22.04	128.3 ± 36.21	1.30	1.31 (0.96 - 1.78)	104.1 ± 17.88	100.1 ± 17.15	0.96	0.96 (0.71 - 1.31)
	Body Wt (g)	297.2 ± 14.54	301.3 ± 15.35			295.1 ± 11.42	291.6 ± 11.48		
Comparison of Genistein at 35 mg/kg/d									
1	Blotted (mg)	85.9 ± 13.10	151.6 ± 13.14	1.77	1.78* (1.46 - 2.18)	103.5 ± 5.50	175.3 ± 10.67	1.69	1.67* (1.42 - 1.97)
	Body Wt (g)	272.5 ± 20.75	270.4 ± 14.70			258.9 ± 25.70	268.9 ± 14.45		
12	Blotted (mg)	98.6 ± 22.04	152.0 ± 24.29	1.54	1.56* (1.09 - 2.21)	104.1 ± 17.88	163.7 ± 36.09	1.57	1.57* (1.13 - 2.20)
	Body Wt (g)	297.2 ± 14.54	297.2 ± 17.70			295.1 ± 11.42	276.7 ± 9.19		
Comparison of Methoxychlor at 500 mg/kg/d									
1	Blotted (mg)	84.9 ± 8.03	197.1 ± 46.05	2.32	2.32* (1.86 - 2.89)	103.5 ± 5.50	202.6 ± 23.52	1.96	1.96* (1.67 - 2.30)
	Body Wt (g)	255.9 ± 10.19	248.8 ± 11.64			258.9 ± 25.70	253.5 ± 9.82		
3	Blotted (mg)	85.5 ± 8.57	212.0 ± 27.48	2.48	2.42* (1.96 - 2.98)	91.5 ± 7.63	194.0 ± 28.42	2.12	2.11* (1.64 - 2.71)
	Body Wt (g)	278.5 ± 11.15	264.2 ± 12.98			267.4 ± 11.90	259.8 ± 12.23		
12	Blotted (mg)	98.6 ± 22.04	185.0 ± 22.30	1.88	1.95* (1.45 - 2.62)	104.1 ± 17.88	155.4 ± 28.55	1.49	1.49* (1.10 - 2.03)
	Body Wt (g)	297.2 ± 14.54	288.7 ± 9.24			295.1 ± 11.42	294.5 ± 13.77		

Table 25c (continued). Protocol C: Within-laboratory comparison of dose-response and coded single-dose results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Nonylphenol at 80 mg/kg/d									
8	Blotted (mg)	82.2 ± 8.89	96.5 ± 7.50	1.17	1.17 (0.98 - 1.39)	77.8 ± 7.41	125.6 ± 22.83	1.61	1.59* (1.19 - 2.13)
	Body Wt (g)	286.7 ± 21.81	290.8 ± 13.88			283.5 ± 9.40	287.0 ± 13.43		
12	Blotted (mg)	98.6 ± 22.04	129.6 ± 20.50	1.31	1.33* (1.02 - 1.73)	104.1 ± 17.88	143.3 ± 26.56	1.38	1.38* (1.01 - 1.90)
	Body Wt (g)	297.2 ± 14.54	297.1 ± 16.15			295.1 ± 11.42	285.1 ± 8.76		

Table 25d. Protocol D: Within-laboratory comparison of dose-response and coded single-dose results at the same test substance dose

Lab #	Metric	Dose Response				Coded Single-dose			
		Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio	Control	Test substance	Absolute Ratio	Body Wt Adjusted Ratio
Comparison of Bisphenol A 300 mg/kg/d									
2	Blotted (mg)	86.2 ± 13.56	306.8 ± 18.43	3.56	3.74* (2.89 - 4.8)	86.9 ± 13.27	320.2 ± 22.44	3.69	3.91* (3.21 - 4.76)
	Body Wt (g)	274.6 ± 15.93	236.2 ± 10.71			276.6 ± 25.82	248.2 ± 12.13		
Comparison of *o,p'*-DDT at 100 mg/kg/d									
3	Blotted (mg)	86.3 ± 4.86	109.7 ± 9.24	1.27	1.18* (1.028 - 1.36)	88.5 ± 9.40	101.2 ± 17.76	1.14	1.15 (0.92 - 1.43)
	Body Wt (g)	290.6 ± 12.69	282.6 ± 6.80			281.7 ± 14.59	276.3 ± 12.16		
11	Blotted (mg)	71.5 ± 14.84	98.8 ± 5.91	1.38	1.48* (1.17 - 1.87)	75.7 ± 10.09	98.6 ± 20.14	1.30	1.32* (1.03 - 1.68)
	Body Wt (g)	235.0 ± 8.47	224.1 ± 9.60			234.6 ± 15.31	228.7 ± 16.69		
Comparison of Genistein at 35 mg/kg/d									
1	Blotted (mg)	87.2 ± 15.17	189.3 ± 22.55	2.17	2.20* (1.78 - 2.73)	89.2 ± 6.56	188.7 ± 19.43	2.12	2.11* (1.68 - 2.65)
	Body Wt (g)	281.5 ± 19.95	277.2 ± 14.23			293.8 ± 20.46	284.6 ± 17.91		
Comparison of Methoxychlor at 500 mg/kg/d									
1	Blotted (mg)	90.8 ± 15.92	211.9 ± 41.21	2.33	2.38* (1.76 - 3.22)	89.2 ± 6.56	199.7 ± 26.68	2.24	2.23* (1.77 - 2.80)
	Body Wt (g)	272.1 ± 14.66	246.5 ± 17.59			293.8 ± 20.46	280.4 ± 38.58		
3	Blotted (mg)	92.6 ± 5.87	228.2 ± 38.44	2.46	2.46* (1.98 - 3.03)	88.5 ± 9.40	222.3 ± 29.45	2.51	2.67* (2.03 - 3.51)
	Body Wt (g)	282.7 ± 19.53	254.7 ± 8.95			281.7 ± 14.59	250.4 ± 10.42		
Comparison of Nonylphenol at 80 mg/kg/d - No direct comparisons are available									

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval). Superscript numbers indicate the numbers of animals that died prior to necropsy or were not weighed

* , statistically significant at $p<0.05$ using body weight adjusted ratio; **, statistically significant at $p<0.05$ using absolute uterine weights as body weights were not recorded at necropsy

111. An alternative approach is to examine those instances where the same laboratory performed both the dose response and the coded single-dose studies with the same chemical and the same Protocol and where one study would achieve statistical significance and the other would not. There were 9 instances among the 58 comparisons that did not agree with respect to statistical significance (Table 26). None were with GEN or MC where the selected doses were well above the MEDs in all Protocols. One instance with DDT in Protocol A was due to animal mortality. A second instance with NP in Protocol A also appears to be due to animal mortality where 4 of 6 animals died in both studies. In three instances (Laboratory #13 with BPA in Protocol A, Laboratory #11 with DDT in Protocol C, Laboratory #3 with DDT in Protocol D) the results were in close accordance, and it appears that one study achieved statistical significance and the other did not, simply on the basis of chance.

112. The other four instances are Laboratory #12 with BPA in Protocol A, Laboratory #12 with BPA in Protocol B, Laboratory #21 with NP in Protocol B, and Laboratory #8 with NP in Protocol C. Laboratory #12 with BPA in Protocol A had three mortalities in coded single-dose studies and one of the three data points was an extreme outlier with a recorded blotted weight of only 3.8 mg. This appears to be either a recording or animal anomaly and not of the protocol. Laboratory #12 with BPA in Protocol B in the dose response experiments had a coefficient of variation of nearly 24% in the treatment group with two individual values below or near the vehicle control mean, and the group variability appears to be the source of not achieving statistical significance. Laboratory #21 with NP in Protocol B had a vehicle control mean of 53.6 mg, among the highest vehicle control mean values, with little evidence for any response. This instance was noted previously and is discussed further in the section on sources of variability. Laboratory #8 with NP in Protocol C was discussed in the previous section, appearing to be a true instance of non-reproducibility.

113. In conclusion, a reduction in group size due to mortality will decrease the assay's power. Substantial increases in the vehicle control means will reduce the responsiveness of the assay, and large variability within a group will reduce the statistical power with increases in the standard deviation and the coefficient of variation. All these circumstances appear to introduce variabilities that may lead to a decrease in reproducibility, particularly in the lower region of the dose response curve. In this respect, 7 of the 9 instances were indeed in the lower portion of their curve with maximum uterine weight increases in one of the two studies of 60% or less.

Table 26. Differences in statistical significance between dose-response and coded single-dose studies at the same dose

Lab	BPA		DDT		NP	
	DR	**CSD**	**DR**	**CSD**	**DR**	**CSD**
	Protocol A					
	600 mg/kg/d		**300 mg/kg/d**			
12	1.63[1]* (1.29 - 2.06)	1.08[3] (0.43 - 2.71)	3.45[3]* (2.41 - 4.94)	xx[6]	2.95*[4] (2.02 - 4.32)	1.97[4] (0.73 - 5.33)
13	1.17[1] (0.79 - 1.72)	1.25* (1.01 - 1.56)				
	Protocol B					
	300 mg/kg/d				**80 mg/kg/d**	
12	1.33 (0.88 - 1.99)	1.60* (1.12 - 2.31)				
21					1.59[#]*	1.04[#]
	Protocol C					
			100 mg/kg/d		**80 mg/kg/d**	
8					1.17 (0.98 - 1.39)	1.59* (1.19 - 2.13)
11			1.25 (0.98 - 1.59)	1.24* (1.05 - 1.47)		
	Protocol D					
			100 mg/kg/d			
3			1.18* (1.03 - 1.36)	1.14 (0.92 - 1.43)		

Ratio of blotted uterine weights to vehicle control weights after log transformation, and with body weights at necropsy as a covariable (95% confidence interval). DR – dose response; CSD – coded single-dose.
Superscript numbers indicate the numbers of animals that died prior to necropsy or were not weighed
* significant at $p < 0.05$
[#] body weights at day 3 used; computations not performed for NP coded single-dose

114. The final approach to comparing the dose response and the coded single-dose experiments is to examine those cases from the same lab where both the dose-response and coded single-dose experiments were conducted with the same chemical and the same dose level and analyse whether mean relative uterine weights were statistically different from one another. Only 16 groups out of 114 test substance, vehicle, and reference EE groups were statistically different (Table 27). These are divided into three subgroups: 1) where vehicle controls were statistically different (3 of the 16 instances), 2) where EE dose response were significantly different (6 of the 16 instances), and 3) where weak agonist responses were statistically different (7 of the 16 instances). In Table 27, the mean of the coded single-dose relative to the mean of the dose response is calculated for the same protocol, chemical and dose groups with the upper and lower 95% confidence intervals. If the 95% lower confidence interval is $\geq$ to 1.0, then the results are statistically significant (note: rounding is not appropriate, 0.9996 would not be statistically significant).

Table 27: Experiments from those individual laboratories that had statistically significant differences between coded single-dose and dose response studies

Lab	Protocol	Group	CDS Mean Relative to DR Mean		
			Mean	LCL	UCL
1	C	Control - (GEN)	1.24	1.06	1.46
1	C	Control - (MC)	1.22	1.10	1.35
18	B	Control	1.33	1.20	1.48
8	B	Low dose EE	1.24	1.16	1.32
8	C	Low dose EE	1.17	1.06	1.30
13	A	Low dose EE	5.07	2.93	8.76
13	A	High dose EE	1.75	1.18	2.61
18	B	Low dose EE	1.25	1.09	1.42
18	B	High dose EE	1.20	1.05	1.37
1	B	MC	1.33	1.11	1.56
1	C	GEN	1.17	1.11	1.22
8	C	NP	1.30	1.07	1.57
12	B	BPA	1.70	1.16	2.48
12	B	MC	1.43	1.18	1.74
18	B	BPA	1.42	1.24	1.62
18	B	NP	1.13	1.00	1.28

115. The statistical difference in the vehicle controls occurred in two laboratories As there would be random groups of animals in the same lab at different time points, this implies random variation between groups that could generate false positives and false negatives should such differences occur between a vehicle and treated group. This is consistent with three groups of the negative chemical being statistically greater than controls and two groups being statistically less than controls (Table 17). In that case, four instances occurred with immature animals in Protocol B and one with adult OVX animals in Protocol C. Here one instance is with immature animals in Protocol B and two are with adult OVX animals in Protocol C. Again, no protocol can be identified as superior.

116. The statistical differences in the EE reference doses occurs in protocols A, B, and C, and occurred at the low EE dose in 4 cases and two at the high EE dose in three cases. For Laboratory #13, the coded single-dose results in Protocol A for both the low and high EE doses were very different from those observed in either the dose response or the Phase-1 data on both an absolute basis and on a relative basis (see Table 27). This is a clear instance of lack of reproducibility. For Laboratory #8, however, the difference in relative values is modest for both Protocol B (see Table 27) and for Protocol C (see Table 27). The results for Laboratory #18, discussed below, further suggest variation in the absolute weights may not be reflected in the relative response.

117. The statistical differences in the weak agonist doses occurred among four labs, in both Protocols B and C, and with four different chemicals. However, the relative increases of the blotted weights were

not statistically different for MC in Protocol B for Laboratory #1, for GEN in Protocol C for Laboratory #1, for MC in Protocol B in Laboratory #12, or the two chemicals in Protocol B for Laboratory #18 discussed below. This again suggests variation in the absolute weights are not always be reflected in the relative response, and supports the use of the relative response.

118. Five, or almost one third, of the instances of statistical difference occurred in one lab, Laboratory #18. Here the difference is driven by absolute differences amongst the groups, i.e., for the dose response animals the initial mean body weight was 40.6 g, the mean necropsy body weight was 52.1g, and the mean blotted uterine weight was 21.3 mg, and for the coded single-dose, the values were 45.3 g, 55.4 g, and 27.2 mg. The result was that the coded single-dose results for EE, BPA, and NP are then lower in absolute terms. However, the relative increase values were similar, e.g., 2.19 in the dose response and 2.28 in the coded single-dose for BPA and 1.97 and 1.69, respectively, for NP. The body weight adjusted relative values were 2.12 and 2.32 for BPA and 1.93 and 1.73 for NP. This again indicates that the considerable heterogeneity in the absolute immature numbers does not automatically indicate that similar heterogeneity exists in the relative responses. Instead, this suggests that the relative responses may be similar and reproducible even when the starting absolute weights are statistically different.

Comparison of immature (Protocol B) and OVX (Protocol C) dose-responses within labs

119. The responses in Protocols B and C afford the opportunity for a direct comparison of immature and OVX animals because the dose levels, routes, and dosing regimens are the same. The summary results for this comparison are shown in Tables 28a-e. Protocol C appears to achieve statistical significance and to have higher magnitude responses at lower doses with BPA (Table 28a). In contrast, Protocol B achieves statistical significance and higher responses at lower doses with GEN and MC (Tables 28c and d). In the case of NP, the data at the 80 and 100 mg/kg/d doses suggest that Protocol B is more responsive (Table 28e). The data are insufficient to draw any conclusions with DDT (Table 28b). In the coded single-dose studies with the negative chemical, DBP, both Protocol B and C encountered statistically significant differences at approximately the same rates (see Table 17). In conclusion, these data suggest that:

- neither the immature or the OVX version of the assay is consistently better than the other, and
- the overall results are interchangeable between the immature and the OVX versions.

Table 28a. Bisphenol A: Comparison of immature (Protocol B) and OVX (Protocol C) rats

Dose	Lab. #2		Lab. #6		Lab. #7		Lab. #8		Lab. #12	
Protocol	**B**	**C**	**B**	**C**	**B**	**C**	**B**	**C**	**B**	**C**
10	1.12	1.13	1.01	1.03	ND	ND	1.17	0.98	ND	ND
100	1.67*	1.89*	1.31*	1.67*	1.18	2.05*	1.47*	1.60*	1.47	2.03*
300	2.30*	2.79*	1.95*	3.44*	1.37*	2.41*	1.91*	2.65*	1.33	2.72*
600	3.30*	3.08*	3.47*	3.85*	1.75*	3.92*	2.13*	2.85*	2.51*	3.24*
800	4.00*	3.03*	3.66*	3.67*	ND	ND	3.01*	2.79*	ND	ND

Table 28b. *o,p'*-DDT: Comparison of immature (Protocol B) and OVX (Protocol C) rats.

Dose	Lab. #3		Lab. #11		Lab. #12	
Protocol	**B**	**C**	**B**	**C**	**B**	**C**
5	0.88	1.13	1.06	0.97	ND	ND
25	1.03	1.17	1.06	1.15	1.33	1.07
50	1.02	1.30*	1.04	1.25	1.30	1.10
100	1.01	1.43*	1.08	1.25	1.47*	1.31
200	1.31*	1.85*	1.36*	1.34*	ND	ND

Table 28c. Genistein: Comparison of immature (Protocol B) and OVX (Protocol C) rats.

Dose	Lab. #1		Lab. #9		Lab. #12	
Protocol	**B**	**C**	**B**	**C**	**B**	**C**
1	1.20	0.90	1.18*	0.99	ND	ND
15	1.79*	1.53*	1.91*	1.57*	1.48*	1.31
35	2.33*	1.78*	2.57*	1.87*	2.28*	1.56*
50	2.91*	1.68*	3.10*	2.08*	2.70*	1.62*
80	3.30*	1.89*	3.50*	1.98*	ND	ND

Table 28d. Methoxychlor: Comparison of immature (Protocol B) and OVX (Protocol C) rats.

Dose	Lab. #1		Lab. #3		Lab. #12	
Protocol	**B**	**C**	**B**	**C**	**B**	**C**
20	1.16	0.95	1.21	1.08	ND	ND
100	1.36*	1.28*	2.21*	1.72*	2.86*	1.63*
250	1.94*	1.85*	2.88*	1.99*	3.53*	1.79*
500	2.47*	2.32*	2.98*	2.42*	3.34*	1.95*
800	2.69*	2.42*	3.52*	2.59*	ND	ND

Table 28e. Nonylphenol: Comparison of immature (Protocol B) and OVX (Protocol C) rats.

Dose	Lab. #6		Lab. #7		Lab. #8		Lab. #9		Lab. #12	
Protocol	**B**	**B**	**C**	**C**	**B**	**C**	**B**	**C**	**B**	**C**
5	ND	1.05	1.21	ND	0.93	0.97	1.06	1.00	ND	ND
15	0.84	1.22	1.12	1.02	1.02	0.98	1.11	0.99	1.05	1.09
35	1.03	1.12	1.27*	1.14	1.15	1.15	1.33*	1.08	1.31	1.08
80	1.24	1.68*	1.64*	1.16	1.44*	1.17	1.86*	1.23	2.02*	1.33*
100	ND	2.25*	1.52*	ND	1.54*	1.42*	2.38*	1.61*	ND	ND

* , statistically significant increase at $p<0.05$
ND, not done

Comparison of 17α-ethinyl oestradiol values from Phase-1 and Phase-2 experiments

120. Phase-2 of the validation program was designed to assess the reproducibility of the uterotrophic bioassay by comparing three sets of EE results from:

- the dose response of Phase-1,
- the two EE doses selected as references controls in the dose response studies of Phase-2, and
- the two EE doses included in the coded single-dose series of Phase-2,

121. The reference control EE doses and the doses included in the coded single-dose series were identical for a given Protocol and were also used and found to be active in Phase-1. The data from Phase-1 have been reanalysed here to yield the ratio of the geometric means of the uterine weights (treated relative to the vehicle control) after adjusting for the body weight of the animal at necropsy with respective lower and upper 95% confidence levels in order to make comparisons with the Phase-2 analysed data. In

addition, an overall mean of the ratio and its standard deviation have been calculated for each data set to further compare the reproducibility of the assay. The combined results from the Phase-1 and Phase-2 studies for Protocols A, B, C, and D are in Tables 29a-d, respectively.

122. The comparison is made using only laboratories that performed EE analyses in Phase-1 and one or both of the EE analyses in Phase-2 for the same Protocol. If direct comparisons were not available, then those data are not included (e.g., Laboratories #4, 5, 6, and 14 evaluated EE in Protocol B in Phase 2, but not Phase 1, so the Protocol B data for these labs are not included).

Protocol A

123. The large majority of the labs produced internally consistent mean relative blotted uterus weight results with Protocol A in Phases-1 and across the dose response and coded single-dose studies in Phase-2. Consistency is defined here where the means of various experiments fall within the 95% confidence levels of the other studies.

124. There were several exceptions, which tended to occur more often at the lower 1 μg/kg/d EE dose and in accord with the lower portion of the dose response curve being more uncertain and more variable. The most obvious was Laboratory #1, which was less responsive at both the low 1 and high 3 μg/kg/d doses of EE in Phase-2 compared both to its own results in Phase-1 and all other laboratories performing Protocol A in both Phase-1 and Phase-2. In fact, Laboratory #1 was the only lab not to achieve statistical significance in Protocol A with the 1 μg/kg/d EE dose in two separate dose response experiments. There is no apparent explanation. Laboratory #1 used the same rat strain as most other labs (Sprague-Dawley), used the same diet as other labs (and the diet lost was similar in phytoestrogen content to other labs, used a similar vehicle, and the vehicle control weights were 30-38 mg.

125. Other possible exceptions included the following: Laboratory #4, whose relatve mean uterine increase response with 1 μg/kg/d EE in Phase-1 was 1.17, but 3.20 and 2.81 in the dose response and coded single dose studies, respectively in Phase-2 (the responses at the higher 3 μg/kg/d EE dose were, however, similar, see Table 29a); Laboratory #7, where one dose response value (3.15) was noticeably higher than Phase-1 (2.14) and another Phase-2 dose response value (2.16); Laboratory #8 where both the 1 and 3 μg/kg/d EE dose responses were noticeably higher in Phase-2 than Phase-1, and Laboratory #13 whose coded single-dose response at both the 1 and 3 μg/kg/d EE doses in Phase-2 (4.74 and 4.66, respectively) were noticeably higher than either Phase-1 (1.33 and 2.37) or the dose response in Phase-2 (1.44 and 2.55).

Table 29a. Protocol A: Comparison of the EE Phase-1 and Phase-2 blotted weight responses

Lab.	1.0 µg/kg/day			3.0 µg/kg/day		
	Phase-1	Phase-2		Phase-1	Phase-2	
		DR	CSD		DR	CSD
1 [a]		0.99 (0.77-1.26)[b]			1.64* (1.29 - 2.10)	
1	2.26* (1.67 - 3.05)	1.10 (0.92 - 1.32)		4.06* (3.00 - 5.49)	1.50* (1.25 - 1.79)	
2	2.29* (1.77 - 2.97)	3.17* (2.52 - 3.99)	2.78* (2.19 - 3.53)	3.69* (2.85 - 4.77)	4.13* (3.27 - 5.22)	4.41* (3.47 - 5.60)
3	1.85* (1.51 - 2.28)	2.25* (1.77 - 2.86)	1.52* (1.24 - 1.88)	3.43* (2.79 - 4.21)	2.55* (2.00 - 3.25)	2.79* (2.27 - 3.44)
3		1.53* (1.20 - 1.96)			2.80* (2.20 - 3.58)	
4	1.17* (1.01 - 1.39)	3.20* (2.36 - 4.35)	2.81* (2.18 - 3.61)	4.00* (3.36 - 4.75)	4.04* (2.97 - 5.48)	3.70* (2.88 - 4.76)
5	1.40* (1.04 - 1.89)	1.40* (1.04 - 1.90)	1.36* (1.07 - 1.74)	2.83* (1.98 - 4.05)	1.91* (1.41 - 2.59)	1.80* (1.40 - 2.31)
7	2.14* (1.72 - 2.67)	2.16* (1.73 - 2.69)				
7		3.15* (2.57 - 3.85)				
8	1.93* (1.31 - 2.85)	3.09* (2.55 - 3.73)	3.31* (2.73 - 4.00)	3.15* (2.14 - 4.65)	4.69* (3.88 - 5.66)	5.02* (4.15 - 6.08)
9	1.55* (1.28 - 1.96)	2.19* (1.72 - 2.79)		3.01* (2.44 - 3.72)	5.19* (4.10 - 6.58)	
11	2.60* (2.25 - 3.01)	3.04* (2.42 - 3.83)	2.88* (2.26 - 3.68)	3.88* (3.35 - 4.49)	4.52* (3.54 - 5.78)	4.29* (3.36 - 5.48)
12	1.98* (1.27 - 3.10)	2.85* (2.21 - 3.67)	2.98* (1.47 - 6.03)	3.42* (2.19 - 5.33)	4.68* (3.63 - 6.02)	
13	1.33* (1.13 - 1.58)	1.44* (1.06 - 1.95)	4.74* (3.88 - 5.80)	2.37* (2.02 - 2.79)	2.55* (2.06 - 3.17)	4.66* (3.83 - 5.66)
14	2.99* (2.31 - 3.85)	3.11* (2.44 - 3.98)	2.44* (1.61 - 3.70)	3.96* (3.09 - 5.07)	4.69* (3.74 - 5.89)	4.20* (2.73 - 6.47)
mean ± s.d.	**1.97 ± 0.53**	**2.31 ± 0.83**	**2.79 ± 0.94**	**3.44 ± 0.55**	**3.45 ± 1.32**	**3.95 ± 1.04**

[a] Two listings of the same laboratory are used when the laboratory conducted two separate EE control studies in the Phase-2 dose-response protocol.

[b] Mean ratio of blotted uterine weights to vehicle control weights, and with body weights at necropsy as a covariable (95% confidence interval).

DR – dose response; CSD – coded single-dose; * , statistically significant at $p<0.05$.

Mean ± s.d. of the ratios.

Protocol B

126. Again, the large majority of the labs produced internally consistent results with Protocol B in Phases-1 and across the dose response and coded single-dose studies in Phase-2. The exceptions were confined to the lower 0.3 µg/kg/d EE dose, which is again consistent with the lower portion of the dose response curve being more uncertain and more variable. Laboratory #9 observed a more marked increase at the lower 0.3 µg/kg/d dose in Phase-2 (4.22) than in Phase-1 1.73). The observations in labs 15 and 18 showed a strikingly similar pattern (see Table 29b). Laboratory #17, however, had somewhat lower responses at both doses in Phase-2. Otherwise, the similarity between Phase-1 and Phase-2 results in the other labs was remarkably good.

Table 29b. Protocol B: Comparison of the EE Phase-1 and Phase-2 blotted weight responses

Lab.	0.3 µg/kg/day			1.0 µg/kg/day		
	Phase-1	Phase-2		Phase-1	Phase-2	
		DR	CSD		DR	CSD
1[a]	2.44* (1.96 - 3.05)	2.17* (1.80-2.62)[b]		4.07* (3.25 - 5.09)	4.19* (3.47 - 5.05)	
1		2.49* (2.12 - 2.93)			4.07* (3.46 - 4.80)	
2	2.91* (2.40 - 3.53)	2.11* (1.70 - 2.62)	2.25* (1.80 - 2.82)	4.82* (3.98 - 5.85)	4.44* (3.60 - 5.48)	4.42* (3.52 - 5.54)
3	2.49* (2.08 - 2.97)	2.00* (1.68 - 2.38)	2.42* (1.96 - 3.00)	4.24* (3.54 - 5.07)	3.82* (3.17 - 4.60)	4.63* (3.74 - 5.73)
3		2.31* (1.89 - 2.81)			3.79* (3.11 - 4.63)	
7	1.95* (1.54 - 2.47)	1.73* (1.48 - 2.01)		4.89* (3.86 - 6.20)	4.06* (3.49 - 4.72)	
7		1.79* (1.52 - 2.10)			4.16* (3.53 - 4.90)	
8	2.25* (1.86 - 2.71)	2.65* (2.37 - 2.97)	2.99* (2.40 - 3.72)	4.13* (3.43 - 4.99)	4.96* (4.43 - 5.55)	4.76* (3.81 - 5.95)
9	1.73* (1.38 - 2.17)	4.22* (3.63 - 4.91)		4.18* (3.33 - 5.25)	4.26* (3.64 - 4.98)	
11	2.74* (2.22 - 3.37)	3.50* (2.83 - 4.34)	3.24* (2.48 - 4.24)	4.53* (3.66 - 5.61)	4.58* (3.70 - 5.69)	4.26* (3.17 - 5.71)
12	1.83* (1.30 - 2.58)	1.68* (1.11 - 2.53)	1.32 (0.91 - 1.90)	3.07* (2.18 - 4.32)	3.64* (2.43 - 5.45)	
15	2.41* (1.84 - 3.15)	4.45* (3.46 - 5.71)		4.75* (3.64 - 6.19)	4.95* (3.66 - 6.69)	
17	2.99* (2.39 - 3.73)		1.83* (1.29 - 2.61)	4.55* (3.63 - 5.72)		3.50* (2.45 - 5.00)
18	2.11* (1.58 - 2.8)	3.81* (3.28 - 4.50)	3.51* (2.86 - 4.32)	5.99* (4.47 - 8.04)	5.62* (4.89 - 6.49)	5.12* (4.18 - 6.29)
mean ± s.d.	**2.35 ± 0.42**	**2.69 ± 0.97**	**2.51 ± 0.79**	**4.47 ± 0.71**	**4.35 ± 0.56**	**4.45 ± 0.55**

[a] Two listings of the same laboratory are used when the laboratory conducted two separate EE control studies in the Phase-2 dose-response protocol.

[b] Mean ratio of blotted uterine weights to vehicle control weights, and with body weights at necropsy as a covariable (95% confidence interval).

DR – dose response; CSD – coded single-dose; * , statistically significant at $p<0.05$. Mean ± s.d. of the ratios.

Protocol C

127. For 6 of the 7 laboratories, the results were internally consistent results with Protocol C between Phases-1 and across the dose response and coded single-dose studies in Phase-2. The exception was Laboratory #19, which had failed to reach statistical significance at the 0.3 µg/kg/d EE dose and only attained marginal significance at the 1 µg/kg/d EE dose in Phase-1. In Phase-2, Laboratory #19 achieved significance at the lower dose, but not at the higher dose, where the increase (1.97) was not dissimilar to that of the high dose in Phase-1 (1.35) (see Table 29c). This leads to the speculation of whether the doses were possibly reversed.

128. Consistent with observations made during Phase-1 (10)(11) there was a reduction in body weight in the high dose EE group relative to controls across laboratories and in both the dose response and the coded single-dose studies in the OVX animals in Protocol C. These consistent body weight reductions were not seen with the immature animals in Protocols A or B.

Table 29c. Protocol C: Comparison of the EE Phase-1 and Phase-2 blotted weight responses

Lab.	0.3 µg/kg/day			1.0 µg/kg/day		
	Phase-1	Phase-2		Phase-1	Phase-2	
		DR	CSD		DR	CSD
1 [a]	1.94* (1.64 - 2.28)	1.98* (1.70-2.30) [b]		3.24* (2.75 - 3.82)	3.13* (2.66 - 3.67)	
1		2.14* (1.88 - 2.43)			3.77* (3.27 - 4.35)	
2	1.84* (1.52 - 2.22)	2.41* (2.06 - 2.81)	2.45* (1.87 - 3.19)	2.93* (2.47 - 3.48)	3.19* (2.69 - 3.78)	3.68* (2.77 - 4.88)
3	1.86* (1.45 - 2.38)	2.43* (2.14 - 2.77)	2.35* (1.84 - 3.02)	2.83* (2.18 - 3.68)	3.57* (3.06 - 4.18)	3.54* (2.74 - 4.57)
3		2.96* (2.36 - 3.71)			3.61* (2.84 - 4.59)	
7	1.80* (1.49 - 2.18)	1.78* (1.47 - 2.16)		2.56* (2.11 - 3.11)	3.29* (2.69 - 4.01)	
7		2.71* (2.27 - 3.24)			4.32* (3.55 - 5.25)	
8	2.69* (2.28 - 3.17)	2.16* (1.91 - 2.43)	2.72* (2.03 - 3.63)	3.68* (3.12 - 4.34)	2.70* (2.39 - 3.05)	3.31* (2.47 - 4.42)
11	2.40* (1.99 - 2.89)	3.04* (2.63 - 3.51)	2.92* (2.47 - 3.46)	3.69* (3.05 - 4.46)	3.97* (3.36 - 4.69)	3.92* (3.28 - 4.68)
19	1.04 (0.81 - 1.32)		1.97* (1.62 - 2.40)	1.36* (1.07 - 1.73)		0.96 (0.79 - 1.16)
mean ± s.d.	**1.93 ± 0.52**	**2.40 ± 0.43**	**2.48 ± 0.36**	**2.91 ± 0.81**	**3.51 ± 0.49**	**3.08 ± 1.21**

[a] Two listings of the same laboratory are used when the laboratory conducted two separate EE control studies in the Phase-2 dose-response protocol.

[b] Mean ratio of blotted uterine weights to vehicle control weights, and with body weights at necropsy as a covariable (95% confidence interval).

DR – dose response; CSD – coded single-dose; * , statistically significant at $p<0.05$. Mean ± s.d. of the ratios.

Protocol D

129. The results were internally consistent with Protocol D between Phases-1 and across the dose response and coded single-dose studies in Phase-2 (Table 29d). The possible exception was Laboratory #11, where both the low 0.3 µg/kg/d EE and the high 1 µg/kg/d EE doses were noticeably more responsive in both the dose response (5.16 and 5.85, respectively) and coded single-dose studies (4.82 and 5.47, respectively) of Phase-2, than in Phase-1 (2.52 and 4.23, respectively) (see Table 29d).

130. A reduction in body weight in the high dose EE group relative to controls was observed with the OVX animals in both the dose response and the coded single-dose studies with Protocol D. As with Phase-1, these reductions occurred to a greater extent with the increased number of doses in Protocol D versus C.

Table 29d. Protocol D: Comparison of the EE Phase-1 and Phase-2 blotted weight responses

Lab.	0.3 µg/kg/day			1.0 µg/kg/day		
	Phase-1	Phase-2		Phase-1	Phase-2	
		DR	CSD		DR	CSD
1 [a]	2.37* (1.85 - 3.04)	2.93* (2.38-3.60) [b]		4.00* (3.05 - 5.26)	4.40* (3.45 - 5.61)	
1		2.75* (2.30 - 3.30)			4.11* (3.36 - 5.04)	
3	2.49* (1.94 - 3.21)	2.77* (2.44 - 3.14)	3.05* (2.40 - 3.88)	3.09* (2.28 - 4.19)	3.67* (3.13 - 4.29)	4.41* (3.32 - 5.87)
3		2.85* (2.50 - 3.26)			4.04* (3.49 - 4.69)	
7	2.58* (1.98 - 3.17)	2.45* (1.97 - 3.05)		3.65* (2.75 - 4.85)	4.50* (3.53 - 5.73)	
7		3.28* (2.48 - 4.35)			5.67* (4.15 - 7.74)	
11	2.52* (2.11 - 3.01)	5.16* (3.65 - 7.28)	4.82* (3.61 - 6.42)	4.23* (3.46 - 5.17)	5.85* (4.26 - 8.05)	5.47* (4.14 - 7.21)
mean ± s.d.	**2.49 ± 0.09**	**3.17 ± 0.91**	**3.94 ± 1.25**	**3.74 ± 0.50**	**4.61 ± 0.83**	**4.94 ± 0.75**

[a] Two listings of the same laboratory are used when the laboratory conducted two separate EE control studies in the Phase-2 dose-response protocol.

[b] Mean ratio of blotted uterine weights to vehicle control weights, and with body weights at necropsy as a covariable (95% confidence interval).

DR – dose response; CSD – coded single-dose; * , statistically significant at $p<0.05$. Mean ± s.d. of the ratios.

131. The responses to EE in Protocols B and C afford another opportunity for a direct comparison of immature and OVX animals, again, because the dose levels, routes, and dosing regimens are the same. The means from Table 28b and 28c have been combined in Table 29. The results suggest that the immature and OVX animals provide similar responses in the lower regions of the dose response curve, but that the immature animals may be more responses in the higher regions of the dose response curve. As most weak agonists may better reflect the lower region of the EE dose response curve, these data reinforce the previous conclusions that:

- neither the immature or the OVX version of the assay is consistently better than the other with weak oestrogen agonists, and
- the overall results should be interchangeable between the immature and the OVX versions.

Table 30. Comparison of EE responses of immature (Protocol B) and OVX (Protocol C) rats

Protocol	0.3 µg/kg/day			1.0 µg/kg/day		
	Phase-1	Phase-2		Phase-1	Phase-2	
		DR	CSD		DR	CSD
B	2.35 ± 0.42	2.69 ± 0.97	2.51 ± 0.79	4.47 ± 0.71	4.35 ± 0.56	4.45 ± 0.55
C	1.93 ± 0.52	2.40 ± 0.43	2.48 ± 0.36	2.91 ± 0.81	3.51 ± 0.49	3.08 ± 1.21

DR – dose response; CSD – coded single-dose.

Sources of variability

132. The uterotrophic bioassay has several potential sources of variability. Some sources are common between the immature and the OVX animals and some are unique to one or the other. A partial listing of sources to consider are:

- Dietary phytoestrogen content. Increased levels of phytoestrogens may at sufficient levels begin increase the baseline mean uterine weight, leading to a gradual loss in dynamic range with increasing phytoestrogen levels. As immature rats can consume approximately twice as much food per kg of body weight as young adults, this will effective double the phytoestrogen intake for immature animals consuming the same diet. The diet may then be a source of variation across laboratories, and this is addressed in a later section.
- Dosing errors and losses. The smaller physical size of the immature animals presents a greater challenge for oral gavage and a reduced area for subcutaneous injection.
- The dissection and tissue preparation of the uterus. The skill of individual technicians is paramount, and the immature uterus is about one-third the weight of the young adult size after OVX and thus may encounter increased. variability within the same laboratory and vary across laboratories.
- Tissue desiccation. The small, moist uterine tissue may encounters weight loss via desiccation and require precautionary procedures (e.g., weighing chambers with moistened paper).
- Incorrect animal age could include immature animals in the early stages of puberty, increasing the variability of the uterine weights (25)(26). This could reduce the sensitivity of the bioassay.
- Incomplete OVX will leave ovarian tissue that can produce endogenous oestrogen and retard the regression of the uterine weight. The presence of such tissue can be discovered by gross pathology and histopathology.

133. These factors would be expected to increase the coefficient and generate an association between the coefficient of variation and the laboratory for a protocol. The initial investigation of differences in the coefficient of variation and the influence of phytoestrogens in the diet is presented in following sections.

Comparison of vehicle control uterine weights

134. While the relative increase in uterine weight was reproducible across laboratories, the absolute wet and blotted immature and OVX uterine weights varied. This section examines this variability for both the immature and the OVX animals as well as the minimum time of regression had been increased from 10 to 14 days between Phase-1 and Phase-2, these data were also reviewed.

135. The immature vehicle control blotted uterine weights are plotted against their respective body weights in Figure 3. Four observations can be made. First, there is a substantial range in body weights from about 35 grams to nearly 70 grams. Second, there is a gradual trend for uterine weights to increase modestly with body weights. Third at any given body weight there is an approximate 10-15 milligram range in the mean blotted uterine weight. Fourth, there are six means that are greater than 40 milligrams, and at least five of these appear to lie outside the range observed in the other data.

136. The same plot of blotted vehicle control weights versus body weight is done for the OVX animals in Figure 4. The body weight range is 220-300 grams and a range in uterine weights exists at a given body weight. However, any trend for uterine weights to increase with body weights is reduced in comparison with the immature animals.

137. These data are expressed for each laboratory as mean of the vehicle control blotted uterine weight relative to the body weight in Table 31. The immature animals are relatively variable with the ratio varying from a low of 0.037 in Laboratory #14 to a value three-fold higher of 0.119 in Laboratory #6. The ‘outlying’ points in Figure 3 come from Laboratories #6, 20, 21, where the ratios are high. In contrast, the OVX values are almost constant across laboratories with a range of 0.029 to 0.037 per cent. The highest value is again from Laboratory #6.

Figure 3. Mean blotted uterine weights for immature vehicle control groups relative to their mean body weight

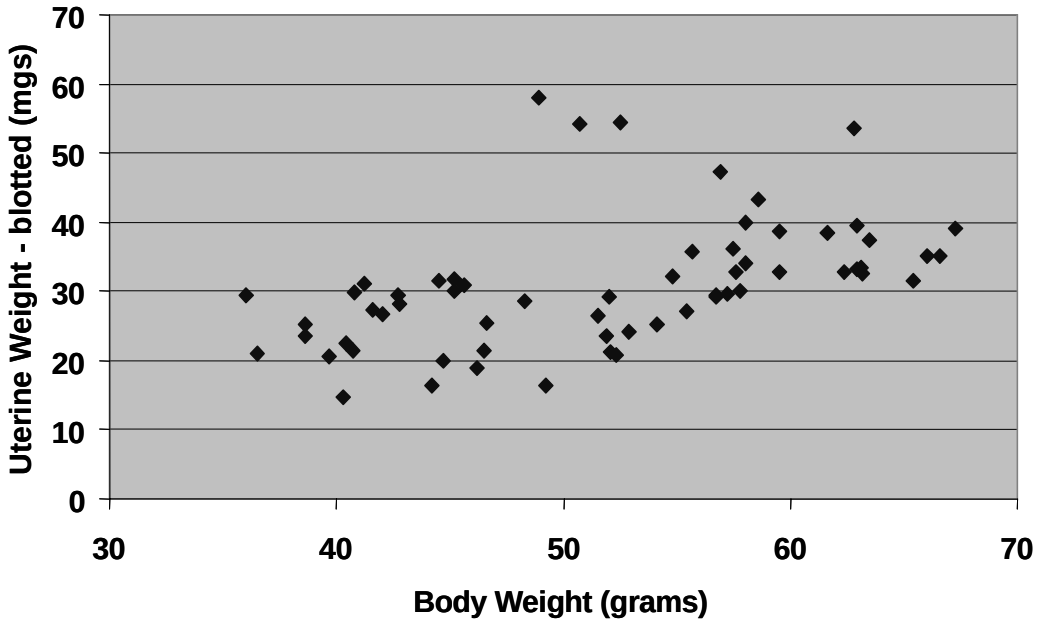


Figure 4. Mean blotted uterine weights for OVX vehicle control groups relative to their mean body weight

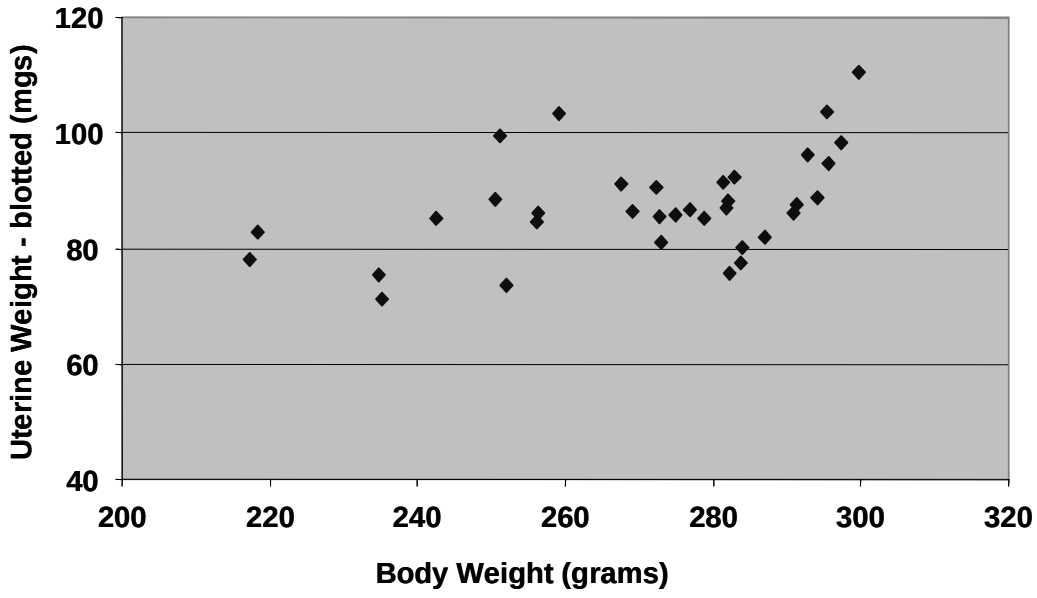


Table 31. Blotted uterine weights as a percentage of body weight in animals treated only with the vehicle control

	Immature rats	OVX rats
Lab.	**Protocols A, B**	**Protocols C, C', D**
1	.057 ± 0.005 (6)	.033 ± 0.004 (6)
2	.055 ± 0.003 (4)	.034 ± 0.004 (4)
3	.051 ± 0.002 (5)	.032 ± 0.001 (6)
4	.071 ± 0.004 (4)	--
5	.065 ± 0.008 (4)	--
6	.119 ± 0.001 (2)	.037 (1)
7	.053 ± 0..009 (4)	.031 ± 0.004 (3)
8	.045 ± 0.005 (6)	.029 ± 0.002 (3)
9	.055 ± 0.006 (2)	.030 ± 0..005 (2)
11	.064 ± 0.005 (4)	.034 ± 0.003 (4)
12	.059 ± 0.015 (4)	.033 ± 0.001 (4)
13	.073 ± 0.011 (4)	--
14	.037 ± 0.003 (4)	--
15	.060 (1)	--
16	.054 (1)	--
17	.065 (1)	--
18	.045 ± 0.006 (2)	--
19	.064 (1)	.033 (1)
20	.105 ± 0.001 (2)	--
21	.086 ± 0.004 (2)[a]	--

mean ± S.D. (number of experiments)

[a] This is an estimate, based on estimated final body weights of 56 and 61 g for the 2 groups

Comparison of vehicle control weight variability

138. The coefficients of variation were calculated for all immature and all OVX vehicle control groups from Protocols A and B and Protocols C and D, respectively. These were then ranked in ascending order and plotted in Figures 5 and 6, respectively. A cautious observation is that CV plot for immature animals has a 'tail' of about ten points that exceeds a CV of 0.25, the maximum CV calculated for the OVX animals. The caution is that several labs performed Protocols A and B or B only, and not C, so the CV's may not be reflective of the Protocols.

Figure 5. Rank order of the coefficients of variation for the immature vehicle control groups from Protocols A and B

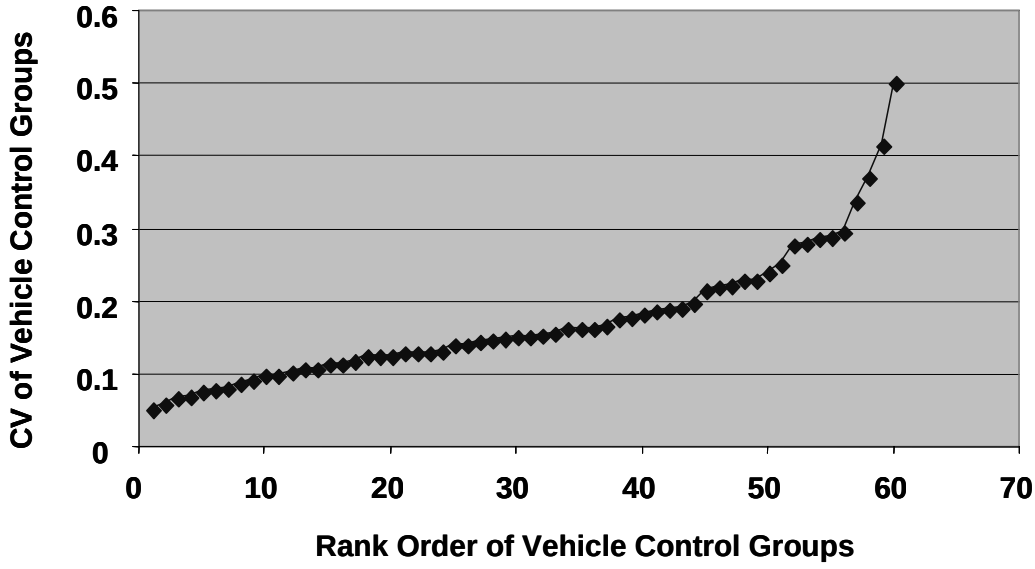
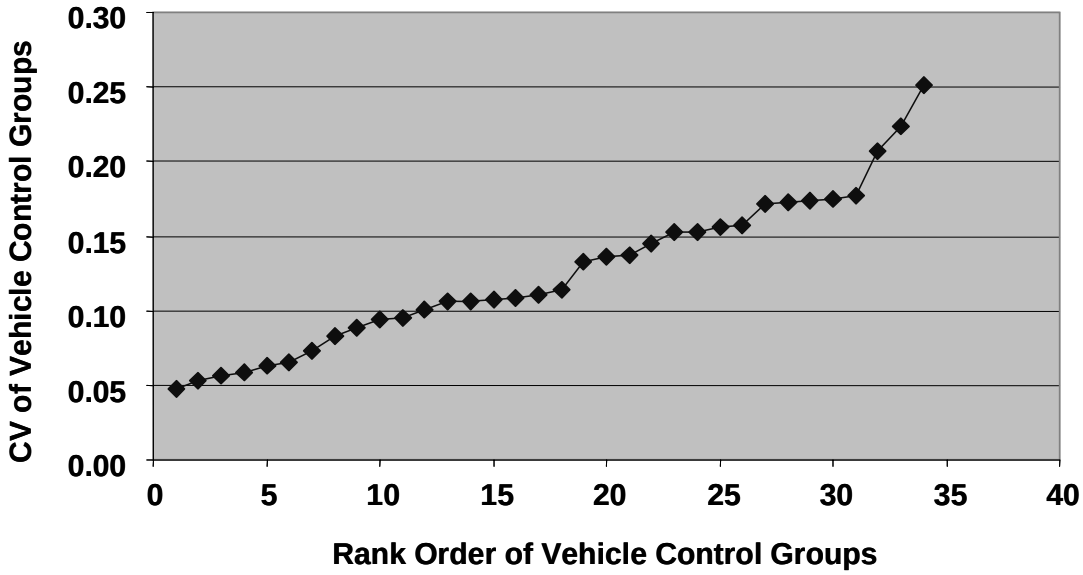


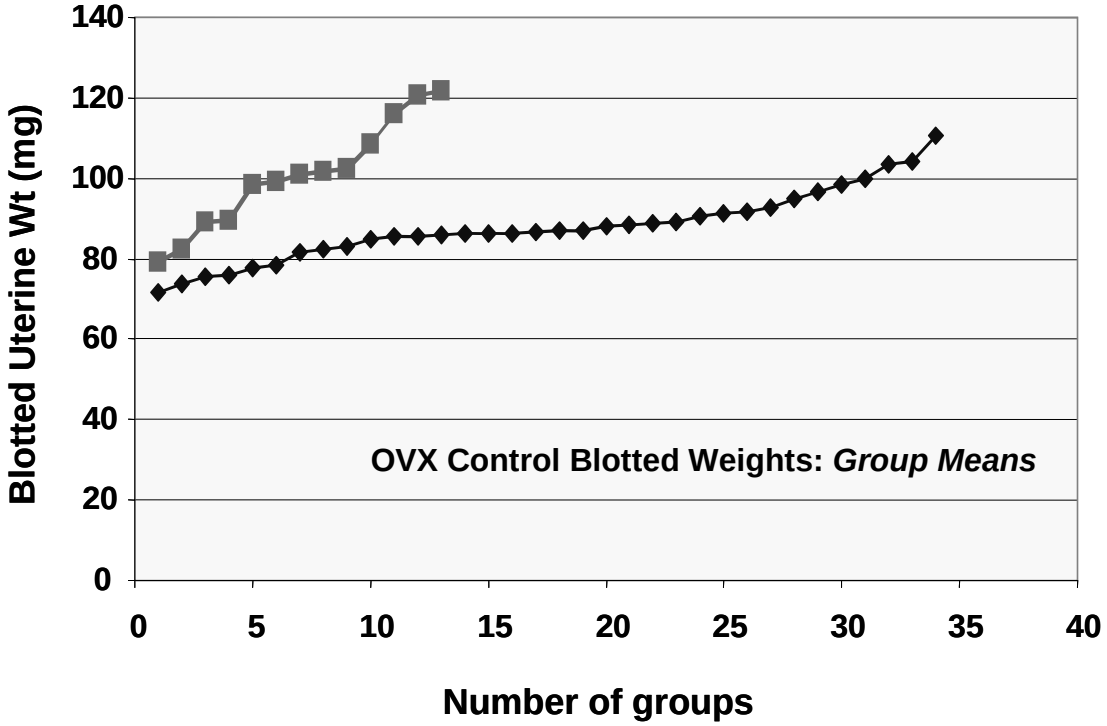
Figure 6. Rank order of the coefficients of variation for the OVX vehicle control groups from Protocols C and D



Regression time for OVX animals

139. The VMG modified the OVX protocols, which in Phase-1 required a minimum 10-day regression time after OVX. In Phase-2, a 14-day regression time was required. To assess the possible impact this change may have had, the vehicle blotted control weights of OVX animals from both Phase-1 and Phase-2 were compared. The results are shown in Figure 7. The results indicate a slight lower overall mean uterine weight in Phase-2 with the increase in regression time to 14 days.

Figure 7. Rank order of the mean blotted uterine weights of the OVX vehicle control groups from Phase-1 (squares) and Phase-2 (diamonds)



DIET ANALYSES

140. One possible factor in the greater range of the immature vehicle control blotted uterine is the phytoestrogen content of the laboratory diet. As noted, there is evidence that, when high levels of phytoestrogens are present in the animal diet, uterine control weights could potentially be increased and thereby reduce the dynamic range of the assay by decreasing uterine responsiveness (49)(508)(reviewed in (25)(26). Therefore, retained diet samples were analysed for their daidzein, genistein, and coumestrol content. The methodological details of the dietary phytoestrogen analyses, the detailed results of the analyses, and the comparison of the dietary phytoestrogen levels to uterotrophic bioassays with weak oestrogen agonists are contained in Annex 3.

141. The phytoestrogen genistein was present in the highest concentrations, followed by daidzein, and these were detected in all samples. Of the 26 diet samples analysed, 8 were below the level of detection of coumestrol. Four laboratories (Laboratories #1, 2, 8, 9) submitted samples from more than one lot of diet. Among laboratories, there were three possible duplicates from the same lot, which were diet codes 4 and 8, diet codes 5 and 9, and diet codes 12 and 13 (Table 32). While not precise split samples, it is of interest to compare the analytical results for these samples. Analyses of samples 4 and 8 closely correspond for all three substances. For samples 5 and 9, the correspondence is excellent for daidzein, but there is an ~20% difference in genistein levels at 216 and 170 µg/g diet, respectively. For samples 12 and 13, the daidzein and coumestrol correspondences are excellent, but the genistein analyses differ at 218 and 180 µg/g diet, respectively. Further, where different lots of the same diet were submitted, the pattern of analytical results were consistent within a given diet except for sample 2, where the daidzein content was somewhat lower.

142. In order to assess the possible interaction between dietary phytoestrogens and uterine weights and the responsiveness of the bioassay, a working assumption was that different phytoestrogens interact in a simple, additive manner. This permits a proxy calculation of total genistein equivalents in the diet. This

assumption of additivity has significant qualifications, which are discussed in detail in the published manuscript (42). To summarise, there are two forms of the oestrogen receptor (ER), α and β, with different tissue distributions and some differences in binding affinity, particularly for phytoestrogens. Further, the ER depends upon coactivators that may be tissue dependent. Therefore, data from the same tissue and endpoint should be used to construct any estimated oestrogen equivalents, e.g., an increase the uterine weight, and extrapolations to other tissues and endpoints done with care. These are published uterotrophic results that question direct additivity and linearity of any equivalency assumptions. Finally, relevant, high quality and comparable *in vivo* uterotrophic data are needed for each chemical, and the phytoestrogen data are fragmented and difficult to compare. Stressing the high degree of uncertainty, the values chosen for equivalency factors are: 0.8 to convert daidzein and 10 to convert CM into genistein units on a weight basis. These factors have been used to calculate a total phytoestrogen content in genistein equivalents for each of the analysed diets. The calculated values are in the right hand column of Table 32. These range from a low of 100 µg total genistein equivalents per g of diet to a high value over 500 µg total genistein equivalents per g of diet.

143. With the phytoestrogen content known, a value for food intake would permit a direct estimation of the actual phytoestrogen intake for each of the vehicle control groups. Eight participating laboratories recorded food consumption for intact, immature females, and three recorded data for OVX females. Intact, immature female displayed a rapid rise in food consumption from approximately 2-4 grams per animal on day one to 6-11 grams per animal on day four before necropsy. As intact, immature animals were group housed with no less than 3 animals per cage, mean food consumption is calculated per animal for a cage. For the OVX females, food consumption was more stable at 14-24 grams per animal on day four among the laboratories. All amounts may include wastage and spillage. Where animals had diminished increases in body weight or even body weight losses, food consumption was lower, and these cases were not considered representative. The approximate food intake ranges were then 130-170 g/kg bw/d for immature animals and 60-75 g/kg bw/d, for OVX animals. These estimates compare favourably with published NTP data where rats consumed a mean of 14.8 grams of diet per day for a 200 gram body weight or 74 g/kg bw/d (51). Importantly, mice in NTP studies consumed a mean of 7.2 grams of diet per day for a 25 gram body weight or 288 g diet/kg bw/d. This indicates a potential phytoestrogen intake in adult mice twice that of the immature rat and four times that of the OVX rat on a body weight basis from the same diet.

Table 32. Comparison of phytoestrogen levels with estimated total genistein equivalents of each diet

Lab	Diet Code	Daidzein (µg/g diet)	Genistein (µg/g diet)	Coumestrol (µg/g diet)	Estimated Total Genistein Equivalents[a] (µg/g diet)
1	4	91.2	206.5	3.1	310
	5	101	216	ND	297
	6	83.7	198.6	ND	266
2	8	84.1	190	ND	257
	9	85	169.8	2.3	261
	10	40.7	123.3	1.8	174
3	20	130.9	221.8	0.8	335
4	2	70.9	151.5	ND	208
5[b]	3	88.1	204.2	2.5	300
6	11	53.9	132.6	ND	176
7	12	101.7	238.3	1.8	338
8	24	48.7	156.8	ND	196
	25	53.2	135.3	ND	178
9	14	85.8	200.1	3.8	307
	15	84.7	175.1	1.3	256
11	13	113.2	180.2	2.8	299
12	7	117	218.3	ND	312
13	1	113.8	239.6	1.3	344
14	17	28.4	72.9	2.6	122
15	19	48	131.9	1.5	185
16	16	84	144.7	2.3	235
17	21	90.1	177.1	2.3	272
18	23	77.8	164.8	0.8	235
19	22	29.2	72.4	0.3	99
20	26	186.6	354.7	0.9	513
21	18	121.2	226.2	4.1	364

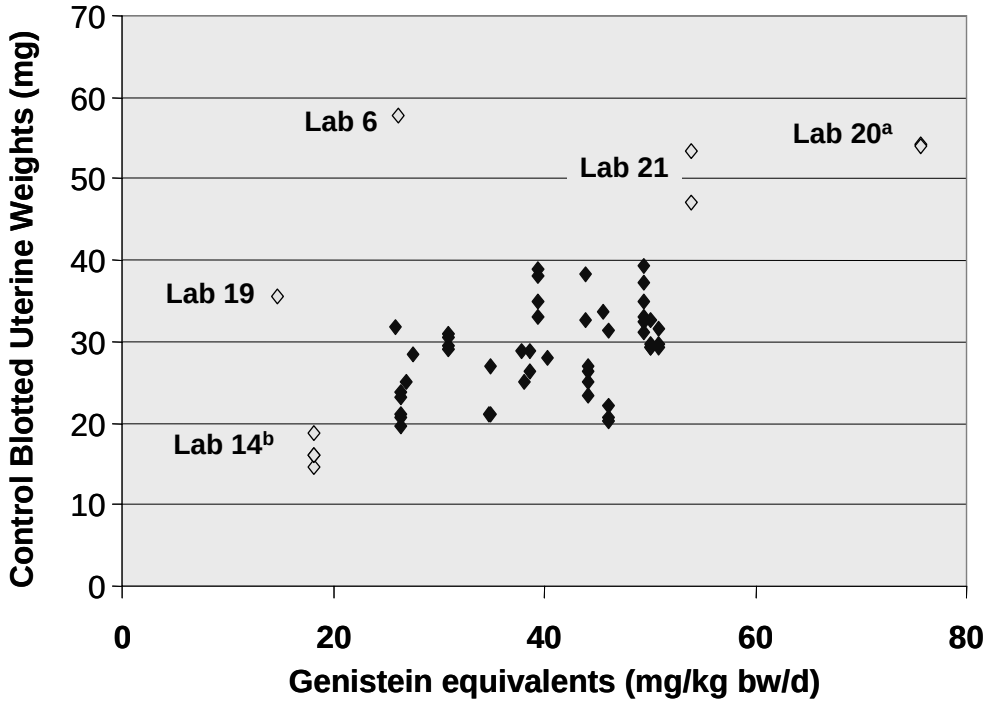
[a] The equivalency factor used to convert daidzein to genistein was 0.8, and the equivalency factor used to convert coumestrol to genistein was 10. The converted µg/g diets were then summed to give total genistein equivalents (TGEs).

[b] In the case of laboratory 5 and diet sample 3, it was discovered that the diet sample had not been used in the uterotrophic studies, but had been used in parallel studies to validate the castrated male or hershberger bioassay and was submitted in error. While the phytoestrogen analyses are reported in Table 2, these data have not been used.

ND – non detectable. Phytoestrogen weights from Annex 3.

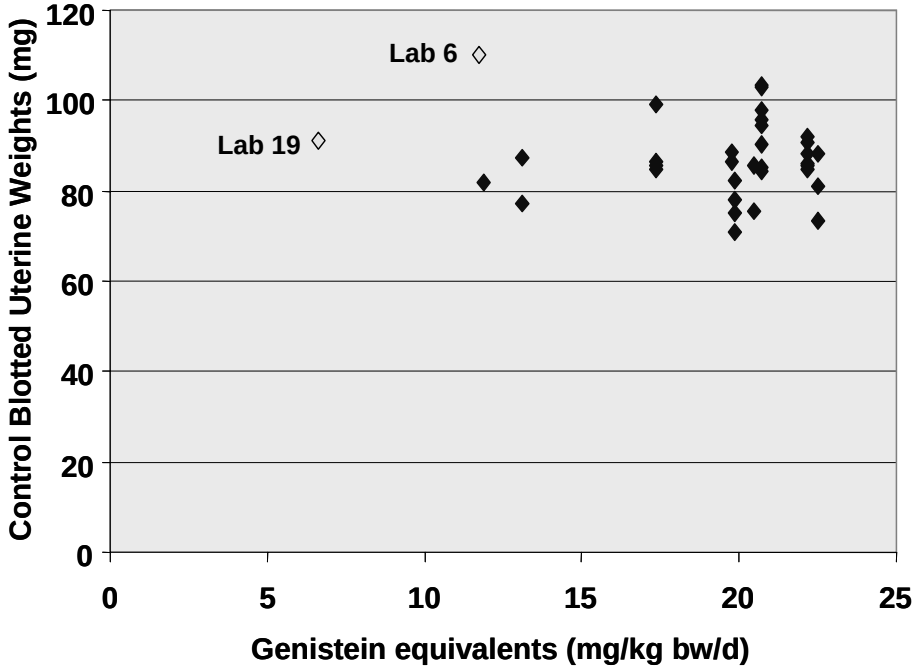
144. The estimated intakes of total genistein equivalents was then compared to the mean vehicle control blotted uterine weights for immature and OVX animals. The results are shown in Figures 8 and 9, respectively. First, the immature intakes ranged from 15 to 75 mg total genistein equivalents per kg body weight per day, and the OVX intakes ranged from 6 to 24 mg total genistein equivalents per kg body weight per day. For the immature animals, several laboratories (Laboratories #6, 19, 20, 21) appear to have 'outlying' values. The values for Laboratory #20 (whose body weights were estimated in the previous section) and #21 suggest along with the other data that a modest slope exists between increasing phytoestrogen intake and the vehicle blotted weight, implying that dietary phytoestrogens do increase the uterine weights. In contrast, there is no suggestion for a similar interaction at the lower intakes with the OVX animals. This is consistent with the published literature that suggests a LOEL of about 40 mg GN/kg/d intake to increase uterine weights (52)(53)(54)(55). This is also consistent with the calculated MEDs for genistein of 20-60 mg/kg/d in the dose response studies (see Table 15c).

Figure 8. Estimated total intake of phytoestrogens as genistein equivalents versus the mean vehicle control blotted weights for immature animals



[a] Two data points
[b] This lab encountered a high level of mortalities.

Figure 9. Estimated total intake of phytoestrogens as genistein equivalents versus the mean vehicle control blotted weights for OVX animals



145. Based on these data, a detailed analysis of the responsiveness of the immature animals with all of the weak agonists is being conducted and published in the peer-reviewed literature (40). The analyses included a calculation of the putative slope using a linear least squares approach and the R-squared value for the estimated dietary intake against the relative uterine response for all the weak agonists in Protocols A, B, and C. An interaction would first require a negative slope. The results are shown in Table 31. The analysis found no evidence to suggest that the OVX animals (Protocol C) were influence by diet phytoestrogens with the current diets as the slopes were positive in four cases, including the three weak agonists (BPA, NP, and GEN). The R-square values did not indicate any significance and ranged from 0.003 to 0.444. The analysis did find evidence for a modest influence of phytoestrogens on the immature animals (Protocols A and B). The slopes were negative in all cases where Laboratories #20 and 21 were included, but the highest R-squared value was only 0.630. This provides only suggestive evidence for a possible decrease in responsiveness to weak agonists such as NP and BPA at intakes of 50 mg/kg/d and above of genistein equivalents or a level of about 350 μg phytoestrogens/g diet. As the validation of the mouse uterotrophic bioassay is being considered, this would extrapolate to 175 μg phytoestrogens/g diet for mice.

Table 33. Linear least squares analyses of dietary total estimated genistein Intake (mg/kg/d) against the ratio of the relative increase of the blotted uterine weights

	Protocol A		Protocol B		Protocol C	
Chemical	**Linear Equation**	**R2**	**Linear Equation**	**R2**	**Linear Equation**	**R2**
Bisphenol A	-0.0111x + 1.8462	0.265	-0.0161x + 2.4539	0.296	0.0299x + 2.2452	0.196
			-0.0089x + 2.2007[1]	0.053		
Genistein	-0.0017x + 2.8993	0.002	-0.0274x + 3.5953	0.630	-0.0365x + 2.4806	0.444
			-0.0231x + 3.4422[1]	0.393		
Methoxychlor	-0.0044x + 3.3632	0.018	-0.0309x + 4.3111	0.519	0.0067x + 2.0387	0.003
			-0.0273x + 4.184[1]	0.325		
Nonylphenol	-0.0049x + 2.4103	0.017	-0.002x + 1.6629	0.007	0.0058x + 1.2591	0.043
			0.013x + 1.167[1]	0.154		
o,p'-DDT	-0.0258x + 4.5469	0.282	-0.0011x + 1.3166	0.003	0.0151x + 1.0125	0.179
			0.0076x + 1.017[1]	0.079		

[1] The values for the high phytoestrogen diet in laboratory 20 were omitted and the least squares analysis performed to assess the influence of these data on the overall trend.

146. A note of caution is necessary, however, in attempting any definitive conclusion. The interpretation that an influence of dietary phytoestrogen at high dietary levels exists largely relies on the results in Laboratory #20. In the NP dose response studies, all relative responses from the test substance responses in Laboratory #20, were ≤ 0.75, meaning that while the mean of the control was 54 mg, the means of the test substances were 34 to 41 mg. This suggests that the control value was an anomaly. In the BPA experiments using the same control, the test substance means ranged from 27 to 97 mg, further indicating the control was an anomaly. In the coded single-dose studies, the vehicle control mean was 54.4 mg; the dibutylphthalate mean was 39 mg and the sc administered DDT mean was 43 mg; and the remaining relative responses were lower than in other laboratories. A general phenomena of increased uterine weights would be expected to have resulted in high blotted means for all groups, and this was not the general case.

147. These findings are consistent with data showing biological activity of GEN at 40-50 mg/kg/d in both the literature (49)(50) as well as the GEN MEDs after oral administration in this report. However, additional dietary factors other than phytoestrogens can lead to premature puberty in the rat (51). Until the role of dietary factors is clarified, laboratories should monitor their control uterine weights for unusual variations and, if these occur, consider their laboratory diets as a possible factor.

DISCUSSION

148. The rat uterotrophic bioassay has been recommended for use as a screening assay to identify substances potentially acting through oestrogen mechanisms. This validation study was conducted in 2000-2001 to demonstrate the ability of the uterotrophic to reliably detect strong and weak oestrogens, to demonstrate the transferability of standardised protocols amongst laboratories, and to quantify the inter- and intralaboratory reproducibility of the assay. The results of this validation study are intended to support the development of an OECD Test Guideline for the uterotrophic bioassay.

149. The first phase (Phase-1) of the validation of the rodent uterotrophic bioassay involved 19 laboratories and measured the animals' responses to a potent reference oestrogen, ethinyl oestradiol. Four protocol versions were used: intact, immature rats treated by oral gavage (Protocol A) and sub-cutaneous injection with a dosing regimen of three consecutive days (Protocol B), and mature, OVX rats treated by subcutaneous injection using two different dosing regimens of three (Protocol C) and seven consecutive days (Protocol D), respectively. Despite differences in rat strain used and different levels of experience among the participating laboratories, there was acceptable agreement among laboratories with respect to the magnitudes of the responses at the different dose levels and the doses at which significant responses were obtained with two versions of the uterotrophic bioassay using the intact, immature rat and the young adult OVX rat, respectively (27)(28). The VMG-mam judged these differences to be acceptable in order to permit the wide practice of the bioassay among OECD member countries.

150. For the Phase-2 of the validation study, it was agreed to retain the four protocol options, with minor modifications and improvements. These protocols would be used to test five weak oestrogen agonists that were three or more orders of magnitude lower in oestrogen receptor binding affinity than EE (bisphenol A, *o,p'*-DDT, genistein, methoxychlor, and nonylphenol). Twenty laboratories participated in the Phase-2 validation studies. The substances were tested in dose-response studies using a series of five pre-determined doses and these same chemicals with the addition of a negative chemical (dibutyl phthalate), using a pre-selected, coded dose equal to one of five doses in the dose-response studies. The potent reference oestrogen, EE, used in the Phase-1 studies would be included in all Phase-2 protocols. So that the results could be compared within and among laboratories from both Phases, two doses from the Phase-1 studies were selected that were judged to approximate an ED_{20} and an ED_{80} dose.

Demonstration of the ability to detect weak oestrogen agonists

151. All Protocols were able to detect each of the five weak oestrogen agonists provided that sufficient doses were administered. The MEDs of the five weak agonists were substantially higher than for the potent reference EE. In Phase-1, the MED for EE in Protocol A using oral gavage was 0.3-1.0 μg/kg/d and in Protocols B, C, and D using subcutaneous injection was 0.1-0.3 μg/kg/d. In Phase-2, the MEDs for the weak agonists using oral gavage ranged from < 20 mg/kg/d for MC to 600 mg/kg/d for BPA, the latter MED being approximately 600,000-fold higher than EE. This demonstrates the capability to detect oestrogen agonists over a substantial concentration range and that this range should encompass the substances of interest to regulatory agencies. The proportion of laboratories achieving statistical significance depended upon the position of the selected dose on the dose-response curve. At doses in the lower regions, of the curve, fewer laboratories achieved statistical significance. Comparing the proportion of laboratories achieving statistical significance at a given dose in the dose response and the coded single-dose studies, the results are reproducible within a given Protocol (Table 23).

Comparison of the immature OVX rat, different protocols and routes of administration

152. The data allow comparisons of the immature and OVX rat, Protocols A through D, and different routes of administration. The immature and the OVX rat are equivalent in this program in their ability to detect weak oestrogen agonists, their responsiveness to both the reference EE and the weak oestrogen agonists, and their lack of response to a negative chemical. This overall equivalence is supported by a

detailed examination of Protocols B and C. For example, Protocol C appeared to be somewhat more sensitive than Protocol B with BPA, but Protocol B somewhat more sensitive with GEN and MC. Thus, both Protocols B and C appeared to have equivalent overall sensitivity to the test substances.

153. The specific characteristics of the individual test substances were important determinants of their response. For example, oral administration in Protocol A appeared to achieve higher and earlier responses with MC and DDT than did sc administration, sc administration achieved higher and earlier responses than oral administration with GEN and BPA, and sc administration was only modestly more sensitive than oral administration with NP. The uterotrophic bioassay is then adaptable to the principle relevant route(s) of exposure of test substances. Extending administration to seven consecutive days (Protocol D) showed no significant or consistent advantage over the 3-day treatment. While increasing the relative response slightly with BPA, no advantage of extending dosing in the magnitude of the response or the MED was obvious with the other weak agonists. In addition, the seven-day treatment protocol would have a modest disadvantage of higher costs as a result of the additional dosing and additional animal maintenance time. The seven-day treatment protocol cannot be recommended for routine use, but may be useful for chemicals that require longer dosing times to reach effective body burden concentrations or to induce specific metabolic enzymes.

Results from the dose response studies

154. A total of 86 chemical/laboratory/protocol dose-response combinations were performed with the five weak oestrogen agonists, and each laboratory included two doses of the potent EE reference as positive controls. Three approaches were used to present and to analyse the data: 1) an analysis of the dose response of the individual agonists, 2) an analysis of the two reference EE doses generated in conjunction with the dose response experiments, and 3) a comparison of the MED achieving statistical significance within each protocol and dose series. For each of these approaches, there was good agreement among laboratories, and across protocols. The magnitude and shape of the dose response curve for each of the five individual weak agonists was similar within a Protocol. The response to the EE reference doses was also similar within a Protocol. An analysis of the MED or the lowest dose of a test substance producing a statistically significant effect reinforces the conclusion of agreement and reproducibility among the laboratories within a Protocol. In several protocols, there was no difference observed in the MED or the MEDs were within a 3- to 4-fold range despite differences in rat strains, diets, and so on.

155. The range of five doses selected for the dose-response studies were estimates based on reports of varying quality and transparency of protocol conditions for these or similar substances. The doses were intended to cover a range sufficient to characterise the complete dose response, i.e., to approximate the full dose-response curve from a NOEL through an ED_{20}, ED_{50}, ED_{80}, to an ED_{100} or maximum plateau. However, the full dose response curve was not covered in all cases. Lower doses of MC and, possibly, GEN and higher doses of DDT were needed in some cases. In other cases, the agonists were extremely weak, such as with BPA, where even a limit dose of 1000 mg/kg/d produced only a minimal 40-70% increase when administered orally. Animal mortalities and body weight losses indicate that a maximum tolerated dose was exceeded for several test substances. This is attributed to the use of adult LC_{50} and MTD data, as all mortalities occurred with very young immature animals soon after they were weaned.

156. The dose-response studies showed a high degree of reproducibility among laboratories within a given Protocol (see Table 9-13 for individual agonists, Tables 14 for low and high EE doses, Tables 15 MEDs, Table 24 for the global analyses, and Tables 25 for the individual laboratories). There were four cases (BPA Protocol E Laboratory #12, DDT Protocol C Laboratory #12, NP Protocol B Laboratory #6, and NP Protocol C Laboratory #6) where statistical significance was not achieved, but only the three intermediate doses, and not the highest dose, were tested. In addition, the CV of the controls in these cases were somewhat higher than average, lowering the sensitivity of the analyses. In the final case of NP Lab. #20, all relative responses with the test substance were ≤ 0.75 and one response were significantly less than the control (see Table 13b); the control blotted mean weight was 54.3 mg and individual values were 40.8,

44.6, 49.2, 54.9, 65.6 and 70.5 mg, substantially above the means for most other vehicle controls (the group mean was number 58 out of 60 when ranked in ascending order); and this control group and the results calculated from it were judged to be an anomaly.

Results from the coded single-dose studies

157. A total of 16 laboratories participated in the coded single-dose studies. The single-doses were selected based on presumed positions in a dose-response curve of ED_{50-80}. In several cases, these judgements were not accurate and some selected doses were at or near the MED in the low region of the dose response curve (e.g., DDT by sc administration). It would be anticipated that some laboratories would not achieve statistical significance under these conditions, and this was the case. However, there was consistent agreement of the repeatability of the relative increase at the selected dose within the same protocol across laboratories in the coded single-dose studies. The putative non-oestrogen was marginally positive in three out of 36 studies (laboratories and protocol variations) in which it was run, indicating a possible false positive rate of about 8%.

158. The inability to select a single dose that would achieve statistical significance, even when some pre-existing data were available, is instructive. The pre-existing data included not only *in vitro* data, but *in vivo* uterotrophic data, albeit from poorly described protocol conditions in some cases. The single-dose selected here was simply used as a comparison with the same dose in a dose response curve in order to assess the repeatability of the assay. In regulatory practice, this strongly suggests, however, a need for several doses and, with the evidence for animal mortality, some need for range finding studies.

Reproducibility of results within laboratories

159. Intra- and inter-laboratory reproducibility were assessed in four ways: the proportion of assays achieving statistical significance when considering the position of the selected dose on the dose response curve (Table 23); analysis as pooled data across all laboratories in a global approach for a given chemical and protocol (Table 24); a restricted comparison to only those laboratories performing experiments on the same chemical in given protocol (Tables 25); and an examination of the EE data from Phase-1 and both the dose response and coded single-dose response of Phase-2 (Tables 29). In addition, specific instances were examined such as differences in achieving statistical significance (Tables 26) and a test for significant difference amongst the data (Table 27). Each approach shows that the relative increase in uterine weights within and across laboratories were reproducible both with all five weak agonists and with the reference EE, taking into account that the magnitude of the relative increase was dose dependent. The analysis of statistical differences based on absolute weights amongst the data is also instructive. Relatively wide variability in the mean blotted uterine weight of the immature animals is evident, not only amongst different laboratories, but even within laboratories using the same strain, supplier, diet and so on. For example, note the difference in three sets of control groups in Table 27. However, when expressed as a relative increase in uterine weight, the results were similar and reproducible in about half of the EE and weak agonist cases in Table 27. This supports the use of the relative increase in uterine weight as the basis for comparison.

160. The instances where results were, first, unconfounded by an absence of a high dose, mortality reducing the power, or questionable data and, second, the results were clearly not reproducible were limited. Laboratory #8 with NP and Protocol B (Table 26), Laboratory #11 with the low dose of EE in Protocol B (Table 29b), Laboratory #19 with both doses of EE in Protocol C (Table 29c), and Laboratory #11 with the low dose of EE in Protocol D (Table 29d) should be noted.

Sources of variability

161. From the results of this validation study it appeared that, not unexpectedly for any bioassay, there is some variability amongst the protocols used and between laboratories. The possible sources of the

observed variability are several. The expertise and care within a laboratory is hypothetically a significant contributor. The difference between immature and OVX protocols in general and, more specifically, the age of the immature animals and the laboratory diet (e.g. phytoestrogen content) are others.

162. An analysis of the phytoestrogen contents of the laboratory diets revealed significant levels in many diets. A review of food consumption indicates that this would lead to different dietary intakes on an approximate ratio for OVX adult rats:immature rats:OVX adult mice of 1:2:4. An examination of the vehicle control weights and the responses to the weak agonists in different laboratories was made relative to an estimated dietary intake of phytoestrogens. The data indicated that no effect was evident for the adult OVX model. However, the data were suggestive of an effect for the immature rat model when GEN intakes would exceed 50 mg/kg/d. This level is consistent with other toxicological studies showing a LOEL in this range as well as the MED values in this study for Protocol A. However, the interpretation that an influence of dietary phytoestrogen interferes with the study results largely relies on the results in Laboratory #20. A close examination of those data reveals that these data are open to question, and any conclusions must be drawn with caution until controlled studies are done with defined diets, defined doses, and sufficient doses of phytoestrogens. However, as a precaution until such data are available, experiments with immature rats or OVX mice should limit the dietary content of phytoestrogens to about 350 µg phytoestrogens/g diet and 175 µg phytoestrogens/g diet, respectively.

163. The statistical analysis conducted did not reveal that any of the factors mentioned above contributed substantially more to the variability of the test results than others. However the statistics used were not specifically designed to study the effect of protocol variables and additional statistical analysis, aimed at the effect of protocol variables, would be needed.

164. Despite the observed variability, results also showed that all weak and strong oestrogen (ant)agonists were detected by 94% of the participating laboratories, thus confirming the highly adequate level of robustness of the bioassay (see Table 34).

Table 34. Overall results of the uterotrophic phase-2 validation studies for all protocols and chemicals: Reported as Positive Identification in the Uterotrophic Assay/Total Number of Trials

Chemical Tested	Coded Single Dose Protocols[a,b]				Dose Response Protocols				
	A	B	C	D	A	B	C	D	E
Bisphenol A	6/10	14/15	7/7	4/4	4/4	10/10	5/5	2/2	0/1
***o,p'*-DDT**	9/9	6/16	4/7	2/2	4/4	4/4	2/3	2/2	1/1
DiButylPhthalate	0/10	2/15	1/7	0/4	NA	NA	NA	NA	NA
EE-low	9/9	12/14	6/6	3/3	13/15	20/20	11/11	9/9	ND
EE-high	8/8	12/13	4/5	3/3	13/13	21/21	11/11	9/9	ND
Genistein	10/10	14/14	6/6	4/4	4/4	4/4	3/3	2/2	1/1
Methoxychlor	10/10	15/15	6/6	4/4	4/4	4/4	3/3	2/2	1/1
Nonylphenol	9/10	12/16	5/6	4/4	4/4	8/10	4/5	2/2	1/1

[a] Protocol E was run only in the dose response studies, so no results are recorded here.
[b] The doses employed for the five weak agonists in the coded single dose studies were either the third or fourth dose in a five dose series used in the dose response studies, so a maximum dose was not used.
NA – Not applicable
ND – Not done

CONCLUSIONS

165. Both the immature and OVX rat protocols were highly robust, reproducible among laboratories, and were able to identify weak oestrogen agonists. It can be concluded that the rat uterotrophic bioassay, using either the immature or OVX procedures, can be used as an effective screen for weak and potent oestrogenic agonists.

RECOMMENDATIONS

The following recommendations are offered:

1. Following the approval of this report of Phase-2 and a report of any additional statistical analysis by the VMG-mammalian, the EDTA and the WNT and subsequent endorsement of the overall validation study by the Joint Meeting, a draft Test Guideline be prepared for this bioassay that allows the use of immature and OVX animals as a screen for identification of substances displaying activity *in vivo* consistent with the characteristics of oestrogenic (ant)agonists.
2. Although the variability of results obtained did not affect the robustness of the bioassay, an additional round of statistical analysis may be conducted to further examine the effects of the laboratory, the immature and the OVX models, and other differences between protocols on the variability of results.
3. Although there is extensive scientific literature supporting the successful use of mice in uterotrophic bioassays, data to clearly demonstrate the reliability and reproducibility of mouse protocols with those of the rat has not been generated at this time. For such a demonstration, bridging data at similar doses and times of administration are needed to establish such factors as the time of regression following OVX, and to demonstrate the ability of the mouse to respond to weak and potent oestrogens.

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ANNEX 1

Participating Laboratories in the OECD Validation of Phase-2 of the Rodent Uterotrophic Bioassay

This information is available to Government Representatives of OECD member countries only.

ANNEX 2

PROTOCOL FOR THE CONDUCT OF THE OECD RODENT UTEROTROPHIC ASSAY

Second stage of the OECD work of the validation of the rodent uterotrophic assay
PROTOCOL C

mature OVARIECTOMISED rats with SUB-CUTANEOUS ADMINISTRATION1 (Contains both multi-chemical and dose-response studies)

[1] Draft taking into account the agreements reached by the OECD Validation Management Group (20-21 January 2000) and subsequent teleconferences on 6 March, 12 and 18 April 2000

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RATIONALE

1. The rodent uterotrophic assay evaluates the ability of a chemical to show biological activities consistent with agonism or antagonism of natural oestrogens. *Uterotrophic* is a term used to describe something which influences the uterus. A chemical causing an increase in weight of the uterus indicates that it has activity consistent with natural oestrogens, and is therefore uterotrophic in nature.

2. This protocol will generate data as part of an overall OECD project to validate the rodent uterotrophic assay. The first step of the OECD validation work on the uterotrophic assay demonstrated the high reliability of the protocol when testing the standard reference chemical – ethinyl oestradiol.

3. The specific aims of the second stage of the validation of the OECD rodent uterotrophic assay is to evaluate the reliability of the protocol when testing weak oestrogenic agonists.

- **Demonstrating the expected increases in uterine weight** in mature ovariectomised female rats following sub-cutaneous injection of 5 weak oestrogen agonists (Methoxychlor, (CAS No 72-43-5) Bisphenol A (CAS No 80-05-7); Genistein (CAS No 446-72-0), o,p'-DDT (CAS No 789 –02-6) and Nonyphenol (CAS No 25154-52-3) and the effects of Dibutyl Phthalate (CAS No 84-742) which is not expected to demonstrate an oestrogenic effect.

- **Comparing** these expected increases in uterine weight **with the reference oestrogen 17-ethinyl oestradiol** (hereafter referred to as EE) (CAS No. 57-63-6).

- **Evaluating the dose-response relationship** between uterine weight increase and the dose of two weak oestrogen agonists – Bisphenol A (CAS No 85-05-7) and Nonylphenol (CAS No: 25154-52-3) and in addition Methoxychlor (CAS No 72-43-5), Genistein (CAS No 446-72-0) and o, p'- DDT (CAS No 789-02-6).

- **Enabling a comparison of the results from a similar protocol** in which very young, immature female rats are exposed by subcutaneous injection **(Protocol B).**

- Assisting in **identifying refinements to the protocol** so that it can be subsequently used to investigate chemicals of unknown oestrogenic activity.

TIME SCHEDULE

4. The time needed for this assay will depend on whether the animals are supplied following ovariectomy or whether this procedure is carried out by the participating laboratory.

TEST SUBSTANCES

Characterisation of test substances

5. Characterisation of the test substances is the responsibility of the chemical suppliers and those managing the chemical repository. It is not the responsibility of the lead or participating laboratories.

6. The test substances will be characterised by name, supplier, batch number, purity, appearance, storage conditions and expiry date.

TEST SYSTEM

Characterisation of the test system

7. The study will be conducted with adult female ovariectomised laboratory rats. These rats will be obtained from a colony maintained under SPF-conditions. The specific strain of rat will be selected by the participating laboratories based on experience and historical control data under their own operating conditions. The participating laboratories will record the strain used their study report(s).

8. At the commencement of the ovariectomy the rats will be 6 weeks old and over.

Animal supply and acclimatisation

9. Upon arrival, the rats will be taken to the room assigned to this study and checked for overt signs of ill health and anomalies. After ovariectomy the adult females will be acclimatised to laboratory conditions for at least 14 days but not more than 28 days.

10. Care should be taken to ensure that complete ovariectomy has been achieved. This may be checked by animals having a small vaginal opening and vaginal smears which lack cellularity, particularly in cornified or nucleated epithelial cells.

Body weight and the selection of animals for the study

11. Variations in body weight may be a source of variation in the weight of tissues of interest. This variation, if present, will increase variability within a group or among groups of animals, decrease assay sensitivity, and possibly lead to false positives or false negatives.

12. Body weights will vary from study to study and different rodent strains. Each participating laboratory should establish its own procedure for limiting the variability in body weight. These procedures will be recorded in the report and should ensure that all groups of animals reflect normal variations expected for healthy animals.

13. As a precautionary measure, any relationship between body weight and uterus weight will be controlled in both the experimental design and data analysis phases of the study.

14. Experimental control is accomplished in two steps. The first step involves selection of animals with relatively small variation in body weight from the larger population. This is done by avoiding unusually small or large animals. Generally this will result in animals that are approximately ±20% of the mean body weight (e.g. 200g ± 40g). While this degree of variability may seem large, it is not expected to affect the outcomes from the study, as long as the animals are healthy.

15. The second part of "experimental" control of body weight involves the assignment of animals to different treatment groups by a randomised complete block approach rather than by completely randomisation.

Allocation of animals into treatment groups

16. Prior to the experimental start date, and following ovariectomy, the animals will be "randomly assigned" to treatment groups such that each group has the same mean weight population within ± 5% probability level. This variable is then included in the data analysis to adjust for differences in body weight.

Identification of the test system

17. The study will be identified with a unique study number and individual rats will be uniquely identified e.g., by ear tags or tail tattoos. Each group of rats will be coded e.g., by a letter and a colour. Each cage will be labelled to show the laboratory code for the group, the animal identification numbers, the cage number, and the study number. The specific identification system used by the participating laboratory will be recorded and included in the study report.

EXPERIMENTAL CONDITIONS

Non-routine health and safety requirements

18. The test substances may be possible reproductive and developmental toxicants therefore appropriate precautions should be taken to protect personnel, e.g., necessary training, labelling and storage procedures, and protective handling procedures during dose preparation and dose administration.

19. Appropriate precautions such as wearing protective gloves, protective clothing and eye protection should be taken when handling the animals, diets, cages, and wastes (e.g. remaining test solutions, faeces, and carcasses). Waste disposal will be in accordance with good practice and existing regulations.

20. To maintain the integrity of the multi-chemical study which will be conducted with coded chemicals, the chemical repository will provide a generic Material Safety Data Sheet giving safety and handling precautions suitable for all the test substances. This will be stringent enough to cover the most hazardous of the chemicals being used in the validation project.

21. Individual Material Safety Data Sheets will also be supplied for all of the coded test substances. In order to maintain the integrity of the coding, these Material Safety Data Sheets should be kept in a sealed envelope in a secure location under the control of a named person within the laboratory. That person should be instructed to keep the Material Safety Data Sheets secure and only to open the sealed envelope in case of emergency involving the coded test substances.

Animal maintenance

22. Appropriate husbandry conditions should be followed. The room will be maintained at a temperature of 22 ± 3 Cº and a relative humidity of between 30% and 70%, other than during room cleaning. Lighting will be artificial with a cycle of 12 hours light and 12 hours dark. Prior to and at the end of the study, the cages and other materials the animals may touch will be cleaned with appropriate agents as specified in the laboratory standard operating procedure. These procedures should be recorded and this information included in the study report.

23. As some bedding materials may contain naturally occurring oestrogenic compounds, the particular bedding used by the participating laboratory should be recorded and details included in the study report.

24. Animals may be caged singly or in groups. In the case of group housing then the group number should not exceed three rats per cage. To avoid overcrowding, two cages of three animals per treatment group is the preferred number.

Feed and drinking water

25. Feed and drinking water (tap or filtered) will be provided *ad libitum.*

26. The rats will be fed the usual rodent diet used by the participating laboratory. Because of the possibility of dietary phytoestrogens the participating laboratory should record the details of the diet, supplier, and the batch used. This information should be included in the study report. Each batch of diet should be analysed by the supplier for nutrients and contaminants according to the supplier's normal practice. The certificate of analysis for the batch used in the study will be included in the study report. The same diet batch should be used throughout the study for all animals.

27. The participating laboratory should maintain a frozen sample of the rodent diet used so that the diet can be further analysed, if necessary, e.g. for phytoestrogens and isoflavones.

EXPERIMENTAL PROCEDURES

Procedure for Ovariectomy

28. A video is available, on request from the lead laboratory which shows the surgical procedure for ovariectomy. Essentially the procedure is as follows. The dorso-lateral abdominal wall should be cut 1 cm lengthways at the mid point between the costal inferior border and the illiac crest, and a few millimetres lateral to the lateral margin of the lumbar muscle. The ovary should be pulled out and disconnected at the junction of the oviduct and the uterine body. After confirming that no massive bleeding is occurring, the abdominal wall should be closed by one suture and the skin closed by autoclips. The ligation points are shown schematically in **Figure 1.**

Administration of the test substances

29. Sub-cutaneous injection will be used to administer all substances.

30. The test substances will be administered once per day on three consecutive days. If the participating laboratory is additionally running a comparative study with longer exposure periods, the test substances will be administered for the longer period as appropriate, e.g., 7 days (Protocol C').

31. The amount administered should be calculated on the body weight of the animal on the day of treatment. Treatment will be at approximately the same time and sequence for each animal. Test dilutions of the test substance will be prepared daily unless information is available which confirms the stability of the test solutions. In the latter case, the dilutions of the test substance can be made before the start of the study consistent with the substance's known stability.

32. The same test vehicle should be used for all test substances. The participating laboratories will record the test vehicle to be used and include this information in their study reports.

33. Instructions on how to prepare each of the test substances will be provided at the time of supply by the central chemical repository. Details of the test vehicle, as well as the vehicle supplier and lot, should be recorded and this information included in the study report. The participating laboratories should preserve a sample of the vehicle, if a further analysis, e.g., of the phytoestrogen content should become necessary.

34. The total amount of s.c. injection per rat will not exceed 4mL/kg/day.

Experimental groups and dose levels

36. Two study designs are to be followed in the overall study - a multi-chemical approach and a dose-response approach. The multi-chemical study is to be carried out using coded test substances and so requires participating laboratories to put specific measures in place to maintain the integrity of the code.

37. Test substances are to be tested at a range of doses, including those where some signs of toxicity may be expected. All test substances will be tested at the doses specified.

38. Both the multi-chemical and dose-response part of the protocol includes a vehicle control group and two positive control groups for Ethinyl oestradiol (at high and low doses). If a participating laboratory is conducting both the multi-chemical and the dose-response approach at the same time, it is preferable that separate control groups are used. However if the two studies are being run at the same time and the participating laboratory wishes to combine control groups, the positive control groups may be deleted in the dose-response part of the study. In this situation data from the positive control groups in the multi-chemical study will be 'decoded' and used at the data analysis stage.

39. When running the multi-chemical and dose-response part of the study at the same time deletion of one or more of the vehicle control groups is not recommended because of the potential loss of statistical power in the data analysis phase.

Multi-chemical approach

40. The multi-chemical approach includes nine groups of six females - 5 oestrogen agonists, a chemical not expected to demonstrate oestrogenic activity, two groups for the standard oestrogen reference chemical - EE and a vehicle control.

41. All test substances, and control substances will be supplied coded along with details of how to make up stock and dosing solutions. To maintain the integrity of the coded study, those persons responsible for making up the stock solutions and administering doses should not reveal the likely identity of the test substance especially to those responsible for recording observations and organ weights at necropsy. The participating laboratory will need to make a written statement on the measures it will take to maintain the integrity of the blind study and a statement that these measures have been followed when it submits its results.

42. All substances will be administered at pre-determined dose levels and will be coded to create a blind study.

43. The design requirements of this study are summarised in Table 1 below. A, B, C, D, E and F represent the coded test substances for this study namely: **Methoxychlor** (CAS No. 72-43-5); **Bisphenol A** (CAS No. 80-05-7); **Genistein** (CAS No. 446-72-0), **o,p'-DDT** (CAS No 789-02-6) and **Nonylphenol** (CAS No 25154-52-3) and **Dibutyl Phthalate** (CAS No.84-74-2).

Table 1. - Details of Experimental Groups and Dose Levels - Multi-Chemical Approach

Groups	N=	Dose		Route	Maximum total s.c. volume/day/rat
		EE (microgram/kg)	Test Substance		
1 (vehicle control)	6		0	s.c.	4ml/kg/day
2	6		A	s.c.	4ml/kg/day
3	6		B	s.c.	4ml/kg/day
4	6		C	s.c.	4ml/kg/day
5	6		D	s.c.	4ml/kg/day
6	6		E	s.c.	4ml/kg/day
7	6		F	s.c.	4ml/kg/day
8	6	Low	EE	s.c.	4ml/kg/day
9	6	High	EE	s.c.	4ml/kg/day
TOTAL	**54**				

Dose- Response Approach

44. The dose-response approach will examine the increase in uterine weight when the test animals are administered increasing dosed of selected test substances. The experimental design for the dose-response study is shown in Table 2. In order to generate a good dose-response curve, doses of chemicals covering the whole of the dose-response range should be given. Based on literature review and discussions with researchers

active in this field the OECD Validation Management Group has identified five dose levels for each of the substances in the study which should provide this spectrum of responses (see Table 3). The same doses must be used by all participating laboratories.

45. If resources are constrained a minimum of three doses which are those likely to be on the ascending part of the dose-response curve should be selected. The same doses must be used by all participating laboratories. Table 3 shows the three minimum doses that must be used (i.e. doses 2,3 and 4 shaded in gray.

46. To generate a good dose-response curve, 8 groups of 6 animals per group are preferred with 5 doses, as shown in Table 3, together with two groups for the standard oestrogen reference chemical – EE and a vehicle control. The minimum number of test groups for the dose-response study will be 6 (3 test substance groups, 2 positive reference dose groups of ethinyl oestradiol and a vehicle control group).

Table 2. - Details of Experimental Groups - Dose-response approach

Groups	N=	Dose		Route	Maximum total s.c. volume/day/rat
		EE (microgram/kg)	Test Substance		
1 (vehicle control)	6	0	0	s.c.	4ml/kg/day
2	6		Dose 1	s.c.	4ml/kg/day
3	6		Dose 2	s.c.	4ml/kg/day
4	6		Dose 3	s.c.	4ml/kg/day
5	6		Dose 4	s.c.	4ml/kg/day
6	6		Dose 5	s.c.	4ml/kg/day
7	6	0.3		s.c.	4ml/kg/day
8	6	1		s.c.	4ml/kg/day
TOTAL	**36**				

Table 3. - Doses to be used for each of the test substances – with three minimum doses for each of the test substances shaded in gray

S.C (mg/kg/day)	**1**	**2**	**3**	**4**	**5**
Methoxychlor	20	100	250	500	800
Genistein	1	15	35	50	80
O p, DDT	5	25	50	100	200
Bisphenol	10	100	300	600	800
Nonylphenol	5	15	35	80	100

Observations, analyses and measurements

Clinical signs

47. Animal observations will be conducted according to the usual routine of the participating laboratory. On working days, all cages will be checked in the morning and afternoon for dead or moribund animals. On Saturdays and Sundays and other non-working days, a minimum of one check per day will be carried out. All abnormalities will be recorded and included in the study report.

Body weight and food consumption

48. The body weight of each rat will be recorded daily to the nearest 0.1 g, starting just prior to initiation of treatment i.e. when the animals are allocated into groups. As an optional measurement, the amount of food consumed during the treatment period may be measured per cage by weighing the feeders. The food consumption results will be expressed in grams per rat per day.

Measurement of uterus weight

49. Both wet and blotted uterus weights are the mandatory endpoints of this test protocol. Measurement of the wet weight includes the uterus and its luminal contents. Blotted weight is measured after the luminal contents of the uterus have been expressed and removed.

50. Twenty-four hours after the last treatment, the rats will be humanely killed in the same sequence as the test substance was administered. The method of humane killing will be the one routinely used by the participating laboratory, and this should be recorded and details included in the study report.

51. Procedures should ensure that the variation in excising and trimming the organs, is minimised. For example, the same prosector should be responsible for the weighing the uteri. If this is not possible, an alternate procedure is to design the necropsy so that each prosector weighs animals from each treatment group, as opposed to having one individual weigh all the tissues from a control group, while someone else is responsible for the treated groups.

52. If the evaluation of each chemical requires necropsy of more rats than is reasonable for a single day, necropsy may be staggered on two consecutive days. In this case the work could be divided so that necropsy of 3 animals per treatment per day (1 cage) takes place on the first day with the dosing and necropsy being delayed by one day in the second half of the animals. If this procedure is necessary, care should be taken so that the treatment of the animals is also staggered and that the age of the animals does not fall outside that needed for the assay. If a staggered necropsy procedure is to be used then a description will be included in the report.

53. The uterus will be carefully dissected and trimmed of fascia and fat to avoid loss of luminal contents. The vagina shall be removed from the uterus at the level of the uterine cervix. Further details for the removal and preparation of uterine tissues for weight measurement are included in the legend to **Figure 2**.

54. The uterus will be transferred to a uniquely marked and weighed container (e.g., a petri-dish) with care to avoid desiccation before weighing. The uterus will be weighed with the luminal contents (wet weight) to the nearest 0.1 mg.

55. Each uterus will then be individually processed to carefully blot the excess fluid. For example, both uterine horns may be pierced or cut longitudinally, placed on moistened filter paper (e.g., Whatman No. 3) and gently pressed to absorb the luminal fluid. The procedure used must have good reproducibility within the laboratory and not be too severe to render the tissue unacceptable for histopathological analysis, as this additional investigation will be undertaken by some by some laboratories.

56. For those laboratories wishing to perform a histopathological examination of the vagina and/or uterus, the uterus and vagina should be fixed in 10% neutral buffered formalin (4% formaldehyde). If histopathology is done, the procedure used must be recorded and included in the study report. As it is known that tissue reactions differ in each portion of the uterus, **Figure 3** shows the points at which histological cross sections should be made. Use of PCNA and BrdU labelling is encouraged as part of the histopathological procedure.

REPORTING REQUIREMENTS

57. Each participating laboratory should record and provide the raw data with the items as listed below. A report of this data and an analysis of the results should be made to the lead laboratory. A standard Excel spreadsheet will be provided and is to be used by each participating laboratory for the reporting of results.

58. A final report will be prepared for each experiment conducted by each participating laboratory including details of:

Laboratory Protocol:

- Including date and approval

Testing facility:

- Address details
- Responsible personnel and their study responsibilities

Test Substance:

- Characterisation of test substances (to be provided by chemical supplier/repository)
- Method and frequency of preparation of dilutions

Vehicle:

- Characterisation of test vehicle (nature, supplier and lot)

Test animals:

- Strain
- Supplier and specific supplier facility
- Age of animal when ovariectomised

- Age of animal when administration of test substance began
- Details of acclimatisation procedure
- Number of animals per cage
- Detail and method of individual animal and group identification.

Test Conditions:

- Details of randomisation process (i.e., method used)
- Record of cage location in laboratory racks
- Diet (name, type, supplier, content)
- Water source (e.g., tap water or filtered water) and supply (by tubing from a large container, in bottles, etc.)
- Bedding
- Record of lighting interval
- Record of air conditioning (filter maintenance)
- Record of room clean up
- Description of necropsy procedure (if necropsy is staggered over 2 days)
- Description of blotting procedure details
- Details of histopathological procedures (including copy of standard operating procedures)

Results

For individual animals:

- Daily body weight - from the day the animals are allocated into groups to the day of necropsy
- Age of each animal (in days counting birth date as day 0) when administration of test compound begins
- Date and time of each s.c. injection
- Calculated amount of each s.c. injection
- Daily record of status of animal, including relevant symptoms and observations
- Suspected cause of death (if found during study in moribund state or dead)
- Date and time of humane killing
- Approximate time interval in hours between last test substance administration and humane killing
- Organ weight at necropsy
- Wet uterine weight per animal and any observations on loss of luminal fluid during dissection and preparation for weighing to the nearest 0.1 mg
- Blotted uterine weight per animal to the nearest 0.1 mg
- If undertaken, histopathological report of uterus and vagina.

For each group of animals

- Daily body weights (from day of allocation into groups to the day of necropsy)
- Uterine weights (both wet and dry) per dose given
- If measured, daily food consumption

STATISTICAL ANALYSIS OF THE RESULTS

59. The lead laboratory will be responsible for making an overall assessment and presentation to the Validation Management Group. The raw data will include body weight, clinical status of animals during the test and before necropsy and uterine weight (wet and blotted). The OECD Validation Management Group will determine the statistical procedures to be used in the evaluation of data taking into account dependent statistical advice.

RETENTION OF RECORDS, SAMPLES AND SPECIMENS

60. The chemical repository or chemical supplier will retain a reference sample of all test substances until the end of the whole project. Samples of diet, and test vehicle should be retained by the participating laboratories, so that further analyses can be carried out if needed, Participating laboratories should retain raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study.

Figure 1 : Schematic diagram showing points of ligation in surgically removing ovaries

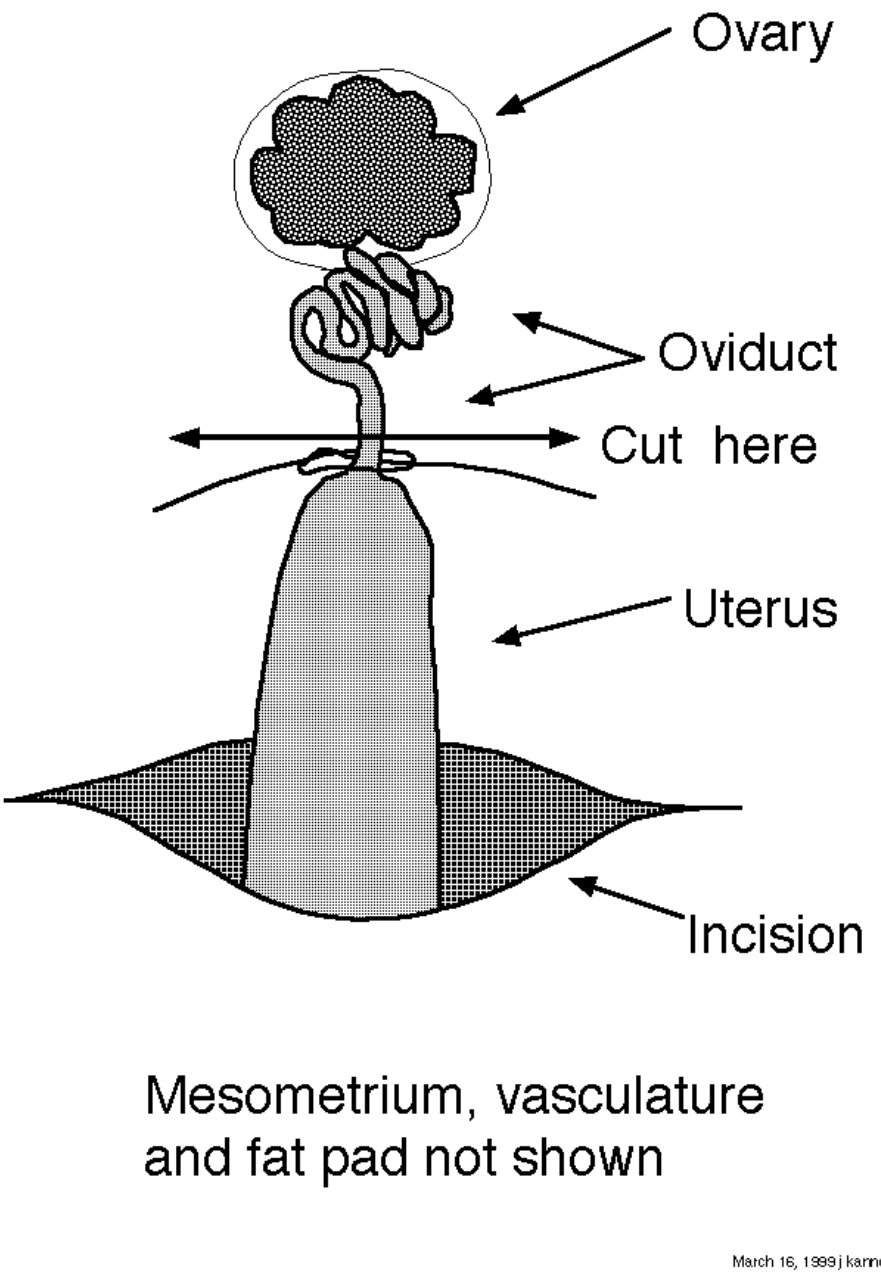
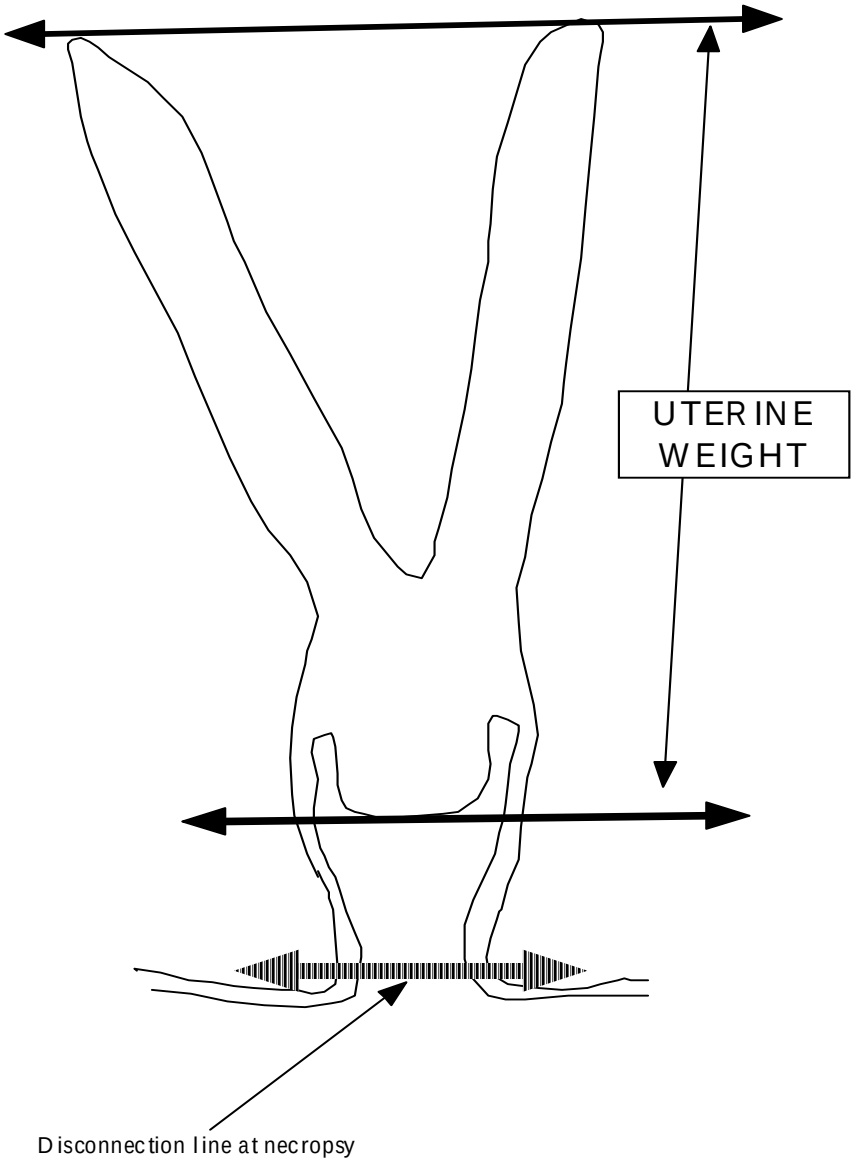
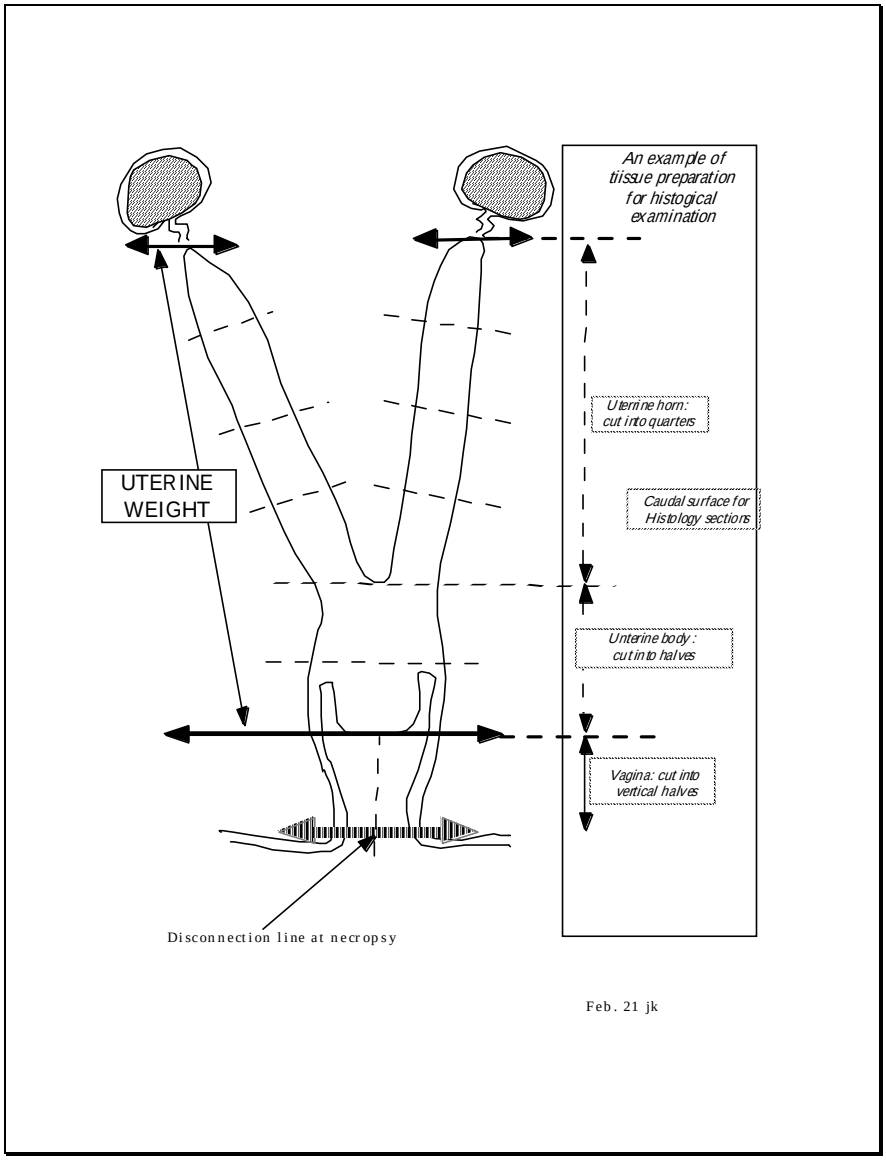


Figure 2: The removal and preparation of the uterine tissues for weight measurement



In detail the procedure is to open the pubic symphysis. Then, each ovary and uterine horn is detached from the dorsal abdominal wall. Urinary bladder and ureters are removed from the ventral and lateral side of uterus and vagina. Fibrous adhesion between the rectum and the vagina is detached until the junction of vaginal orifice and perineal skin is identified. The uterus and vagina is detached from the body by incising the vaginal wall just above the junction between perineal skin as shown in the figure. The excess fat and connective tissue is trimmed away. The vagina is removed from the uterus as shown in the figure for uterus weight measurement. Weight with luminal fluid (wet weight) and without the luminal fluid (blotted weight) are measured.

Figure 3: One example for the preparation of the uterus and vagina for optional histopathological examinations



As it is known that the tissue reaction differs in each portion of uterus. It is recommended to prepare cross sections from different portions of this hollow organ, to observe cell proliferation (for example BrdU labelling) as well as histological changes of the uterine components.

ANNEX 3

OECD PHASE-2 VALIDATION STUDIES OF THE UTEROTROPHIC ASSAY:

ANALYSES OF THE PHYTOESTROGEN CONTENT OF THE DIETS

INTRODUCTION

1. In order to determine the role of the phytoestrogen content of animal diets on the uterotrophic response, the American Chemistry Council generously offered funding for an analysis of the isoflavanoid (coumestrol, genistein, and daidzein) contents in the animal diets used by the Phase-2 laboratories.

2. Diets were sent to Syngenta Central Toxicology Laboratories, Macclesfield, UK, by all of the participating laboratories. The diets were given a numeric code, packaged, and sent to Bioclinical Services International (Dr. M. Morton, Cardiff, UK) for phytoestrogen (isoflavanoid) analysis. Some laboratories sent more than one diet if different batches were used in different studies, or if animals were weaned onto one diet and then fed another diet during the studies. All diets were analysed for genistein, daidzein, and coumestrol. The results of the analyses were provided to the OECD Secretariat, and individual results were provided to the participating laboratories.

METHODS USED

3. The diets were analysed for genistein, daidzein, and coumestrol content by GC-MS. Aliquots of the diets (10 pellets) were ground to a homogenous powder, 100mg was extracted with 80% methanol (80ml) by ultrasonication (3 min) followed by incubation at 60^{o}C for 2h and further ultrasonication (3min). The mixtures were cooled, made up to 100ml with methanol, and 0.1ml samples taken and mixed with 0.05ml methanol containing internal standards (d^4-daidzein, d^4-genistein, d^4-coumestrol, and d^4-dihydroxyflavone). Sodium acetate buffer (1ml; 0.1M; pH 5.0) was added to the samples which were then treated with β-glucuronidase (*Helix pomatia*; 1000 units) to a final volume of 2.5ml, and incubated overnight at 37^{o}C. The products were extracted with ethyl acetate (2 x 4ml) and the combined extracts evaporated to dryness. The residues were reconstituted in chloroform:heptane:methanol (10:10:1), applied to short columns of Sephadex LH20, washed with chloroform:heptane:methanol (10:10:1) (4ml), and eluted with methanol. After evaporation of the methanol, the samples were derivatised with *n*-(*t*-butyldimethylsilyl)-N-methyltrifluoroacetamide containing 1% *t*-butyldimethylsilyl chloride (0.04ml) in acetonitrile (0.04ml) at 65^{o}C for 2h. After evaporation of the solvents the residues were reconstituted in ethyl acetate (0.02ml) for GC-MS analysis.

4. GC-MS was carried out on a DB5 MS bonded silica capillary column (10m x 0.25mm; phase thickness 0.25 μm) using helium as carrier gas and a temperature of 70-300^{o}C at 40^{o}C per minute. Isotope dilution MS was performed using selective ion monitoring at mass 425 for daidzein, 429 for d^4-daidzein, 555 for genistein, 559 for d^4-genistein, 496 for coumestrol and 500 for d^4-coumestrol. Peak area ratios were determined for analytes and internal standards. Calibration curves were constructed and the concentrations of daidzein, genistein, and coumestrol in the samples were determined. All values are the mean of triplicate determinations; the limit of quantitation for all the substances was 50pg/g diet.

RESULTS

5. A total of 27 diet samples were analysed for daidzen, genistein, and coumestrol content (Table 1). Genistein was present in the highest concentrations in all the diets. With the exception of two samples of 72.4 and 72.9 µg/gm, the values were all between 123.3 and 254.7 µg/gm.

6. Daidzein levels tended to be from one-half to one-third those of genistein, and ranged from 28.4 – 186.6 µg/gm. Coumestrol was present at relatively low concentrations, up to 4.1 µg/gm, and was below the level of detection (<0.05 µg/gm) in 8 of the 26 diets analysed.

Table 1. Phytoestrogen levels in the laboratory diets (µg/gm)

Lab. #	Sample*	Daidzein	Genistein	Coumestrol
1	1	48.7	156.8	0
	2	53.2	135.3	0
2	1	28.4	72.9	2.6
3	1	70.9	151.5	0
4	1	113.8	239.6	1.3
5	1	85.8	200.1	3.8
	2	84.7	175.1	1.3
6	1	53.9	132.6	0
7	1	88.1	204.2	2.5
8	1	101.7	238.3	1.8
9	1	84	144.7	2.3
10	1	29.2	72.4	0.3
11	1	121.2	226.2	4.1
12	1	113.2	180.2	2.8
13	1	90.1	177.1	2.3
14	1	91.2	206.5	3.1
	2	101.0	216.0	0
	3	83.7	198.6	0
15	1	130.9	221.8	0.8
16	1	77.8	164.8	0.8
17	1	186.6	254.7	0.9
18	1	84.1	190	0
	2	85	169.8	2.3
	3	40.7	123.3	1.8
19	1	48	131.9	1.5
20	1	117	218.3	0

* Some laboratories sent more than one diet sample if different batches were used in the different protocols; these samples were analysed separately. Some laboratories sent multiple samples of the same diet and batch; these were combined for analysis.

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ANNEX 4

Mean wet and blotted uterine weights and terminal body weights from dose-response and single-dose studies

Table 1a. Weights of wet and blotted uteri, and body weights, in animals administered bisphenol A in Protocol A; dose-response study

Lab.	Weighing	0	60 mg/kg/d	200 mg/kg/d	375 mg/kg/d	600 mg/kg/d	1000 mg/kg/d
4	uterus - wet (mg)	39.0 ± 6.51	39.7 ± 4.93	49.7 ± 20.39	43.3 ± 5.35	43.0 ± 4.30[1]	59.0 ± 9.27
	uterus - blotted (mg)	31.8 ± 3.66	32.0 ± 3.35	32.6 ± 14.60	32.3 ± 3.98	34.4 ± 2.70[1]	49.0 ± 8.72
	body wt. (gm)	41.5 ± 2.74	42.2 ± 3.66	42.3 ± 19.70	39.5 ± 3.78	31.4 ± 3.36[1]	38.0 ± 3.58
8	uterus - wet (mg)	30.9 ± 2.95^	33.1 ± 3.24	36.0 ± 3.46	37.5 ± 4.35	50.9 ± 18.34	52.0 ± 3.19
	uterus - blotted (mg)	29.5 ± 2.95^	32.2 ± 3.13	34.8 ± 3.48	36.1 ± 3.89	49.1 ± 17.77	50.4 ± 2.94
	body wt. (gm)	56.7 ± 1.74^	56.3 ± 3.51	55.0 ± 3.15	54.8 ± 2.75	53.5 ± 3.92	53.5 ± 2.29
18	uterus - wet (mg)	26.5 ± 4.20	26.8 ± 3.23	30.1 ± 1.60	30.4 ± 5.82	37.0 ± 5.54	44.1 ± 8.36[1]
	uterus - blotted (mg)	25.4 ± 4.19	25.7 ± 2.94	29.0 ± 1.86	29.4 ± 5.85	35.8 ± 5.68	42.8 ± 8.32[1]
	body wt. (gm)	46.6 ± 7.14	48.0 ± 5.86	47.7 ± 3.48	42.5 ± 4.59	44.3 ± 4.00	45.3 ± 3.35[1]
20	uterus - wet (mg)	24.2 ± 2.48^	--	29.6 ± 5.75	31.0 ± 1.43	39.1 ± 7.43[1]	--
	uterus - blotted (mg)	20.6 ± 1.81^	--	25.8 ± 5.19	26.8 ± 2.44	33.8 ± 6.04[1]	--
	body wt. (gm)	39.7 ± 3.10^	--	38.5 ± 2.17	33.7 ± 3.82	39.5 ± 6.00[1]	--

Table 1b. Weights of wet and blotted uteri, and body weights, in animals administered bisphenol A in Protocol B; dose-response study

Lab.	Weighing	0	10 mg/kg/d	100 mg/kg/d	300 mg/kg/d	600 mg/kg/d	800 mg/kg/d
1	uterus - wet (mg)	25.2 ± 2.79	29.5 ± 4.42	36.5 ± 5.35	48.1 ± 7.34	53.4 ± 11.59	77.4 ± 16.85
	uterus - blotted (mg)	23.5 ± 2.33	27.8 ± 4.24	34.5 ± 5.07	45.5 ± 7.26	50.7 ± 10.71	70.1 ± 9.13
	body wt. (gm)	51.9 ± 6.75	52.1 ± 7.57	50.8 ± 1.96	52.6 ± 3.59	51.4 ± 7.00	49.6 ± 5.42
4	uterus - wet (mg)	33.4 ± 7.02	37.0 ± 9.27	45.3 ± 6.56	61.5 ± 10.82	112.7 ± 35.60	142.0 ± 25.64
	uterus - blotted (mg)	28.0 ± 3.46	31.2 ± 7.28	38.8 ± 12.95	51.3 ± 15.60	82.2 ± 31.40	104.8 ± 8.80
	body wt. (gm)	45.2 ± 2.32	44.2 ± 3.60	41.5 ± 4.04	45.8 ± 3.06	43.3 ± 3.56	42.3 ± 2.73
6	uterus - wet (mg)	61.1 ± 15.24	--	72.7 ± 17.73	80.6 ± 16.86	131.7 ± 59.15[1]	--
	uterus - blotted (mg)	58.0 ± 14.00	--	69.1 ± 17.38	76.8 ± 15.96	115.5 ± 39.04[1]	--
	body wt. (gm)	48.9 ± 8.15	--	49.0 ± 6.92	47.9 ± 7.07	52.6 ± 6.68[1]	--
8	uterus - wet (mg)	34.5 ± 4.30	34.0 ± 2.82	44.2 ± 4.32	65.9 ± 10.58	161.6 ± 38.51	209.7 ± 35.88
	uterus - blotted (mg)	32.8 ± 4.26	32.9 ± 2.92	42.8 ± 4.22	64.2 ± 9.88	113.0 ± 10.39	119.0 ± 9.64
	body wt. (gm)	57.6 ± 4.26	56.6 ± 3.96	57.2 ± 3.70	57.2 ± 3.57	54.9 ± 2.67	54.7 ± 2.69
11	uterus - wet (mg)	58.0 ± 7.84	81.4 ± 9.96	88.8 ± 8.72	107.7 ± 12.03	120.9 ± 15.32	136.1 ± 13.55
	uterus - blotted (mg)	47.3 ± 6.92	67.7 ± 7.79	71.0 ± 9.08	89.0 ± 11.37	93.4 ± 14.04	113.7 ± 10.19
	body wt. (gm)	51.7 ± 5.72[+]	51.2 ± 4.96*	52.8 ± 7.70*	52.2 ± 3.60*	53.2 ± 3.06*	54.3 ± 1.97*
16	uterus - wet (mg)	25.0 ± 1.66^	30.9 ± 3.00	37.7 ± 3.27	51.1 ± 5.50	98.6 ± 16.36	144.9 ± 44.28
	uterus - blotted (mg)	21.3 ± 1.50^	28.5 ± 3.62	33.8 ± 3.85	46.6 ± 5.28	72.1 ± 5.41	95.0 ± 10.63
	body wt. (gm)	52.1 ± 3.70^	57.1 ± 4.91	53.0 ± 4.55	55.1 ± 3.76	53.8 ± 3.11	52.6 ± 3.02
17	uterus - wet (mg)	57.7 ± 13.08^	36.2 ± 7.20	35.8 ± 4.29	53.7 ± 9.83	90.2 ± 18.97	107.3 ± 30.72
	uterus - blotted (mg)	54.3 ± 11.77^	27.4 ± 7.56	31.6 ± 4.90	50.8 ± 9.08	81.7 ± 13.65	92.9 ± 15.35
	body wt. (gm)	50.7 ± 4.01^	51.6 ± 1.78	52.8 ± 1.74	51.4 ± 1.87	50.4 ± 3.32	51.4 ± 2.84
18	uterus - wet (mg)	28.1 ± 1.98	31.0 ± 2.42	46.2 ± 6.92	62.1 ± 6.24	98.3 ± 27.58	144.5 ± 53.95
	uterus - blotted (mg)	26.5 ± 1.80	29.4 ± 2.44	44.5 ± 6.40	59.8 ± 5.72	88.0 ± 17.01	105.0 ± 15.13
	body wt. (gm)	51.5 ± 2.45	49.9 ± 2.88	51.5 ± 3.56	48.8 ± 3.53	50.5 ± 2.23	49.5 ± 3.85
19	uterus - wet (mg)	33.2 ± 5.56^	35.3 ± 8.19	36.2 ± 4.26	50.2 ± 6.18	82.8 ± 23.64	132.7 ± 43.37
	uterus - blotted (mg)	28.7 ± 5.47^	26.3 ± 4.68	27.3 ± 4.80	36.8 ± 6.91	67.8 ± 13.00	87.5 ± 18.07
	body wt. (gm)	48.3 ± 3.65^	46.3 ± 3.70	46.4 ± 2.38	44.8 ± 3.84	44.3 ± 4.77	46.9 ±
20	uterus - wet (mg)	26.8 ± 6.97^	--	34.7 ± 3.59	32.1 ± 6.64	65.2 ± 23.00	--
	uterus - blotted (mg)	22.4 ± 6.47^	--	31.4 ± 4.47	28.2 ± 6.64	56.3 ± 17.81	--
	body wt. (gm)	40.4 ± 3.38^	--	38.1 ± 5.62	36.8 ± 5.79	39.7 ± 4.06	--

[+] body weights were from day 3, rather than at time of sacrifice.

^ shared controls

Table 1c. Weights of wet and blotted uteri, and body weights, in animals administered bisphenol A in Protocol C; dose-response study

Lab.	Weighing	0	10 mg/kg/d	100 mg/kg/d	300 mg/kg/d	600 mg/kg/d	800 mg/kg/d
1	uterus - wet (mg)	92.5 ± 10.51	90.4 ± 7.82	152.1 ± 29.28	353.9 ± 86.49	355.3 ± 97.07	388.1 ± 113.91
	uterus - blotted (mg)	88.0 ± 9.76	86.0 ± 7.29	139.3 ± 21.94	229.2 ± 35.16	243.4 ± 30.12	239.6 ± 35.55
	body wt. (gm)	291.0 ± 17.09	291.5 ± 17.04	282.3 ± 11.60	281.2 ± 14.29	276.3 ± 21.93	276.5 ± 19.53
6	uterus - wet (mg)	115.5 ± 19.84^	--	236.7 ± 43.08	274.1 ± 69.59	728.8 ± 207.15	--
	uterus - blotted (mg)	110.7 ± 19.60^	--	219.5 ± 45.59	236.1 ± 53.39	393.7 ± 68.46	--
	body wt. (gm)	299.6 ± 29.76^	--	291.6 ± 12.58	269.6 ± 24.97	277.5 ± 8.91	--
8	uterus - wet (mg)	91.4 ± 13.17	93.9 ± 10.84	150.3 ± 24.55	619.1 ± 157.48[1]	764.9 ± 173.18	825.8 ± 240.53
	uterus - blotted (mg)	88.8 ± 12.90	91.5 ± 10.46	146.7 ± 23.53	294.2 ± 23.44[1]	333.3 ± 32.99	318.5 ± 32.10
	body wt. (gm)	250.2 ± 12.36	250.6 ± 13.27	243.3 ± 12.50	229.5 ± 11.72[1]	236.5 ± 9.30	237.9 ± 9.74
18	uterus - wet (mg)	103.9 ± 13.20	116.9 ± 13.00	210.3 ± 62.72	439.1 ± 129.16	588.4 ± 161.90	728.3 ± 201.57
	uterus - blotted (mg)	99.8 ± 10.76	112.5 ± 11.69	188.3 ± 25.51	278.6 ± 35.86	306.7 ± 32.98	301.9 ± 43.25
	body wt. (gm)	250.9 ± 13.24	251.4 ± 9.97	240.2 ± 12.08	238.0 ± 13.90	236.4 ± 11.03	229.9 ± 17.53
20	uterus - wet (mg)	106.0 ± 18.84^	--	225.4 ± 45.83	444.5 ± 89.56	837.0 ± 207.10	--
	uterus - blotted (mg)	98.6 ± 22.04^	--	197.4 ± 33.88	266.3 ± 44.60	314.1 ± 60.01	--
	body wt. (gm)	297.2 ± 14.54^	--	291.8 ± 12.26	299.9 ± 10.99	289.3 ± 23.00	--

Table 1d. Weights of wet and blotted uteri, and body weights, in animals administered bisphenol A in Protocol C'; dose-response study

Lab.	Weighing	0	10 mg/kg/d	100 mg/kg/d	300 mg/kg/d	600 mg/kg/d	800 mg/kg/d
8	uterus - wet (mg)	82.2 ± 2.94	91.1 ± 7.47	192.7 ± 6.30	358.8 ± 109.44	421.4 ± 72.68	525.8 ± 41.04
	uterus - blotted (mg)	80.4 ± 2.70	88.8 ± 7.70	188.8 ± 4.96	314.1 ± 40.32	346.7 ± 41.94	376.9 ± 25.57
	body wt. (gm)	283.7 ± 14.51	285.8 ± 14.66	259.1 ± 11.75	245.7 ± 5.74	249.5 ± 7.28	244.5 ± 6.29
18	uterus - wet (mg)	89.0 ± 13.97	100.8 ± 16.54	214.5 ± 14.44	342.8 ± 42.58	613.0 ± 141.93	484.8 ± 139.04
	uterus - blotted (mg)	86.2 ± 13.56	97.6 ± 16.07	209.7 ± 13.14	306.8 ± 18.43	389.9 ± 57.69	353.9 ± 48.07
	body wt. (gm)	274.6 ± 15.93	269.2 ± 20.29	246.9 ± 9.88	236.2 ± 10.71	242.5 ± 16.54	230.5 ± 24.79

Table 1e. Weights of wet and blotted uteri, and body weights, in animals administered bisphenol A in Protocol D; dose-response study

Lab.	Weighing	0	60 mg/kg/d	200 mg/kg/d	375 mg/kg/d	600 mg/kg/d	1000 mg/kg/d
20	uterus - wet (mg)	101.1 ± 16.93^	--	120.9 ± 11.63	133.7 ± 38.71	130.9 ± 11.92	--
	uterus - blotted (mg)	95.0 ± 16.43^	--	112.4 ± 10.36	125.2 ± 38.35	125.3 ± 10.03	--
	body wt. (gm)	295.5 ± 11.09^	--	281.7 ± 14.55	289.7 ± 11.37	278.5 ± 11.92	--

[+] body weights were from day 3, rather than at time of sacrifice.
^ shared controls

Table 2a. Weights of wet and blotted uteri, and body weights, in animals administered *o,p'*-DDT in Protocol A; dose-response study

Lab.	Weighing	0	10 mg/kg/d	50 mg/kg/d	125 mg/kg/d	300 mg/kg/d	600 mg/kg/d
7	uterus - wet (mg)	45.5 ± 7.68	54.3 ± 11.72	67.3 ± 6.11	87.3 ± 17.05	123.4 ± 40.55[1]	181.2 ± 9.83[4]
	uterus - blotted (mg)	43.3 ± 7.91	52.5 ± 11.61	65.1 ± 5.43	83.4 ± 15.78	100.3 ± 20.64[1]	123.7 ± 21.57[4]
	body wt. (gm)	58.6 ± 5.07	60.5 ± 2.19	58.2 ± 4.38	55.0 ± 6.53	44.3 ± 11.26[1]	35.0 ± 1.27[4]
12	uterus - wet (mg)	29.5 ± 4.35	34.2 ± 3.71	63.7 ± 5.55	67.9 ± 4.38	103.8 ± 31.81	210.3 ± 127.63[3]
	uterus - blotted (mg)	25.3 ± 3.75	31.3 ± 3.87	58.7 ± 5.24	64.2 ± 4.20	87.0 ± 3.90	95.2 ± 5.73[3]
	body wt. (gm)	38.6 ± 3.91	40.6 ± 2.40	41.3 ± 4.01	37.4 ± 2.57	39.5 ± 3.56	28.3 ± 2.30[3]
15	uterus - wet (mg)	34.6 ± 3.93^	44.6 ± 7.09	64.4 ± 4.90	81.3 ± 12.90	101.6 ± 11.55	229.6 ± 65.83[4]
	uterus - blotted (mg)	32.6 ± 4.23^	42.5 ± 7.03	62.0 ± 5.06	78.3 ± 12.46	97.3 ± 11.28	104.1 ± 13.22[4]
	body wt. (gm)	63.2 ± 3.07^	62.7 ± 2.21	62.7 ± 1.77	60.6 ± 2.96	54.3 ± 9.56	36.1 ± 2.69[4]
20	uterus - wet (mg)	24.2 ± 2.48^	--	61.4 ± 9.24	74.0 ± 14.28	129.2 ± 58.42[3]	--
	uterus - blotted (mg)	20.6 ± 1.81^	--	54.6 ± 9.03	67.5 ± 14.06	67.4 ± 16.63[3]	--
	body wt. (gm)	39.7 ± 3.10^	--	40.5 ± 2.54	41.3 ± 5.85	33.0 ± 8.16[3]	--

Table 2b. Weights of wet and blotted uteri, and body weights, in animals administered *o,p'*-DDT in Protocol B; dose-response study

Lab.	Weighing	0	5 mg/kg/d	25 mg/kg/d	50 mg/kg/d	100 mg/kg/d	200 mg/kg/d
7	uterus - wet (mg)	39.0 ± 10.51	44.2 ± 9.83	41.5 ± 15.56	37.7 ± 8.94	44.3 ± 9.77	53.4 ± 8.45
	uterus - blotted (mg)	36.1 ± 10.07	40.5 ± 9.34	38.1 ± 14.74	33.7 ± 8.68	39.5 ± 8.87	48.5 ± 8.89
	body wt. (gm)	57.5 ± 5.69	57.3 ± 4.97	56.7 ± 5.27	56.5 ± 4.62	55.9 ± 6.36	56.7 ± 4.54
12	uterus - wet (mg)	27.1 ± 4.15	27.5 ± 3.55	30.6 ± 4.84	27.1 ± 2.09	28.4 ± 2.82	35.8 ± 8.34
	uterus - blotted (mg)	23.6 ± 3.87	24.7 ± 3.31	25.7 ± 3.47	24.1 ± 2.18	25.1 ± 2.79	32.9 ± 7.99
	body wt. (gm)	38.6 ± 4.11	38.3 ± 3.34	40.0 ± 3.87	38.1 ± 3.21	38.3 ± 3.50	39.3 ± 3.95
15	uterus - wet (mg)	34.2 ± 3.49	35.0 ± 3.74	40.8 ± 7.04	40.2 ± 2.19	40.0 ± 3.62	51.3 ± 8.10
	uterus - blotted (mg)	31.5 ± 3.97	33.2 ± 3.33	39.0 ± 6.85	37.4 ± 1.82	37.8 ± 3.49	49.5 ± 7.97
	body wt. (gm)	65.4 ± 3.13	65.1 ± 4.32	64.3 ± 3.60	65.2 ± 4.67	64.6 ± 1.70	65.0 ± 2.97
20	uterus - wet (mg)	26.8 ± 6.97^	--	30.8 ± 10.00	37.2 ± 6.82	37.6 ± 10.86	--
	uterus - blotted (mg)	22.4 ± 6.47^	--	26.3 ± 9.16	31.8 ± 6.26	33.9 ± 10.49	--
	body wt. (gm)	40.4 ± 3.38^	--	37.6 ± 6.10	42.7 ± 3.54	41.0 ± 5.01	--

Table 2c. Weights of wet and blotted uteri, and body weights, in animals administered *o,p'*-DDT in Protocol C; dose-response study

Lab.	Weighing	0	5 mg/kg/d	25 mg/kg/d	50 mg/kg/d	100 mg/kg/d	200 mg/kg/d
12	uterus - wet (mg)	88.9 ± 6.60	87.2 ± 7.21	98.5 ± 8.16	104.9 ± 12.79	103.6 ± 28.85	112.1 ± 18.41
	uterus - blotted (mg)	78.5 ± 8.38	75.8 ± 7.10	90.5 ± 7.63	97.1 ± 12.07	99.6 ± 28.59	103.4 ± 15.93
	body wt. (gm)	217.0 ± 5.55	216.2 ± 8.56	217.8 ± 8.86	214.4 ± 9.70	214.6 ± 9.22	212.7 ± 6.96
15	uterus - wet (mg)	90.8 ± 8.37	101.2 ± 10.13	105.0 ± 6.55	116.6 ± 5.31	126.7 ± 10.92	170.9 ± 37.58
	uterus - blotted (mg)	85.5 ± 8.57	96.6 ± 9.84	99.6 ± 6.43	111.1 ± 4.86	122.2 ± 10.32	159.6 ± 27.57
	body wt. (gm)	268.9 ± 8.50	267.3 ± 7.50	266.8 ± 14.01	266.5 ± 12.36	264.3 ± 10.76	256.6 ± 17.51
20	uterus - wet (mg)	106.0 ± 18.84^	--	111.8 ± 22.24	116.6 ± 13.98	136.6 ± 37.63	--
	uterus - blotted (mg)	98.6 ± 22.04^	--	104.1 ± 20.63	107.9 ± 16.45	128.3 ± 36.21	--
	body wt. (gm)	297.2 ± 14.54^	--	300.1 ± 21.01	294.3 ± 22.76	301.3 ± 15.35	--

Table 2d. Weights of wet and blotted uteri, and body weights, in animals administered *o,p'*-DDT in Protocol C'; dose-response study

Lab.	Weighing	0	5 mg/kg/d	25 mg/kg/d	50 mg/kg/d	100 mg/kg/d	200 mg/kg/d
12	uterus - wet (mg)	84.9 ± 14.40	80.5 ± 7.12	83.6 ± 8.98	102.0 ± 7.79	104.8 ± 5.54	125.0 ± 35.69
	uterus - blotted (mg)	71.5 ± 14.84	73.9 ± 6.44	76.7 ± 8.14	93.9 ± 8.43	98.8 ± 5.91	117.6 ± 33.48
	body wt. (gm)	235.0 ± 8.47	233.8 ± 11.51	235.3 ± 12.16	232.9 ± 12.09	224.1 ± 9.60	221.8 ± 11.09
15	uterus - wet (mg)	89.9 ± 4.86	91.4 ± 10.00	98.0 ± 11.77	104.2 ± 6.70	113.4 ± 9.35	147.7 ± 13.13
	uterus - blotted (mg)	86.3 ± 4.86	87.5 ± 9.40	93.8 ± 10.54	100.8 ± 6.21	109.7 ± 9.24	142.5 ± 12.25
	body wt. (gm)	290.6 ± 19.95	289.0 ± 11.66	291.3 ± 18.44	282.5 ± 10.96	282.6 ± 6.80	273.8 ± 14.48

Table 2e. Weights of wet and blotted uteri, and body weights, in animals administered *o,p'*-DDT in Protocol D; dose-response study

Lab.	Weighing	0	20 mg/kg/d	60 mg/kg/d	120 mg/kg/d	300 mg/kg/d	500 mg/kg/d
20	uterus - wet (mg)	101.1 ± 16.93^	--	228.1 ± 64.85	472.4 ± 242.66	683.7 ± 143.62	--
	uterus - blotted (mg)	95.0 ± 16.43^	--	191.2 ± 23.03	253.6 ± 70.91	275.6 ± 31.42	--
	body wt. (gm)	295.5 ± 11.09^	--	286.1 ± 16.83	278.1 ± 10.52	269.4 ± 3.92	--

^ shared controls

Table 3a. Weights of wet and blotted uteri, and body weights, in animals administered genistein in Protocol A; dose-response study

Lab.	Weighing	0	20 mg/kg/d	60 mg/kg/d	120 mg/kg/d	300 mg/kg/d	500 mg/kg/d
1	uterus - wet (mg)	23.1 ± 3.25	24.8 ± 3.61	36.2 ± 6.11	51.7 ± 3.99	65.5 ± 9.44	69.8 ± 7.82
	uterus - blotted (mg)	21.4 ± 2.56	22.9 ± 3.16	34.1 ± 5.62	49.6 ± 4.09	61.7 ± 8.50	65.7 ± 7.81
	body wt. (gm)	46.5 ± 5.61	40.8 ± 4.34	44.0 ± 4.49	44.4 ± 4.35	42.9 ± 3.98	42.7 ± 4.73
5	uterus - wet (mg)	29.7 ± 4.54	39.9 ± 6.49	66.1 ± 13.87	77.0 ± 8.68	74.8 ± 7.25	91.0 ± 15.13
	uterus - blotted (mg)	29.2 ± 4.48	39.4 ± 6.53	65.6 ± 13.93	76.3 ± 8.60	74.1 ± 7.23	89.0 ± 13.52
	body wt. (gm)	56.7 ± 2.71	58.0 ± 3.90	57.7 ± 3.61	57.2 ± 2.81	58.1 ± 2.12	56.8 ± 3.31
14	uterus - wet (mg)	45.9 ± 6.29	64.2 ± 12.02	80.6 ± 7.40	82.9 ± 8.35	92.4 ± 8.19	112.3 ± 28.77
	uterus - blotted (mg)	39.1 ± 4.10	55.3 ± 11.49	68.3 ± 7.03	74.7 ± 8.69	81.4 ± 7.85	96.8 ± 24.56
	body wt. (gm)	67.3 ± 2.62	66.3 ± 3.50	66.7 ± 2.81	65.4 ± 5.09	63.6 ± 2.53	63.5 ± 2.30
20	uterus - wet (mg)	24.2 ± 2.48^	--	58.5 ± 10.89	72.2 ± 12.41	82.8 ± 11.95	--
	uterus - blotted (mg)	20.6 ± 1.81^	--	52.4 ± 9.89	64.5 ± 11.94	74.6 ± 10.43	--
	body wt. (gm)	39.7 ± 3.10^	--	40.7 ± 3.30	41.7 ± 4.90	43.4 ± 4.48	--

Table 3b. Weights of wet and blotted uteri, and body weights, in animals administered genistein in Protocol B; dose-response study

Lab.	Weighing	0	1 mg/kg/d	15 mg/kg/d	35 mg/kg/d	50 mg/kg/d	80 mg/kg/d
1	uterus - wet (mg)	22.6 ± 1.40	26.0 ± 2.28	46.5 ± 9.17	58.8 ± 11.38	67.6 ± 10.51	84.3 ± 8.44
	uterus - blotted (mg)	20.9 ± 1.12	24.4 ± 1.87	44.4 ± 8.63	56.4 ± 10.91	64.8 ± 10.18	80.4 ± 7.84
	body wt. (gm)	62.3 ± 5.95	51.1 ± 5.78	51.9 ± 5.34	51.1 ± 5.10	51.6 ± 4.42	52.6 ± 4.92
5	uterus - wet (mg)	34.9 ± 3.47^	41.3 ± 8.49	65.9 ± 4.95	89.9 ± 4.69	106.7 ± 7.71	145.3 ± 29.46
	uterus - blotted (mg)	34.1 ± 3.67^	40.0 ± 8.20	64.7 ± 5.18	88.7 ± 4.61	104.1 ± 7.12	120.0 ± 13.10
	body wt. (gm)	58.0 ± 2.27^	57.1 ± 3.54	57.7 ± 3.30	59.3 ± 3.89	57.2 ± 1.99	58.4 ± 2.84
14	uterus - wet (mg)	38.2 ± 10.47	44.5 ± 11.46	62.8 ± 6.75	82.9 ± 14.09	105.2 ± 16.99	120.2 ± 20.31
	uterus - blotted (mg)	33.4 ± 9.32	39.6 ± 10.26	58.0 ± 5.64	75.4 ± 11.61	94.2 ± 10.91	105.9 ± 14.33
	body wt. (gm)	63.1 ± 4.45	62.4 ± 3.10	62.8 ± 3.36	62.0 ± 3.18	62.5 ± 3.50	60.8 ± 3.14
20	uterus - wet (mg)	26.8 ± 6.97^	--	41.7 ± 8.36	55.9 ± 13.68	66.2 ± 14.75	--
	uterus - blotted (mg)	22.4 ± 6.47^	--	35.2 ± 9.19	50.6 ± 10.96	59.4 ± 12.36	--
	body wt. (gm)	40.4 ± 3.38^	--	42.1 ± 5.40	40.6 ± 4.57	40.3 ± 3.58	--

Table 3c. Weights of wet and blotted uteri, and body weights, in animals administered genistein in Protocol C; dose-response study

Lab.	Weighing	0	1 mg/kg/d	15 mg/kg/d	35 mg/kg/d	50 mg/kg/d	80 mg/kg/d
5	uterus - wet (mg)	87.2 ± 11.74^	85.9 ± 10.39	136.9 ± 23.21	161.4 ± 7.49	181.0 ± 17.13	172.6 ± 13.57
	uterus - blotted (mg)	86.3 ± 11.88^	85.0 ± 10.35	135.8 ± 22.92	160.1 ± 7.33	179.4 ± 16.71	170.6 ± 11.92
	body wt. (gm)	256.1 ± 8.87^	257.9 ± 10.05	258.4 ± 9.90	255.2 ± 11.14	253.3 ± 12.09	253.6 ± 10.56
14	uterus - wet (mg)	93.5 ± 12.30	84.0 ± 7.86	144.7 ± 18.42	162.6 ± 13.97	151.0 ± 14.35	177.6 ± 40.11
	uterus - blotted (mg)	85.9 ± 13.10	77.2 ± 6.44	131.5 ± 15.40	151.6 ± 13.14	142.1 ± 11.98	163.4 ± 33.74
	body wt. (gm)	272.5 ± 20.75	277.0 ± 13.53	275.5 ± 14.15	270.4 ± 14.70	267.4 ± 10.96	272.4 ± 15.03
20	uterus - wet (mg)	106.0 ± 18.84^	--	146.1 ± 33.23	162.2 ± 26.36	183.3 ± 57.85	--
	uterus - blotted (mg)	98.6 ± 22.04^	--	133.9 ± 30.80	152.0 ± 24.29	168.3 ± 54.04	--
	body wt. (gm)	297.2 ± 14.54^	--	303.6 ± 12.80	297.2 ± 17.70	303.4 ± 17.97	--

Table 3d. Weights of wet and blotted uteri, and body weights, in animals administered genistein in Protocol C'; dose-response study

Lab.	Weighing	0	1 mg/kg/d	15 mg/kg/d	35 mg/kg/d	50 mg/kg/d	80 mg/kg/d
5	uterus - wet (mg)	76.8 ± 4.59^	90.9 ± 9.17	157.5 ± 21.51	193.5 ± 12.44	209.9 ± 11.29	243.8 ± 76.43
	uterus - blotted (mg)	75.9 ± 4.97^	89.7 ± 9.13	156.7 ± 21.76	192.4 ± 11.73	208.7 ± 10.70	215.3 ± 37.35
	body wt. (gm)	282.1 ± 12.40^	282.6 ± 11.72	276.9 ± 12.39	280.2 ± 10.44	277.1 ± 12.44	275.3 ± 12.18
14	uterus - wet (mg)	96.4 ± 17.25	96.9 ± 15.94	161.8 ± 20.55	207.8 ± 29.01	222.0 ± 29.72	394.0 ± 75.24
	uterus - blotted (mg)	87.2 ± 15.17	85.8 ± 11.09	145.8 ± 19.66	189.3 ± 22.50	200.0 ± 25.22	303.6 ± 24.41
	body wt. (gm)	281.5 ± 19.95	268.8 ± 13.92	270.2 ± 14.68	264.2 ± 14.56	266.4 ± 12.93	265.3 ± 12.31

Table 3e. Weights of wet and blotted uteri, and body weights, in animals administered genistein in Protocol D; dose-response study

Lab.	Weighing	0	20 mg/kg/d	60 mg/kg/d	120 mg/kg/d	300 mg/kg/d	500 mg/kg/d
20	uterus - wet (mg)	101.1 ± 16.93^	--	194.5 ± 50.41	191.7 ± 43.16	270.9 ± 92.51	--
	uterus - blotted (mg)	95.0 ± 16.43^	--	172.6 ± 38.92	178.9 ± 39.60	195.1 ± 20.90	--
	body wt. (gm)	295.5 ± 11.09^	--	291.2 ± 12.85	285.7 ± 6.30	283.4 ± 12.36	--

^ shared controls

Table 4a. Weights of wet and blotted uteri, and body weights, in animals administered methoxychlor in Protocol A; dose-response study

Lab.	Weighing	0	20 mg/kg/d	50 mg/kg/d	120 mg/kg/d	300 mg/kg/d	500 mg/kg/d
2	uterus - wet (mg)	18.4 ± 2.19	58.3 ± 16.27	55.2 ± 8.01	65.4 ± 8.20[1]	63.4 ± 11.41[1]	62.0 ± 4.58[3]
	uterus - blotted (mg)	14.8 ± 2.28	51.0 ± 12.98	45.2 ± 8.13	59.2 ± 9.65[1]	55.6 ± 8.20[1]	51.7 ± 3.79[3]
	body wt. (gm)	40.3 ± 6.83	45.8 ± 4.26	40.5 ± 4.69	48.2 ± 5.81[1]	46.3 ± 5.47[1]	47.6 ± 4.17[3]
14	uterus - wet (mg)	44.3 ± 6.44	88.2 ± 17.18	104.5 ± 13.59	103.4 ± 11.55	112.6 ± 8.44	114.3 ± 6.37
	uterus - blotted (mg)	38.4 ± 6.50	76.4 ± 16.74	87.1 ± 11.13	86.2 ± 7.74	93.1 ± 7.50	98.8 ± 5.14
	body wt. (gm)	61.6 ± 2.49	61.4 ± 3.78	61.1 ± 1.84	61.1 ± 3.57	59.2 ± 2.21	58.0 ± 2.96
15	uterus - wet (mg)	34.6 ± 3.93^	74.7 ± 10.74	90.7 ± 10.65	98.5 ± 9.11	108.8 ± 9.62	98.0 ± 16.90
	uterus - blotted (mg)	32.6 ± 4.23^	72.2 ± 10.22	90.8 ± 9.67	94.7 ± 8.91	105.0 ± 9.75	94.8 ± 15.37
	body wt. (gm)	62.9 ± 1.64^	62.2 ± 3.38	62.0 ± 3.04	62.1 ± 3.44	58.4 ± 3.31	58.0 ± 4.00
20	uterus - wet (mg)	24.2 ± 2.48^	--	89.3 ± 26.37	88.3 ± 17.06	86.3 ± 10.42	--
	uterus - blotted (mg)	20.6 ± 1.81^	--	79.6 ± 24.95	77.9 ± 14.63	78.6 ± 10.02	--
	body wt. (gm)	39.7 ± 3.10^	--	39.8 ± 5.76	39.2 ± 3.38	38.3 ± 3.30	--

Table 4b. Weights of wet and blotted uteri, and body weights, in animals administered methoxychlor in Protocol B; dose-response study

Lab.	Weighing	0	20 mg/kg/d	100 mg/kg/d	250 mg/kg/d	500 mg/kg/d	800 mg/kg/d
2	uterus - wet (mg)	18.3 ± 3.61	23.5 ± 4.18	40.8 ± 14.58	58.2 ± 13.33	74.3 ± 18.48	72.6 ± 21.34
	uterus - blotted (mg)	16.3 ± 3.78	17.8 ± 4.54	27.7 ± 6.28	44.3 ± 12.34	61.8 ± 13.12	60.2 ± 7.60
	body wt. (gm)	44.2 ± 5.25	44.7 ± 4.52	46.4 ± 4.97	40.7 ± 9.15	44.7 ± 5.00	46.8 ± 4.49
14	uterus - wet (mg)	39.7 ± 7.58	44.9 ± 9.18	52.4 ± 9.38	77.0 ± 13.82	99.3 ± 17.57	103.4 ± 6.86
	uterus - blotted (mg)	35.2 ± 6.34	40.9 ± 8.42	48.0 ± 9.43	68.0 ± 11.28	86.3 ± 10.15	93.5 ± 6.15
	body wt. (gm)	66.6 ± 4.48	65.0 ± 3.87	65.4 ± 4.20	65.5 ± 3.69	64.7 ± 2.92	64.8 ± 3.84
15	uterus - wet (mg)	34.2 ± 3.49	47.1 ± 6.83	86.2 ± 19.85	108.9 ± 20.26	121.9 ± 30.68	139.7 ± 10.78
	uterus - blotted (mg)	31.5 ± 3.97	45.1 ± 6.82	81.0 ± 16.97	101.4 ± 16.06	107.4 ± 17.90	132.2 ± 9.09
	body wt. (gm)	63.5 ± 3.92	63.2 ± 2.13	62.6 ± 1.54	61.5 ± 3.38	62.3 ± 2.59	63.8 ± 1.54
20	uterus - wet (mg)	26.8 ± 6.97^	--	71.3 ± 10.13	89.2 ± 8.98	83.2 ± 10.60	--
	uterus - blotted (mg)	22.4 ± 6.47^	--	62.3 ± 9.23	76.2 ± 38.60	72.0 ± 9.42	--
	body wt. (gm)	40.4 ± 3.38^	--	39.7 ± 6.98	39.6 ± 1.66	38.7 ± 5.27	--

Table 4c. Weights of wet and blotted uteri, and body weights, in animals administered methoxychlor in Protocol C; dose-response study

Lab.	Weighing	0	20 mg/kg/d	100 mg/kg/d	250 mg/kg/d	500 mg/kg/d	800 mg/kg/d
14	uterus - wet (mg)	92.1 ± 8.86	88.5 ± 6.03	118.3 ± 15.22	173.3 ± 34.90	256.7 ± 80.39	282.1 ± 90.49
	uterus - blotted (mg)	84.9 ± 8.03	81.4 ± 6.04	108.3 ± 13.16	156.5 ± 25.31	197.1 ± 46.05	203.3 ± 34.71
	body wt. (gm)	255.9 ± 10.19	259.4 ± 16.10	251.7 ± 9.52	249.3 ± 9.53	248.8 ± 11.64	246.6 ± 13.26
15	uterus - wet (mg)	90.8 ± 8.37	97.1 ± 8.47	155.7 ± 19.42	184.4 ± 33.95	298.4 ± 126.20	298.9 ± 56.93
	uterus - blotted (mg)	85.5 ± 8.57	92.5 ± 8.93	149.1 ± 18.59	173.6 ± 30.05	212.0 ± 27.48	226.2 ± 23.49
	body wt. (gm)	278.5 ± 11.15	278.7 ± 10.33	272.1 ± 15.33	272.4 ± 10.41	264.2 ± 12.98	264.5 ± 12.62
20	uterus - wet (mg)	106.0 ± 18.84^	--	170.2 ± 31.93	196.8 ± 48.65	214.6 ± 24.88	--
	uterus - blotted (mg)	98.6 ± 22.04^	--	155.8 ± 32.81	171.7 ± 36.49	185.0 ± 22.30	--
	body wt. (gm)	297.2 ± 14.54^	--	287.4 ± 16.77	290.0 ± 12.36	288.7 ± 9.24	--

Table 4d. Weights of wet and blotted uteri, and body weights, in animals administered methoxychlor in Protocol C'; dose-response study

Lab.	Weighing	0	20 mg/kg/d	100 mg/kg/d	250 mg/kg/d	500 mg/kg/d	800 mg/kg/d
14	uterus - wet (mg)	98.1 ± 15.43	90.6 ± 11.93	128.1 ± 28.27	167.9 ± 32.11	254.0 ± 61.79	246.9 ± 30.58
	uterus - blotted (mg)	90.8 ± 15.92	84.8 ± 10.43	121.2 ± 26.36	155.9 ± 28.39	211.9 ± 41.21	216.7 ± 27.12
	body wt. (gm)	272.1 ± 14.66	271.6 ± 16.86	237.2 ± 14.85	251.4 ± 14.90	246.5 ± 17.59	245.8 ± 14.41
15	uterus - wet (mg)	96.8 ± 6.73	104.3 ± 5.76	151.0 ± 26.12	237.7 ± 29.06	238.8 ± 40.63	252.0 ± 34.97
	uterus - blotted (mg)	92.6 ± 5.87	99.3 ± 4.97	144.6 ± 25.61	221.2 ± 20.14	228.2 ± 38.44	246.9 ± 30.58
	body wt. (gm)	282.7 ± 19.53	277.6 ± 17.55	265.2 ± 18.00	256.6 ± 14.68	254.7 ± 8.95	250.9 ± 10.80

Table 4e. Weights of wet and blotted uteri, and body weights, in animals administered methoxychlor in Protocol D; dose-response study

Lab.	Weighing	0	20 mg/kg/d	50 mg/kg/d	120 mg/kg/d	300 mg/kg/d	500 mg/kg/d
20	uterus - wet (mg)	101.1 ± 16.93^	--	247.1 ± 20.31	301.8 ± 54.21	388.6 ± 18.89	--
	uterus - blotted (mg)	95.0 ± 16.43^	--	194.7 ± 33.94	217.1 ± 16.75	231.8 ± 23.71	--
	body wt. (gm)	295.5 ± 11.09^	--	278.7 ± 12.96	275.0 ± 11.90	279.0 ± 9.69	--

^ shared controls

Table 5a. Weights of wet and blotted uteri, and body weights, in animals administered nonylphenol in Protocol A; dose-response study

Lab.	Weighing	0	15 mg/kg/d	75 mg/kg/d	125 mg/kg/d	250 mg/kg/d	350 mg/kg/d
3	uterus - wet (mg)	31.3 ± 10.31	30.8 ± 5.42	50.5 ± 10.54	52.5 ± 5.72	62.3 ± 8.02[2]	--[6]
	uterus - blotted (mg)	29.3 ± 10.91	28.7 ± 4.68	45.2 ± 5.98	49.3 ± 5.89	60.3 ± 6.99[2]	--[6]
	body wt. (gm)	42.7 ± 2.91	38.8 ± 7.12	39.7 ± 3.49	36.3 ± 5.00	28.5 ± 1.55[2]	--[6]
5	uterus - wet (mg)	29.7 ± 4.54	36.9 ± 7.82	42.5 ± 5.30	60.2 ± 11.17	58.1 ± 9.28	60.6 ± 4.03[3]
	uterus - blotted (mg)	29.2 ± 4.48	36.4 ± 7.80	42.0 ± 5.36	59.7 ± 11.14	57.6 ± 8.98	60.0 ± 4.22[3]
	body wt. (gm)	56.7 ± 2.71	59.0 ± 2.29	58.3 ± 3.16	57.0 ± 3.11	47.1 ± 9.55	33.8 ± 3.83[3]
8	uterus - wet (mg)	31.4 ± 2.47^	33.0 ± 4.42	44.8 ± 7.36	49.8 ± 4.24	65.3 ± 10.10[1]	69.2 ± 8.66[3]
	uterus - blotted (mg)	30.0 ± 2.30^	31.8 ± 4.34	43.5 ± 7.07	48.1 ± 4.13	62.8 ± 9.44[1]	64.6 ± 8.30[3]
	body wt. (gm)	57.8 ± 3.50^	56.6 ± 4.97	54.3 ± 4.61	55.5 ± 4.50	49.8 ± 7.12[1]	43.8 ± 4.57[3]
20	uterus - wet (mg)	24.2 ± 2.48^	--	50.5 ± 10.39	45.0 ± 9.30	66.9 ± 20.29[4]	--
	uterus - blotted (mg)	20.6 ± 1.81^	--	45.5 ± 9.22	42.0 ± 7.85	62.2 ± 18.95[4]	--
	body wt. (gm)	39.7 ± 3.10^	--	44.1 ± 1.91	40.5 ± 3.44	39.9 ± 2.05[4]	--

Table 5b. Weights of wet and blotted uteri, and body weights, in animals administered nonylphenol in Protocol B; dose-response study

Lab.	Weighing	0	5 mg/kg/d	15 mg/kg/d	35 mg/kg/d	80 mg/kg/d	100 mg/kg/d
1	uterus - wet (mg)	26.0 ± 3.06	23.9 ± 2.69	26.1 ± 2.13	29.2 ± 1.59	37.0 ± 9.72	38.8 ± 5.26
	uterus - blotted (mg)	24.2 ± 2.77	22.3 ± 2.50	24.5 ± 1.83	27.5 ± 1.45	36.2 ± 9.30	36.9 ± 4.80
	body wt. (gm)	52.9 ± 6.02	52.0 ± 4.96	51.0 ± 5.77	51.4 ± 4.34	50.3 ± 4.84	50.4 ± 4.42
3	uterus - wet (mg)	34.7 ± 3.47	34.3 ± 7.89	29.7 ± 2.88	37.2 ± 2.40	64.5 ± 18.51	53.5 ± 13.49
	uterus - blotted (mg)	34.1 ± 3.67	31.3 ± 7.53	28.2 ± 3.76	34.0 ± 4.00	61.5 ± 17.41	51.2 ± 13.23
	body wt. (gm)	45.6 ± 3.93	45.3 ± 3.88	44.1 ± 3.88	44.8 ± 2.73	44.4 ± 3.26	44.0 ± 2.91
5	uterus - wet (mg)	34.9 ± 3.47^	37.1 ± 6.88	38.8 ± 6.28	46.3 ± 5.79	65.1 ± 9.37	82.8 ± 13.92
	uterus - blotted (mg)	34.1 ± 3.67^	36.4 ± 6.67	38.1 ± 6.32	45.2 ± 5.89	63.9 ± 9.31	80.5 ± 13.10
	body wt. (gm)	58.0 ± 2.27^	58.1 ± 3.84	58.2 ± 3.50	57.1 ± 1.85	58.3 ± 2.75	56.6 ± 2.30
6	uterus - wet (mg)	61.1 ± 15.24	--	52.7 ± 17.14	66.7 ± 12.35	79.5 ± 37.03	--
	uterus - blotted (mg)	58.0 ± 14.00	--	50.5 ± 16.83	62.4 ± 12.38	75.5 ± 33.32	--
	body wt. (gm)	48.9 ± 8.15	--	49.2 ± 11.85	50.8 ± 6.52	48.8 ± 8.97	--
8	uterus - wet (mg)	30.7 ± 4.18	32.2 ± 3.35	37.1 ± 5.53	34.9 ± 7.49	52.5 ± 13.23	68.1 ± 7.85
	uterus - blotted (mg)	29.6 ± 3.85	31.0 ± 3.29	35.8 ± 5.36	33.8 ± 7.27	50.9 ± 12.73	66.3 ± 7.69
	body wt. (gm)	57.2 ± 4.01	57.0 ± 3.13	56.8 ± 4.30	57.4 ± 4.17	57.4 ± 4.20	57.0 ± 3.02
11	uterus - wet (mg)	58.0 ± 7.84	84.8 ± 17.77	75.4 ± 15.29	83.2 ± 11.67	82.9 ± 15.24	78.3 ± 20.80
	uterus - blotted (mg)	47.3 ± 6.92	66.6 ± 15.54	64.2 ± 16.29	68.2 ± 10.19	75.2 ± 15.77	60.0 ± 15.00
	body wt. (gm)	51.7 ± 5.72^{+}	53.7 ± 4.41*	55.8 ± 3.66*	52.3 ± 2.34*	53.7 ± 2.16*	51.7 ± 1.75*
16	uterus - wet (mg)	25.0 ± 1.66^	20.7 ± 2.82	23.1 ± 2.97	28.2 ± 4.57	52.4 ± 5.42	72.9 ± 6.30
	uterus - blotted (mg)	21.3 ± 1.50^	19.0 ± 2.42	19.8 ± 3.35	24.9 ± 5.27	41.9 ± 4.00	64.4 ± 6.70
	body wt. (gm)	52.1 ± 3.70^	54.4 ± 4.81	56.5 ± 3.86	57.2 ± 4.23	55.2 ± 3.20	58.1 ± 2.54
17	uterus - wet (mg)	57.7 ± 13.08^	41.9 ± 20.16	43.8 ± 8.70	45.3 ± 10.93	49.4 ± 12.65	50.5 ± 10.88
	uterus - blotted (mg)	54.3 ± 11.77^	37.1 ± 8.88	33.7 ± 7.91	37.7 ± 11.77	41.3 ± 10.96	39.0 ± 10.01
	body wt. (gm)	50.7 ± 4.01^	51.8 ± 3.00	50.1 ± 3.12	52.2 ± 5.09	51.2 ± 3.16	50.4 ± 1.95
19	uterus - wet (mg)	33.2 ± 5.56^	31.3 ± 5.43	33.5 ± 2.43	30.3 ± 4.50	42.8 ± 9.77	57.0 ± 17.52
	uterus - blotted (mg)	28.7 ± 5.47^	27.0 ± 3.35	23.5 ± 3.56	24.7 ± 4.08	35.2 ± 7.76	43.8 ± 13.82
	body wt. (gm)	48.3 ± 3.65^	49.1 ± 2.49	48.9 ± 2.50	47.3 ± 5.28	48.1 ± 3.89	47.0 ± 2.59
20	uterus - wet (mg)	26.8 ± 6.97^	--	32.4 ± 5.92	33.3 ± 14.40	49.1 ± 8.42	--
	uterus - blotted (mg)	22.4 ± 6.47^	--	27.4 ± 5.34	27.8 ± 15.41	42.5 ± 5.94	--
	body wt. (gm)	40.4 ± 3.38^	--	43.6 ± 2.47	37.1 ± 7.50	40.2 ± 2.46	--

Table 5c. Weights of wet and blotted uteri, and body weights, in animals administered nonylphenol in Protocol C; dose-response study

Lab.	Weighing	0	5 mg/kg/d	15 mg/kg/d	35 mg/kg/d	80 mg/kg/d	100 mg/kg/d
1	uterus - wet (mg)	86.1 ± 9.20	84.7 ± 10.17	84.8 ± 8.03	99.5 ± 12.82	101.1 ± 7.13	122.2 ± 15.96
	uterus - blotted (mg)	82.2 ± 8.89	80.0 ± 9.91	81.2 ± 8.20	94.7 ± 12.02	96.5 ± 7.50	117.0 ± 14.52
	body wt. (gm)	286.7 ± 21.81	291.2 ± 17.60	289.3 ± 18.64	289.3 ± 17.75	290.8 ± 13.88	287.8 ± 15.55
5	uterus - wet (mg)	87.2 ± 11.74^	86.3 ± 5.17	89.0 ± 14.35	94.5 ± 20.04	106.9 ± 14.18	140.0 ± 22.49
	uterus - blotted (mg)	86.3 ± 11.88^	85.6 ± 4.91	87.5 ± 14.47	93.4 ± 19.85	105.6 ± 13.74	137.7 ± 21.83
	body wt. (gm)	256.1 ± 8.87^	255.3 ± 8.93	260.1 ± 9.96	254.0 ± 11.51	256.1 ± 11.48	254.4 ± 12.33
6	uterus - wet (mg)	115.5 ± 19.84^	--	123.6 ± 18.60	130.8 ± 8.90	136.7 ± 33.99	--
	uterus - blotted (mg)	110.7 ± 19.60^	--	112.5 ± 16.77	123.8 ± 12.21	131.2 ± 33.16	--
	body wt. (gm)	299.6 ± 29.76^	--	300.3 ± 17.75	297.2 ± 14.12	302.6 ± 22.87	--
8	uterus - wet (mg)	75.7 ± 6.68	92.0 ± 12.94	84.4 ± 11.52	96.5 ± 9.82	124.5 ± 20.06	115.5 ± 22.27
	uterus - blotted (mg)	73.9 ± 6.58	89.9 ± 12.43	83.0 ± 11.56	94.2 ± 9.67	122.0 ± 18.56	113.5 ± 21.29
	body wt. (gm)	251.5 ± 10.11	252.4 ± 10.26	251.0 ± 15.54	253.0 ± 11.48	252.9 ± 8.20	249.9 ± 10.75
20	uterus - wet (mg)	106.0 ± 18.84^	--	117.6 ± 18.15	118.1 ± 18.76	146.7 ± 26.86	--
	uterus - blotted (mg)	98.6 ± 22.04^	--	105.3 ± 17.70	105.1 ± 15.22	129.6 ± 20.50	--
	body wt. (gm)	297.2 ± 14.54^	--	301.4 ± 15.13	299.1 ± 16.72	297.1 ± 16.15	--

Table 5d. Weights of wet and blotted uteri, and body weights, in animals administered nonylphenol in Protocol C'; dose-response study

Lab.	Weighing	0	5 mg/kg/d	15 mg/kg/d	35 mg/kg/d	80 mg/kg/d	100 mg/kg/d
5	uterus - wet (mg)	76.8 ± 4.59^	80.4 ± 9.84	88.0 ± 10.30	106.3 ± 16.24	140.2 ± 14.09	160.6 ± 18.76
	uterus - blotted (mg)	75.9 ± 4.97^	79.5 ± 10.14	87.3 ± 10.09	105.4 ± 16.29	139.0 ± 13.58	158.0 ± 17.42
	body wt. (gm)	282.1 ± 12.40^	282.3 ± 14.46	278.9 ± 13.94	274.9 ± 13.89	271.6 ± 19.50	271.2 ± 12.52
8	uterus - wet (mg)	81.5 ± 12.75	84.9 ± 9.93	88.1 ± 4.53	106.8 ± 11.92	171.8 ± 17.90	158.9 ± 29.05
	uterus - blotted (mg)	83.6 ± 13.41	83.0 ± 9.98	85.7 ± 4.94	104.7 ± 12.12	166.9 ± 17.97	154.4 ± 29.48
	body wt. (gm)	272.8 ± 14.89	273.1 ± 15.08	268.9 ± 13.94	274.9 ± 13.89	271.6 ± 19.50	271.2 ± 12.52

Table 5e. Weights of wet and blotted uteri, and body weights, in animals administered nonylphenol in Protocol D; dose-response study

Lab.	Weighing	0	15 mg/kg/d	75 mg/kg/d	125 mg/kg/d	250 mg/kg/d	350 mg/kg/d
20	uterus - wet (mg)	101.1 ± 16.93^	--	163.5 ± 24.57	179.0 ± 30.90	179.2 ± 15.50	--
	uterus - blotted (mg)	95.0 ± 16.43^	--	153.8 ± 23.74	168.7 ± 28.77	168.9 ± 16.16	--
	body wt. (gm)	295.5 ± 11.09^	--	284.5 ± 10.86	283.7 ± 13.93	280.4 ± 11.60	--

* body weights were from day 3, rather than at time of sacrifice.
^ shared controls

Table 6. Weights of wet and blotted uteri, and body weights, from animals administered the vehicle control; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	22.0 ± 3.06	27.5 ± 4.75	82.0 ± 8.25		
	terus -blotted (mg)	20.0 ± 2.65	25.3 ± 4.77	77.8 ± 7.41		
	ody wt. (gm)	44.7 ± 2.54	54.1 ± 3.45	283.5 ± 9.40		
2	terus - wet (mg)	21.5 ± 8.43[2]	18.3 ± 3.61			
	terus -blotted (mg)	19.0 ± 5.66[2]	16.3 ± 3.78			
	ody wt. (gm)	46.2 ± 8.80[2]	49.2 ± 4.30			
3	terus - wet (mg)	31.3 ± 5.79	36.0 ± 6.57			
	terus -blotted (mg)	29.8 ± 5.49	31.2 ± 4.92			
	ody wt. (gm)	40.8 ± 4.67	41.2 ± 4.42			
4	terus - wet (mg)	33.2 ± 8.08	34.2 ± 3.97			
	terus -blotted (mg)	30.0 ± 6.66	29.5 ± 3.21			
	ody wt. (gm)	34.5 ± 4.85	36.0 ± 2.97			
7	terus - wet (mg)	43.1 ± 7.22	39.0 ± 4.18			
	terus -blotted (mg)	40.0 ± 7.05	32.8 ± 4.11			
	ody wt. (gm)	58.0 ± 4.25	59.5 ± 4.21			
8	terus - wet (mg)		32.0 ± 7.47			
	terus -blotted (mg)		28.3 ± 7.11			
	ody wt. (gm)		42.8 ± 4.91			
9	terus - wet (mg)		25.6 ± 6.79			
	terus -blotted (mg)		21.5 ± 6.22			
	ody wt. (gm)		40.7 ± 6.01			
10	terus - wet (mg)		45.7 ± 21.61	98.4 ± 14.16		
	terus -blotted (mg)		35.8 ± 18.02	91.8 ± 12.47		
	ody wt. (gm)		55.7 ± 6.57	281.1 ± 18.17		
11	terus - wet (mg)		67.2 ± 7.69			
	terus -blotted (mg)		53.6 ± 4.26			
	ody wt. (gm)		56.3 ± 2.80 [a]			
12	terus - wet (mg)	29.9 ± 3.30	32.4 ± 4.38	87.7 ± 5.13	81.6 ± 9.65	
	terus -blotted (mg)	27.3 ± 3.44	26.6 ± 3.75	83.0 ± 4.91	75.7 ± 10.09	
	ody wt. (gm)	41.6 ± 3.79	42.0 ± 3.21	218.1 ± 7.03	234.0 ± 13.77	
13	terus - wet (mg)		32.3 ± 14.47			
	terus -blotted (mg)		28.3 ± 7.11			
	ody wt. (gm)		42.8 ± 4.92			
14	terus - wet (mg)	34.3 ± 2.05	40.5 ± 11.21	112.6 ± 5.02	93.6 ± 7.80	
	terus -blotted (mg)	32.8 ± 1.93	38.6 ± 10.87	103.5 ± 5.50	89.2 ± 6.56	
	ody wt. (gm)	62.4 ± 4.95	59.5 ± 3.18	258.9 ± 25.70	293.8 ± 20.46	
15	terus - wet (mg)	36.7 ± 5.62	34.7 ± 5.23	95.6 ± 7.41	92.6 ± 10.04	
	terus -blotted (mg)	35.2 ± 5.74	33.2 ± 5.06	91.5 ± 7.63	88.5 ± 9.40	
	ody wt. (gm)	66.0 ± 3.83	62.9 ± 1.87	267.4 ± 11.90	281.7 ± 14.59	
16	terus - wet (mg)		29.5 ± 2.19			
	terus -blotted (mg)		27.2 ± 2.22			
	ody wt. (gm)		55.4 ± 3.00			
17	terus - wet (mg)		57.4 ± 7.17			
	terus -blotted (mg)		54.4 ± 7.73			
	ody wt. (gm)		52.5 ± 5.76			

Table 6. Weights of wet and blotted uteri, and body weights, from animals administered the vehicle control; single-dose study, Continued

18	terus - wet (mg)	30.3 ± 2.76	33.7 ± 6.28	88.0 ± 10.14	89.4 ± 13.20	
	terus -blotted (mg)	29.2 ± 2.73	32.1 ± 6.43	85.6 ± 9.76	86.9 ± 13.27	
	ody wt. (gm)	52.0 ± 3.62	54.8 ± 2.52	242.3 ± 14.13	276.6 ± 25.82	
20	terus - wet (mg)	25.1 ± 7.25	36.3 ± 6.59	112.5 ± 18.02		102.7 ± 24.26
	terus -blotted (mg)	21.0 ± 8.75	31.6 ± 6.11	104.1 ± 17.88		96.5 ± 24.27
	ody wt. (gm)	36.5 ± 3.62	44.5 ± 2.85	295.5 ± 11.42		292.6 ± 12.03

Table 7a. Weights of wet and blotted uteri, and body weights, from animals administered bisphenol A; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	41.7 ± 8.19	50.4 ± 11.91	275.1 ± 64.40		
	terus -blotted (mg)	38.9 ± 7.99	47.3 ± 10.81	223.6 ± 30.14		
	ody wt. (gm)	45.8 ± 7.43	51.4 ± 2.77	273.7 ± 18.42		
2	terus - wet (mg)	32.0 ± 10.71[2]	48.8 ± 5.71			
	terus -blotted (mg)	27.8 ± 5.91[2]	42.7 ± 2.88			
	ody wt. (gm)	45.3 ± 9.52[2]	46.1 ± 4.57			
3	terus - wet (mg)	42.5 ± 4.37	57.2 ± 9.26			
	terus -blotted (mg)	40.7 ± 4.37	53.2 ± 8.38			
	ody wt. (gm)	42.9 ± 3.14	39.8 ± 4.29			
4	terus - wet (mg)	35.6 ± 4.10[1]	50.7 ± 13.49			
	terus -blotted (mg)	32.6 ± 5.50[1]	46.3 ± 12.50			
	ody wt. (gm)	27.8 ± 2.17[1]	36.5 ± 2.81			
7	terus - wet (mg)	48.8 ± 10.67	68.6 ± 5.87			
	terus -blotted (mg)	46.4 ± 10.36	65.5 ± 5.28			
	ody wt. (gm)	54.0 ± 10.05	57.6 ± 4.64			
8	terus - wet (mg)		71.0 ± 22.24			
	terus -blotted (mg)		62.6 ± 16.42			
	ody wt. (gm)		39.8 ± 5.46			
9	terus - wet (mg)		78.4 ± 90.12			
	terus -blotted (mg)		45.7 ± 23.85			
	ody wt. (gm)		38.3 ± 5.12			
10	terus - wet (mg)			310.7 ± 94.13		
	terus -blotted (mg)			214.3 ± 25.56		
	ody wt. (gm)			269.3 ± 14.30		
11	terus - wet (mg)		112.0±19.64			
	terus -blotted (mg)		89.4 ±12.51			
	ody wt. (gm)		55.2 ± 3.31[a]			
12	terus - wet (mg)	41.7 ± 4.39	49.7 ± 9.71	535.3 ± 186.07	321.1 ± 73.54	
	terus -blotted (mg)	38.1 ± 3.96	45.4 ± 9.19	272.8 ± 31.09	278.5 ± 39.93	
	ody wt. (gm)	40.7 ± 3.88	36.7 ± 4.10	206.1 ± 10.03	206.7 ± 9.43	
13	terus - wet (mg)		71.1 ± 22.24			
	terus -blotted (mg)		62.6 ± 16.42			
	ody wt. (gm)		39.8 ± 5.46			

Table 7a. Weights of wet and blotted uteri, and body weights, from animals administered bisphenol A; single-dose study, Continued

14	terus - wet (mg)	38.3 ± 5.12	61.5 ± 6.17	499.4 ± 194.48	397.7 ± 63.79	
	terus -blotted (mg)	36.7 ± 4.61	59.4 ± 6.30	270.3 ± 35.71	327.4 ± 38.45	
	ody wt. (gm)	62.6 ± 3.80	59.6 ± 4.42	254.6 ± 14.91	257.7 ± 12.58	
15	terus - wet (mg)	49.9 ± 6.93	67.1 ± 10.48	435.3 ± 133.18	303.6 ± 78.67	
	terus -blotted (mg)	48.2 ± 6.66	64.9 ± 10.38	264.7 ± 30.93	276.8 ± 56.52	
	ody wt. (gm)	64.0 ± 3.56	61.6 ± 4.41	257.8 ± 10.07	254.6 ± 7.80	
16	terus - wet (mg)		68.4 ± 11.79			
	terus -blotted (mg)		62.0 ± 10.17			
	ody wt. (gm)		52.4 ± 4.19			
17	terus - wet (mg)		64.4 ± 11.88			
	terus -blotted (mg)		60.0 ± 11.48			
	ody wt. (gm)		49.8 ± 4.03			
18	terus - wet (mg)	44.1 ± 7.31	57.5 ± 13.07	307.5 ± 58.15	394.8 ± 83.81	
	terus -blotted (mg)	42.5 ± 7.10	55.6 ± 12.65	223.3 ± 28.92	320.2 ± 22.44	
	ody wt. (gm)	51.7 ± 3.23	51.7 ± 2.40	231.5 ± 9.17	248.2 ± 12.13	
20	terus - wet (mg)	27.8 ± 16.06[3]	54.9 ± 10.57	470.7 ± 262.33		141.2 ± 46.49
	terus -blotted (mg)	24.1 ± 17.99[3]	50.4 ± 10.25	240.6 ± 56.38		132.6 ± 44.07
	ody wt. (gm)	30.4 ± 3.39	44.2 ± 4.48	282.1 ± 9.52		280.2 ± 6.45

Table 7b. Weights of wet and blotted uteri, and body weights, from animals administered *o,p'*-DDT; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	95.7 ± 28.21	28.4 ± 3.52	95.1 ± 7.85		
	terus -blotted (mg)	74.8 ± 8.12	26.2 ± 3.49	90.6 ± 8.55		
	ody wt. (gm)	39.5 ± 5.86	52.5 ± 3.29	279.2 ± 17.50		
2	terus - wet (mg)	178.5 ± 17.00[2]	19.5 ± 3.78			
	terus -blotted (mg)	70.3 ± 3.40[2]	18.2 ± 3.31			
	ody wt. (gm)	39.5 ± 3.13[2]	46.9 ± 3.34			
3	terus - wet (mg)	184.5 ± 68.73	50.3 ± 5.61			
	terus -blotted (mg)	107.8 ± 12.27	48.7 ± 6.19			
	ody wt. (gm)	37.1 ± 5.61	41.9 ± 4.24			
4	terus - wet (mg)	232.8 ± 52.09	64.8 ± 28.29			
	terus -blotted (mg)	103.7 ± 14.40	55.8 ± 21.54			
	ody wt. (gm)	26.3 ± 5.89	35.3 ± 3.44			
7	terus - wet (mg)	107.9 ± 19.26	34.6 ± 4.03			
	terus -blotted (mg)	100.1 ± 13.23	31.2 ± 3.78			
	ody wt. (gm)	48.8 ± 3.23	58.6 ± 3.30			
8	terus - wet (mg)		43.7 ± 7.21			
	terus -blotted (mg)		39.7 ± 7.45			
	ody wt. (gm)		41.8 ± 5.06			
9	terus - wet (mg)		31.5 ± 5.94			
	terus -blotted (mg)		26.3 ± 5.53			
	ody wt. (gm)		38.6 ± 4.29			
10	terus - wet (mg)		45.3 ± 10.85	99.6 ± 9.22		
	terus -blotted (mg)		37.0 ± 10.49	93.7 ± 8.56		
	ody wt. (gm)		57.4 ± 5.05	270.3 ± 11.67		

Table 7b. Weights of wet and blotted uteri, and body weights, from animals administered *o,p'*-DDT; single-dose study, Continued

11	terus - wet (mg)		107.2 ±11.53			
	terus -blotted (mg)		79.9 ±5.54			
	ody wt. (gm)		55.5 ± 4.23 [a]			
12	terus - wet (mg)	136.5 ± 60.46	29.9 ± 4.42	110.8 ± 13.75	106.2 ± 20.52	
	terus -blotted (mg)	94.8 ± 13.57	26.0 ± 4.46	102.8 ± 13.47	98.6 ± 20.14	
	ody wt. (gm)	37.9 ± 2.85	37.7 ± 2.78	215.2 ± 6.93	228.7 ± 16.69	
13	terus - wet (mg)		43.7 ± 7.21			
	terus -blotted (mg)		39.7 ± 7.45			
	ody wt. (gm)		41.8 ± 5.06			
14	terus - wet (mg)	118.1 ± 77.02	80.7 ± 14.90	185.5 ± 17.48	97.3 ± 18.81	
	terus -blotted (mg)	88.7 ± 17.09	77.9 ± 14.25	171.8 ± 13.96	94.5 ± 18.34	
	ody wt. (gm)	56.9 ± 2.89	59.6 ± 3.62	262.9 ± 16.37	289.4 ± 21.87	
15	terus - wet (mg)	110.3 ± 32.79	41.7 ± 7.09	124.7 ± 31.32	105.2 ± 18.55	
	terus -blotted (mg)	99.2 ± 14.89	40.1 ± 6.74	120.4 ± 31.06	101.2 ± 17.76	
	ody wt. (gm)	58.3 ± 3.69	64.0 ± 3.15	269.1 ± 11.45	276.3 ± 12.16	
16	terus - wet (mg)		29.5 ± 1.93			
	terus -blotted (mg)		26.2 ± 1.33			
	ody wt. (gm)		53.3 ± 2.39			
17	terus - wet (mg)		47.5 ± 8.07			
	terus -blotted (mg)		43.3 ± 6.83			
	ody wt. (gm)		53.6 ± 2.38			
15	terus - wet (mg)	136.1 ± 18.33	36.6 ± 3.83	126.5 ± 20.43	107.4 ± 11.10	
	terus -blotted (mg)	101.8 ± 5.47	35.2 ± 3.57	122.6 ± 20.27	104.0 ± 10.47	
	ody wt. (gm)	46.8 ± 4.69	52.9 ± 2.17	241.3 ± 11.51	273.0 ± 14.96	
20	terus - wet (mg)	xx[6]	51.7 ± 12.07	109.1 ± 15.58		783.9 ± 193.53
	terus -blotted (mg)	xx[6]	47.3 ± 10.59	100.1 ± 17.15		290.6 ± 57.07
	ody wt. (gm)	xx[6]	44.0 ± 3.40	291.6 ± 11.48		270.6 ± 7.32

Table 7c. Weights of wet and blotted uteri, and body weights, from animals administered dibutyl phthalate; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	21.3 ± 2.30	26.3 ± 2.70	109.0 ± 57.94		
	terus -blotted (mg)	19.4 ± 1.91	24.8 ± 2.77	103.6 ± 54.48		
	ody wt. (gm)	43.6 ± 5.03	53.2 ± 3.21	280.8 ± 10.91		
2	terus - wet (mg)	21.2 ± 9.28	23.7 ± 4.46			
	terus -blotted (mg)	17.3 ± 8.19	21.5 ± 2.35			
	ody wt. (gm)	44.4 ± 7.57	47.4 ± 2.02			
3	terus - wet (mg)	32.3 ± 6.74	27.5 ± 2.59			
	terus -blotted (mg)	30.0 ± 6.03	26.2 ± 2.23			
	ody wt. (gm)	42.5 ± 2.94	42.8 ± 4.83			
4	terus - wet (mg)	24.7 ± 2.66	34.7 ± 3.39			
	terus -blotted (mg)	21.7 ± 2.07	29.2 ± 3.31			
	ody wt. (gm)	26.7 ± 4.63	36.8 ± 3.76			
7	terus - wet (mg)	44.5 ± 9.71	37.8 ± 2.47			
	terus -blotted (mg)	40.8 ± 8.48	34.7 ± 2.36			
	ody wt. (gm)	57.1 ± 3.87	58.7 ± 7.05			

Table 7c. Weights of wet and blotted uteri, and body weights, from animals administered dibutyl phthalate; single-dose study, Continued

8	terus - wet (mg)		27.5 ± 3.27			
	terus -blotted (mg)		24.0 ± 3.76			
	ody wt. (gm)		41.8 ± 4.53			
9	terus - wet (mg)		23.1 ± 3.86			
	terus -blotted (mg)		18.5 ± 4.03			
	ody wt. (gm)		38.8 ± 4.44			
10	terus - wet (mg)		35.3 ± 9.33	80.9 ± 8.30		
	terus -blotted (mg)		27.4 ± 7.27	75.6 ± 7.84		
	ody wt. (gm)		59.4 ± 6.01	276.6 ± 10.06		
11	terus - wet (mg)		90.4 ± 5.14			
	terus -blotted (mg)		66.2 ± 8.50			
	ody wt. (gm)		53.7 ± 3.27 [a]			
12	terus - wet (mg)	29.1 ± 6.39	40.7 ± 3.28	90.7 ± 13.49	83.0 ± 14.47	
	terus -blotted (mg)	26.0 ± 5.96	35.7 ± 3.03	82.3 ± 12.69	75.1 ± 13.41	
	ody wt. (gm)	39.8 ± 3.01	40.0 ± 2.19	217.1 ± 10.85	229.9 ± 13.19	
13	terus - wet (mg)		27.5 ± 3.27			
	terus -blotted (mg)		24.0 ± 3.76			
	ody wt. (gm)		41.8 ± 4.53			
14	terus - wet (mg)	31.9 ± 5.80	39.0 ± 10.91	159.1 ± 23.81	85.4 ± 10.46	
	terus -blotted (mg)	30.4 ± 5.75	37.6 ± 9.85	143.9 ± 18.81	81.8 ± 9.65	
	ody wt. (gm)	63.6 ± 2.78	60.1 ± 3.84	264.7 ± 16.70	290.7 ± 18.49	
15	terus - wet (mg)	35.8 ± 5.06	35.2 ± 5.31	85.5 ± 5.54	92.8 ± 5.90	
	terus -blotted (mg)	34.5 ± 4.75	33.8 ± 5.23	81.9 ± 4.87	88.9 ± 5.31	
	ody wt. (gm)	64.5 ± 1.67	63.4 ± 4.58	270.6 ± 14.37	280.1 ± 12.16	
16	terus - wet (mg)		23.2 ± 3.19			
	terus -blotted (mg)		20.3 ± 3.35			
	ody wt. (gm)		55.6 ± 2.42			
17	terus - wet (mg)		41.6 ± 6.40			
	terus -blotted (mg)		39.0 ± 7.20			
	ody wt. (gm)		48.0 ± 4.49			
18	terus - wet (mg)	29.0 ± 4.20	34.6 ± 4.23	87.5 ± 12.63	93.5 ± 10.91	
	terus -blotted (mg)	27.9 ± 4.48	33.0 ± 3.57	84.6 ± 11.76	90.0 ± 10.74	
	ody wt. (gm)	47.8 ± 7.50	54.3 ± 3.80	238.6 ± 13.11	279.9 ± 16.22	
20	terus - wet (mg)	18.9 ± 4.29[3]	36.1 ± 7.54	103.2 ± 16.19		104.3 ± 11.83
	terus -blotted (mg)	15.4 ± 1.54[3]	32.2 ± 7.71	96.3 ± 17.09		92.9 ± 12.52
	ody wt. (gm)	33.0 ± 9.14[3]	46.0 ± 3.17	291.7 ± 12.12		289.1 ± 7.06

Table 7d. Weights of wet and blotted uteri, and body weights, from animals administered genistein; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	66.5 ± 7.63	66.6 ± 4.05	158.8 ± 20.15		
	terus -blotted (mg)	62.8 ± 6.89	63.2 ± 4.61	151.7 ± 19.29		
	ody wt. (gm)	41.6 ± 5.29	53.8 ± 2.41	281.0 ± 22.15		
2	terus - wet (mg)	63.2 ± 8.32[1]	59.3 ± 8.29			
	terus -blotted (mg)	58.2 ± 6.06[1]	52.7 ± 8.52			
	ody wt. (gm)	49.4 ± 5.48[1]	46.2 ± 6.37			
3	terus - wet (mg)	79.7 ± 6.53	72.7 ± 9.05			
	terus -blotted (mg)	76.0 ± 6.13	70.5 ± 9.42			
	ody wt. (gm)	41.0 ± 6.62	4.06 ± 4.01			
4	terus - wet (mg)	86.0 ± 17.06	71.0 ± 10.26			
	terus -blotted (mg)	75.8 ± 13.56	66.2 ± 10.15			
	ody wt. (gm)	32.7 ± 2.50	35.5 ± 3.27			
7	terus - wet (mg)	67.2 ± 2.79	80.2 ± 9.04			
	terus -blotted (mg)	61.6 ± 3.73	75.7 ± 8.19			
	ody wt. (gm)	56.1 ± 3.31	63.9 ± 3.36			
9	terus - wet (mg)		79.0 ± 12.25			
	terus -blotted (mg)		66.9 ± 8.07			
	ody wt. (gm)		40.7 ± 2.55			
11	terus - wet (mg)		105.8 ± 21.76			
	terus -blotted (mg)		95.4 ± 20.07			
	ody wt. (gm)		53.8 ± 2.40 [a]			
12	terus - wet (mg)	84.8 ± 6.61	65.7 ± 6.86	149.5 ± 5.37	194.9 ± 26.66	
	terus -blotted (mg)	77.8 ± 5.91	58.9 ± 5.83	142.9 ± 5.43	182.5 ± 23.85	
	ody wt. (gm)	41.6 ± 3.15	37.4 ± 3.36	216.1 ± 9.40	228.4 ± 12.52	
14	terus - wet (mg)	81.0 ± 5.42	82.2 ± 6.66	189.9 ± 10.64	199.4 ± 11.75	
	terus -blotted (mg)	78.5 ± 5.21	79.9 ± 6.25	175.3 ± 10.67	188.7 ± 19.43	
	ody wt. (gm)	62.6 ± 3.81	58.6 ± 5.09	268.9 ± 14.45	284.6 ± 17.91	
15	terus - wet (mg)	97.9 ± 10.31	92.0 ± 11.88	157.8 ± 22.53	167.7 ± 24.48	
	terus -blotted (mg)	95.5 ± 10.14	89.8 ± 11.82	153.0 ± 20.98	162.8 ± 23.10	
	ody wt. (gm)	66.2 ± 2.09	63.3 ± 3.52	269.2 ± 14.14	276.2 ± 6.76	
16	terus - wet (mg)		73.4 ± 13.43			
	terus -blotted (mg)		68.8 ± 13.57			
	ody wt. (gm)		53.9 ± 2.09			
17	terus - wet (mg)		78.9 ± 11.95			
	terus -blotted (mg)		72.8 ± 10.92			
	ody wt. (gm)		53.2 ± 2.25			
18	terus - wet (mg)	78.1 ± 20.14[1]	95.4 ± 12.26	181.4 ± 5.48	205.0 ± 18.60	
	terus -blotted (mg)	74.8 ± 19.46[1]	92.8 ± 11.82	176.0 ± 5.85	200.4 ± 18.52	
	ody wt. (gm)	52.4 ± 3.24[1]	53.9 ± 3.50	236.8 ± 14.70	275.6 ± 27.69	
20	terus - wet (mg)	75.8 ± 9.94	75.5 ± 12.66	184.6 ± 39.17		272.1 ± 56.01
	terus -blotted (mg)	67.3 ± 10.63	70.2 ± 12.08	163.7 ± 36.09		211.5 ± 33.50
	ody wt. (gm)	34.4 ± 6.05	45.0 ± 3.53	276.7 ± 9.19		278.1 ± 7.44

Table 7e. Weights of wet and blotted uteri, and body weights, from animals administered methoxychlor; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	77.9 ± 7.00	77.9 ± 16.10	177.7 ± 30.83		
	terus -blotted (mg)	73.5 ± 5.60	71.7 ± 14.30	159.5 ± 20.58		
	ody wt. (gm)	43.8 ± 6.19	52.0 ± 3.17	268.3 ± 15.62		
2	terus - wet (mg)	57.8 ± 10.23	74.2 ± 9.30			
	terus -blotted (mg)	44.0 ± 9.93	63.8 ± 8.61			
	ody wt. (gm)	44.1 ± 0.66	47.8 ± 5.44			
3	terus - wet (mg)	94.3 ± 18.66	112.8 ± 13.88			
	terus -blotted (mg)	88.7 ± 15.74	103.2 ± 7.36			
	ody wt. (gm)	39.7 ± 4.81	40.8 ± 2.90			
4	terus - wet (mg)	124.8 ± 30.78	112.2 ± 24.97			
	terus -blotted (mg)	97.3 ± 13.94	87.7 ± 13.71			
	ody wt. (gm)	34.2 ± 4.75	37.7 ± 4.68			
7	terus - wet (mg)	121.6 ± 10.47	126.6 ± 17.22			
	terus -blotted (mg)	113.3 ± 9.55	117.6 ± 15.03			
	ody wt. (gm)	53.1 ± 3.36	57.8 ± 4.25			
8	terus - wet (mg)		114.4 ± 47.85			
	terus -blotted (mg)		91.5 ± 24.29			
	ody wt. (gm)		42.6 ± 3.28			
9	terus - wet (mg)		145.2 ± 25.24			
	terus -blotted (mg)		88.9 ± 13.60			
	ody wt. (gm)		40.0 ± 1.98			
11	terus - wet (mg)		129.2 ± 16.11			
	terus -blotted (mg)		105.0 ± 11.64			
	ody wt. (gm)		49.3 ± 1.03 [a]			
12	terus - wet (mg)	109.5 ± 17.99	75.3 ± 32.80	517.4 ± 66.64	245.8 ± 36.57	
	terus -blotted (mg)	90.1 ± 9.80	61.9 ± 18.10	250.3 ± 14.41	233.0 ± 37.15	
	ody wt. (gm)	36.3 ± 2.12	38.2 ± 2.59	204.8 ± 8.75	210.0 ± 17.46	
13	terus - wet (mg)		114.4 ± 47.85			
	terus -blotted (mg)		91.5 ± 24.29			
	ody wt. (gm)		42.6 ± 3.28			
14	terus - wet (mg)	100.4 ± 11.07	113.7 ± 11.64	261.7 ± 52.81	210.3 ± 29.00	
	terus -blotted (mg)	97.2 ± 10.94	101.7 ± 8.11	202.6 ± 23.52	199.7 ± 26.68	
	ody wt. (gm)	58.5 ± 3.40	59.2 ± 2.46	253.5 ± 9.82	280.4 ± 38.58	
15	terus - wet (mg)	95.7 ± 7.76	92.0 ± 15.21	232.7 ± 60.15	231.8 ± 28.40	
	terus -blotted (mg)	93.6 ± 7.70	88.9 ± 14.81	194.0 ± 28.42	222.3 ± 29.45	
	ody wt. (gm)	62.9 ± 3.33	63.2 ± 3.18	259.8 ± 12.23	250.4 ± 10.42	
16	terus - wet (mg)		108.1 ± 18.82			
	terus -blotted (mg)		87.4 ± 13.21			
	ody wt. (gm)		55.9 ± 3.24			
17	terus - wet (mg)		113.5 ± 18.20			
	terus -blotted (mg)		95.8 ± 8.63			
	ody wt. (gm)		52.7 ± 3.11			
18	terus - wet (mg)	93.3 ± 11.11	98.5 ± 7.59	222.7 ± 109.60	236.5 ± 27.96	
	terus -blotted (mg)	89.2 ± 9.60	93.0 ± 7.44	185.8 ± 61.61	220.9 ± 28.38	
	ody wt. (gm)	49.0 ± 3.19	52.0 ± 3.55	229.5 ± 9.20	243.5 ± 17.02	

Table 7e. Weights of wet and blotted uteri, and body weights, from animals administered methoxychlor; single-dose study, Continued

20	terus - wet (mg)	74.1 ± 22.48[3]	112.0 ± 13.17	164.9 ± 27.28		454.1 ± 88.06
	terus -blotted (mg)	58.4 ± 12.54[3]	96.7 ± 12.05	155.4 ± 28.55		229.4 ± 38.31
	ody wt. (gm)	34.7 ± 5.44[3]	43.9 ± 2.87	294.5 ± 13.77		278.8 ± 11.62

Table 7f. Weights of wet and blotted uteri, and body weights, from animals administered nonylphenol; single-dose study

				Protocol		
Lab.	**Weighing**	**A**	**B**	**C**	**C'**	**D**
1	terus - wet (mg)	57.6 ± 4.70	34.1 ± 3.35	132.0 ± 24.18		
	terus -blotted (mg)	54.6 ± 3.52	32.4 ± 3.38	125.6 ± 22.83		
	ody wt. (gm)	36.3 ± 5.88	52.3 ± 3.89	287.0 ± 13.43		
2	terus - wet (mg)	41.5 ± 0.71[4]	35.2 ± 22.51			
	terus -blotted (mg)	36.0 ± 2.83[4]	29.2 ± 12.19			
	ody wt. (gm)	43.3 ± 5.87[4]	49.3 ± 3.96			
3	terus - wet (mg)	61.0 ± 7.07[4]	47.5 ± 8.53			
	terus -blotted (mg)	49.5 ± 0.71[4]	45.2 ± 8.33			
	ody wt. (gm)	26.0 ± 1.27[4]	41.8 ± 4.40			
4	terus - wet (mg)	66.6 ± 8.02[1]	35.0 ± 3.90			
	terus -blotted (mg)	59.0 ± 4.74[1]	32.0 ± 1.67			
	ody wt. (gm)	28.8 ± 7.09[1]	36.5 ± 4.04			
7	terus - wet (mg)	63.2 ± 18.12[2]	56.9 ± 8.25			
	terus -blotted (mg)	60.1 ± 17.66[2]	53.7 ± 8.31			
	ody wt. (gm)	47.3 ± 6.30[2]	58.3 ± 4.67			
8	terus - wet (mg)		73.7 ± 17.09			
	terus -blotted (mg)		68.0 ± 16.72			
	ody wt. (gm)		41.2 ± 4.44			
9	terus - wet (mg)		31.4 ± 2.95			
	terus -blotted (mg)		26.0 ± 2.73			
	ody wt. (gm)		38.3 ± 5.21			
10	terus - wet (mg)		54.5 ± 13.80	132.8 ± 16.84		
	terus -blotted (mg)		45.4 ± 13.48	125.6 ± 16.97		
	ody wt. (gm)		59.8 ± 3.63	273.7 ± 21.37		
11	terus - wet (mg)		71.8 ± 7.09			
	terus -blotted (mg)		55.6 ± 6.18			
	ody wt. (gm)		55.2 ± 3.06 [a]			
12	terus - wet (mg)	68.9 ± 26.69[2]	57.0 ± 9.78	124.8 ± 20.78	157.1 ± 28.60	
	terus -blotted (mg)	57.7 ± 14.01[2]	51.6 ± 9.30	114.2 ± 19.57	147.2 ± 27.27	
	ody wt. (gm)	28.7 ± 6.17[2]	37.5 ± 3.67	214.7 ± 9.57	220.3 ± 9.96	
13	terus - wet (mg)		73.7 ± 17.09			
	terus -blotted (mg)		68.0 ± 16.72			
	ody wt. (gm)		41.2 ± 4.44			
14	terus - wet (mg)	57.7 ± 6.48	65.0 ± 6.18	161.3 ± 20.23	149.2 ± 29.56	
	terus -blotted (mg)	55.9 ± 6.07	62.6 ± 5.83	149.3 ± 15.91	139.5 ± 27.00	
	ody wt. (gm)	57.9 ± 4.17	60.4 ± 3.75	260.3 ± 13.89	287.4 ± 16.19	

Table 7f. Weights of wet and blotted uteri, and body weights, from animals administered nonylphenol; single-dose study,Continued

15	terus - wet (mg)	58.9 ± 11.10	60.9 ± 9.64	130.6 ± 18.86	154.9 ± 15.35	
	terus -blotted (mg)	57.4 ± 10.66	58.8 ± 9.32	125.9 ± 18.06	149.0 ± 15.16	
	ody wt. (gm)	55.8 ± 13.64	61.8 ± 4.20	266.8 ± 12.13	268.4 ± 10.46	
16	terus - wet (mg)		53.3 ± 6.10			
	terus -blotted (mg)		45.8 ± 3.84			
	ody wt. (gm)		52.4 ± 2.82			
17	terus - wet (mg)		65.6 ± 11.02			
	terus -blotted (mg)		58.8 ± 11.55			
	ody wt. (gm)		52.8 ± 3.13			
18	terus - wet (mg)	52.1 ± 6.26[1]	43.4 ± 7.01	110.7 ± 16.61	167.7 ± 25.94	
	terus -blotted (mg)	50.5 ± 5.85[1]	41.8 ± 6.54	106.8 ± 15.51	159.7 ± 24.17	
	ody wt. (gm)	35.5 ± 9.68[1]	52.6 ± 2.22	242.2 ± 14.10	270.7 ± 12.26	
20	terus - wet (mg)	45.4 ± 1.13[4]	57.6 ± 13.42	156.0 ± 28.94		170.2 ± 21.98
	terus -blotted (mg)	38.5 ± 2.69[4]	52.8 ± 12.44	143.3 ± 26.56		157.7 ± 25.55
	ody wt. (gm)	36.7 ± 1.98[4]	42.1 ± 3.96	285.1 ± 8.76		272.6 ± 20.39

[a] body weights at day 3

Table 8a. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol A

		dose-response		single dose	
Lab.	**Weighing**	**1.0 µg/kg/d**	**3.0 µg/kg/d**	**1.0 µg/kg/d**	**3.0 µg/kg/d**
1	uterus - wet (mg)			141.3 ± 19.87	71.7 ± 10.45
	uterus - blotted (mg)			99.2 ± 10.95	65.5 ± 8.69
	body wt. (gm)			43.0 ± 4.00	42.9 ± 5.60
2	uterus - wet (mg)	62.3 ± 8.33	120.8 ± 61.23[1]	52.5 ± 15.02	117.2 ± 23.18[1]
	uterus - blotted (mg)	51.5 ± 6.83	73.4 ± 15.04[1]	47.7 ± 14.46	77.4 ± 4.93[1]
	body wt. (gm)	48.6 ± 7.49	44.0 ± 4.69[1]	46.6 ± 4.44	46.0 ± 3.51[1]
3	uterus - wet (mg)	104.0 ± 19.49	172.7 ± 44.93	102.8 ± 27.26	159.0 ± 74.51
	uterus - blotted (mg)	90.0 ± 14.44	113.0 ± 14.52	84.2 ± 12.34	111.0 ± 20.57
	body wt. (gm)	42.3 ± 3.67	42.2 ± 1.68	42.3 ± 4.22	41.7 ± 2.10
4	uterus - wet (mg)	56.0 ± 6.87	127.0 ± 31.99	335.0 ± 63.22	318.0 ± 29.93
	uterus - blotted (mg)	47.3 ± 6.02	82.5 ± 15.83	129.7 ± 19.77	140.0 ± 11.14
	body wt. (gm)	47.0 ± 2.00	42.8 ± 2.48	30.3 ± 3.20	35.5 ± 1.97
5	uterus - wet (mg)	67.5 ± 14.10	199.7 ± 15.29		
	uterus - blotted (mg)	66.3 ± 13.86	153.2 ± 18.82		
	body wt. (gm)	58.4 ± 3.82	57.6 ± 3.41		
7	uterus - wet (mg)	62.8 ± 10.27	91.9 ± 31.15	59.0 ± 6.78	70.0 ± 5.45
	uterus - blotted (mg)	60.0 ± 10.21	82.8 ± 20.24	54.0 ± 6.73	67.6 ± 5.69
	body wt. (gm)	57.8 ± 2.84	57.6 ± 3.53	57.8 ± 3.79	54.8 ± 5.17
12	uterus - wet (mg)	-[a]	-[a]	96.1 ± 27.95	206.4 ± 29.90
	uterus - blotted (mg)	-[a]	-[a]	78.5 ± 15.16	118.1 ± 11.57
	body wt. (gm)	-[a]	-[a]	40.1 ± 4.27	42.9 ± 5.71
14	uterus - wet (mg)	55.4 ± 21.80	74.3 ± 15.23	50.6 ± 8.01	94.9 ± 29.48
	uterus - blotted (mg)	38.0 ± 7.07	62.1 ± 13.83	48.1 ± 7.24	87.5 ± 22.72
	body wt. (gm)	61.7 ± 3.10	61.0 ± 3.35	61.4 ± 3.10	62.9 ± 2.87
14	uterus - wet (mg)	48.4 ± 5.54	66.4 ± 11.30		
	uterus - blotted (mg)	40.4 ± 4.06	55.9 ± 10.68		
	body wt. (gm)	65.2 ± 3.15	65.2 ± 3.00		

Table 8a. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol A, Continued

15	uterus - wet (mg)	61.9 ± 7.64	126.1 ± 19.20	55.0 ± 3.73	111.7 ± 31.22
	uterus - blotted (mg)	59.7 ± 7.80	109.0 ± 12.01	53.2 ± 3.89	97.8 ± 15.79
	body wt. (gm)	62.5 ± 3.66	62.8 ± 4.96	66.1 ± 3.00	65.5 ± 3.37
15	uterus - wet (mg)	92.2 ± 15.57	109.4 ± 13.56		
	uterus - blotted (mg)	88.4 ± 14.45	100.0 ± 6.49		
	body wt. (gm)	63.3 ± 3.86	64.0 ± 4.41		
17	uterus - wet (mg)	117.0 ± 10.24	175.4 ± 28.26		
	uterus - blotted (mg)	99.3 ± 9.74	127.9 ± 21.10		
	body wt. (gm)	52.5 ± 5.01	50.9 ± 3.28		
18	uterus - wet (mg)	94.6 ± 34.83	142.2 ± 39.39	201.4 ± 56.24	92.1 ± 29.01
	uterus - blotted (mg)	86.7 ± 25.15	111.9 ± 14.63	132.1 ± 15.73	82.8 ± 15.77
	body wt. (gm)	48.5 ± 4.71	49.2 ± 3.53	54.1 ± 3.91	52.7 ± 2.35
20	uterus - wet (mg)	66.9 ± 12.99	139.2 ± 33.73	23.2 ± 5.27[b]	64.2 ±11.97
	uterus - blotted (mg)	59.3 ± 14.87	97.3 ± 13.34	18.6 ± 4.45[b]	57.5 ± 11.47
	body wt. (gm)	40.5 ± 2.98	39.3 ± 3.04	36.7 ± 3.97[b]	35.9 ± 3.29

Table 8b. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol B

		dose-response		**single dose**	
Lab.	**Weighing**	**0.3 µg/kg/d**	**1.0 µg/kg/d**	**0.3 µg/kg/d**	**1.0 µg/kg/d**
1	uterus - wet (mg)	63.0 ± 4.23	141.5 ± 18.05	149.6 ± 16.17	77.7 ± 2.85
	uterus - blotted (mg)	60.0 ± 3.99	112.9 ± 13.71	115.0 ± 8.92	73.0 ± 3.14
	body wt. (gm)	50.8 ± 4.49	51.4 ± 3.56	51.5 ± 5.89	52.4 ± 3.37
2	uterus - wet (mg)	49.6 ± 17.87	123.0 ± 24.66	45.7 ± 12.21	113.3 ± 24.43
	uterus - blotted (mg)	39.2 ± 7.73	76.8 ± 11.63	42.8 ± 9.00	74.8 ± 15.22
	body wt. (gm)	39.2 ± 10.04	47.1 ± 5.61	47.9 ± 5.35	46.4 ± 4.65
3	uterus - wet (mg)	97.7 ± 42.70	200.3 ± 44.64	61.0 ± 19.28	140.5 ± 47.45
	uterus - blotted (mg)	84.2 ± 28.41	132.5 ± 17.31	57.7 ± 17.83	106.8 ± 23.28
	body wt. (gm)	44.5 ± 2.09	44.3 ± 5.09	41.4 ± 5.18	42.3 ± 3.69
4	uterus - wet (mg)	51.8 ± 12.07	138.5 ± 48.01	375.2 ± 66.91	324.2 ± 54.32
	uterus - blotted (mg)	46.2 ± 12.51	100.0 ± 30.34	144.8 ± 22.32	153.0 ± 21.86
	body wt. (gm)	43.2 ± 2.99	41.2 ± 6.91	33.7 ± 5.47	35.3 ± 3.93
5	uterus - wet (mg)	215.9 ± 72.60	245.0 ± 64.46		
	uterus - blotted (mg)	143.7 ± 13.41	145.0 ± 14.97		
	body wt. (gm)	57.8 ± 1.65	59.2 ± 1.54		
6	uterus - wet (mg)	107.6 ± 39.93	168.1 ± 35.36		
	uterus - blotted (mg)	99.6 ± 33.89	135.3 ± 20.02		
	body wt. (gm)	52.5 ± 5.88	50.7 ± 8.28		
7	uterus - wet (mg)	156.0 ± 26.99**	266.5 ± 23.79**	72.8 ± 9.84	162.0 ± 30.30
	uterus - blotted (mg)	125.9 ± 15.09**	155.9 ± 13.93**	68.0 ± 9.05	137.3 ± 29.22
	body wt. (gm)	57.1 ± 4.63	53.6 ± 8.14	59.1 ± 4.69	58.9 ± 3.71
8	uterus - wet (mg)			57.3 ± 14.47	144.4 ± 27.37
	uterus - blotted (mg)			52.0 ± 14.51	100.7 ± 12.10
	body wt. (gm)			42.7 ± 3.42	44.9 ± 1.96

Table 8b. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol B,Continued

9	uterus - wet (mg)			36.9 ± 8.05	100.6 ± 55.26
	uterus - blotted (mg)			30.6 ± 6.65	68.6 ± 27.30
	body wt. (gm)			35.2 ± 6.26	36.1 ± 4.15
10	uterus - wet (mg)			42.0 ± 6.99	35.9 ± 10.33
	uterus - blotted (mg)			32.3 ± 6.13	28.4 ± 8.78
	body wt. (gm)			57.5 ± 3.21	58.1 ± 3.98
11	uterus - wet (mg)	205.0 ± 25.70	109.9 ± 9.96	65.8 ± 11.30	166.1 ± 26.90
	uterus - blotted (mg)	151.1 ± 18.39	93.1 ± 7.04	51.7 ± 8.84	131.1 ± 21.36
	body wt. (gm)	51.3 ± 3.20	53.5 ± 4.37	52.3 ± 2.07	53.0 ± 3.63
11	uterus - wet (mg)	255.8 ± 86.82	103.4 ± 7.50		
	uterus - blotted (mg)	166.6 ± 17.54	84.3 ± 8.55		
	body wt. (gm)	54.5 ± 5.58	55.0 ± 4.73		
12	uterus - wet (mg)	- [a]	- [a]	100.9 ± 18.00	174.6 ± 37.43
	uterus - blotted (mg)	- [a]	- [a]	84.9 ± 12.99	104.2 ± 11.80
	body wt. (gm)	- [a]	- [a]	40.7 ± 2.17	36.5 ± 2.56
13	uterus - wet (mg)			57.3 ± 14.47	144.4 ± 27.37
	uterus - blotted (mg)			52.0 ± 14.51	100.7 ± 12.10
	body wt. (gm)			42.7 ± 3.42	44.9 ± 1.96
14	uterus - wet (mg)	91.0 ± 5.53	205.1 ± 18.89	73.1 ± 14.05	39.2 ± 9.16#
	uterus - blotted (mg)	83.8 ± 4.74	136.8 ± 18.49	71.0 ± 13.50	37.5 ± 9.18#
	body wt. (gm)	65.3 ± 3.66	64.9 ± 2.88	60.5 ± 2.94	60.5 ± 3.44
14	uterus - wet (mg)	78.1 ± 8.57	193.4 ± 22.39		
	uterus - blotted (mg)	70.4 ± 6.33	132.3 ± 8.95		
	body wt. (gm)	63.0 ± 2.88	62.3 ± 2.30		
15	uterus - wet (mg)	89.5 ± 11.44	204.4 ± 48.64	85.0 ± 10.38	206.1 ± 34.55
	uterus - blotted (mg)	86.3 ± 11.06	140.2 ± 20.53	82.0 ± 9.79	156.7 ± 21.76
	body wt. (gm)	63.6 ± 2.14	62.6 ± 2.14	64.1 ± 3.11	64.0 ± 3.50
15	uterus - wet (mg)	78.3 ± 8.32	186.8 ± 16.53		
	uterus - blotted (mg)	76.0 ± 7.83	145.7 ± 11.46		
	body wt. (gm)	65.6 ± 2.80	66.3 ± 2.69		
16	uterus - wet (mg)	104.7 ± 22.05	207.5 ± 29.79	115.6 ± 16.00	228.4 ± 51.84
	uterus - blotted (mg)	77.8 ± 9.21	119.1 ± 12.03	97.0 ± 10.10	139.1 ± 12.67
	body wt. (gm)	57.0 ± 2.84	53.0 ± 3.46	57.1 ± 3.71	55.2 ± 3.17
17	uterus - wet (mg)			176.3 ± 53.55	109.6 ± 62.38 [I(m)]
	uterus - blotted (mg)			132.2 ± 23.57	98.1 ± 9.05 [I(m)]
	body wt. (gm)			52.9 ± 5.65	54.5 ± 3.08 [I(m)]
18	uterus - wet (mg)	56.0 ± 14.61	157.0 ± 42.30	188.1 ± 26.16	73.8 ± 9.57
	uterus - blotted (mg)	53.9 ± 14.18	118.0 ± 18.54	138.1 ± 10.61	71.4 ± 9.33
	body wt. (gm)	49.3 ± 3.64	51.3 ± 5.00	53.5 ± 3.92	54.6 ± 2.68
19	uterus - wet (mg)	28.0 ± 8.21	32.9 ± 2.68		
	uterus - blotted (mg)	12.1 ± 1.55	12.7 ± 1.08		
	body wt. (gm)	43.8 ± 1.71	41.6 ± 2.05		
19	uterus - wet (mg)	26.1 ± 7.63	32.9 ± 2.68		
	uterus - blotted (mg)	11.9 ± 1.64	12.68 ± 1.08		
	body wt. (gm)	44.1 ± 1.65	41.6 ± 2.05		
20	uterus - wet (mg)	40.5 ± 12.48	100.7 ± 33.22	41.1 ± 12.18 [c]	46.2 ± 15.73
	uterus - blotted (mg)	35.6 ± 10.24	83.3 ± 25.98	36.2 ± 10.48 [c]	41.8 ± 14.58
	body wt. (gm)	38.7 ± 4.32	40.9 ± 4.23	45.2 ± 2.54 [c]	43.2 ± 3.22

Table 8c. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol C

		dose-response		single dose	
Lab.	**Weighing**	**0.3 µg/kg/d**	**1.0 µg/kg/d**	**0.3 µg/kg/d**	**1.0 µg/kg/d**
1	uterus - wet (mg)			371.3 ± 106.12	242.5 ± 15.49
	uterus - blotted (mg)			257.6 ± 39.84	210.4 ± 12.92
	body wt. (gm)			278.5 ± 12.57	281.3 ± 16.97
5	uterus - wet (mg)	404.7 ± 129.18	829.3 ± 118.68		
	uterus - blotted (mg)	254.1 ± 45.73	325.3 ± 26.93		
	body wt. (gm)	248.3 ± 9.07	236.6 ± 9.48		
6	uterus - wet (mg)	318.4 ± 169.85	656.1 ± 162.32		
	uterus - blotted (mg)	275.2 ± 125.05	396.8 ± 77.90		
	body wt. (gm)	289.5 ± 11.40	285.8 ± 13.71		
10	uterus - wet (mg)			200.1 ± 32.11	112.8 ± 42.62
	uterus - blotted (mg)			177.0 ± 28.14	87.8 ± 11.02
	body wt. (gm)			272.5 ± 10.75	281.9 ± 16.40
12	uterus - wet (mg)	- [a]	- [a]	367.7 ± 38.86	714.7 ± 165.54
	uterus - blotted (mg)	- [a]	- [a]	238.2 ± 14.64	313.3 ± 34.83
	body wt. (gm)	- [a]	- [a]	211.7 ± 8.07	204.6 ± 10.46
14	uterus - wet (mg)	211.3 ± 18.45	764.0 ± 161.24	272.5 ± 28.08	763.3 ± 137.08
	uterus - blotted (mg)	180.5 ± 19.38	307.8 ± 24.13	233.8 ± 24.67	340.8 ± 60.20
	body wt. (gm)	253.0 ± 11.40	242.5 ± 13.92	263.1 ± 16.56	255.8 ± 9.27
14	uterus - wet (mg)	182.2 ± 12.99	536.9 ± 78.74		
	uterus - blotted (mg)	166.4 ± 10.60	256.2 ± 27.49		
	body wt. (gm)	268.1 ± 21.63	256.7 ± 15.85		
15	uterus - wet (mg)	277.7 ± 43.47	589.6 ± 189.03	244.4 ± 59.44	676.8 ± 177.56
	uterus - blotted (mg)	240.3 ± 30.17	290.6 ± 66.36	218.4 ± 44.21	325.0 ± 50.80
	body wt. (gm)	269.4 ± 14.30	265.2 ± 9.95	263.4 ± 11.90	254.5 ± 11.38
15	uterus - wet (mg)	217.3 ± 26.75	520.1 ± 93.56		
	uterus - blotted (mg)	196.9 ± 19.41	278.1 ± 12.91		
	body wt. (gm)	262.1 ± 11.32	251.5 ± 11.96		
18	uterus - wet (mg)	265.4 ± 25.79	665.3 ± 102.82	677.6 ± 71.33	229.9 ± 28.00
	uterus - blotted (mg)	240.0 ± 19.07	319.8 ± 36.51	312.7 ± 22.11	208.2 ± 13.03
	body wt. (gm)	244.3 ± 9.72	237.5 ± 9.25	224.0 ± 12.02	235.4 ± 13.34
20	uterus - wet (mg)	221.0 ± 39.28	665.6 ± 88.06	113.0 ± 16.34 [c]	251.8 ± 36.43
	uterus - blotted (mg)	192.6 ± 34.17	302.5 ± 22.07	100.0 ± 15.10 [c]	204.5 ± 24.49
	body wt. (gm)	292.5 ± 23.31	290.8 ± 17.06	287.0 ± 13.70 [c]	279.3 ± 14.43

Table 8d. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol C'

		dose-response		single dose	
Lab.	**Weighing**	**0.3 µg/kg/d**	**1.0 µg/kg/d**	**0.3 µg/kg/d**	**1.0 µg/kg/d**
5	uterus - wet (mg)	313.5 ± 46.54	360.9 ± 45.66		
	uterus - blotted (mg)	302.1 ± 40.16	336.6 ± 39.46		
	body wt. (gm)	256.4 ± 9.43	249.2 ± 12.11		
12	uterus - wet (mg)	- [a]	- [a]	343.3 ± 33.38	461.3 ± 224.67
	uterus - blotted (mg)	- [a]	- [a]	323.9 ± 30.60	374.8 ± 55.00
	body wt. (gm)	- [a]	- [a]	201.3 ± 7.54	205.5 ± 15.62
14	uterus - wet (mg)	288.8 ± 24.70	408.9 ± 20.95	237.0 ± 31.13	367.4 ± 40.14
	uterus - blotted (mg)	250.0 ± 22.86	375.7 ± 30.64	224.6 ± 22.81	339.5 ± 44.39
	body wt. (gm)	260.6 ± 16.42	250.4 ± 14.18	280.7 ± 17.53	267.8 ± 10.39
14	uterus - wet (mg)	265.9 ± 38.33	374.4 ± 52.93		
	uterus - blotted (mg)	233.8 ± 34.65	326.1 ± 25.27		
	body wt. (gm)	265.9 ± 10.65	253.1 ± 14.03		
15	uterus - wet (mg)	270.2 ± 37.19	355.7 ± 37.98	285.9 ± 42.23	377.4 ± 48.62
	uterus - blotted (mg)	239.8 ± 17.46	335.5 ± 41.15	261.6 ± 35.72	365.0 ± 46.79
	body wt. (gm)	268.0 ± 15.91	258.8 ± 13.56	263.8 ± 8.82	246.9 ± 14.35
14	uterus - wet (mg)	261.5 ± 26.43	341.3 ± 25.72		
	uterus - blotted (mg)	252.4 ± 25.69	326.9 ± 25.15		
	body wt. (gm)	269.6 ± 12.74	253.8 ± 9.66		
18	uterus - wet (mg)	340.0 ± 74.45	524.9 ± 312.45	402.3 ± 52.44	299.6 ± 34.01
	uterus - blotted (mg)	308.5 ± 37.84	394.9 ± 50.64	386.7 ± 52.01	276.1 ± 24.82
	body wt. (gm)	255.2 ± 10.91	241.6 ± 7.56	242.7 ± 24.80	257.0 ± 17.83

Table 8e. Weights of wet and blotted uteri, and body weights, in animals administered EE in Protocol D

		dose-response		single dose	
Lab.	**Weighing**	**1.0 µg/kg/d**	**3.0 µg/kg/d**	**1.0 µg/kg/d**	**3.0 µg/kg/d**
20	uterus - wet (mg)	111.9 ± 15.10	114.4 ± 9.02	95.2 ± 16.50	107.6 ± 20.69
	uterus - blotted (mg)	105.3 ± 13.84	107.4 ± 8.96	85.5 ± 14.79	98.5 ± 18.88
	body wt. (gm)	289.8 ± 11.79	293.5 ± 9.16	289.0 ± 7.02	293.2 ± 5.93

[a] shared EE values in dose-response and single-dose experiments

[b] EE dose was 0.075 µg/kg/d

[c] EE dose was 0.023 µg/kg/d

** Laboratory used EE at 1.0 ug and 3.0 ug

EE results questioned by test laboratory

[1(m)] one animal found to be male at autopsy