替）に比例した分子数のスパイクRNAを添加することで，サンプルの細胞当たりのmRNA絶対量（コピー数）の指標をサンプル中に導入するものである（図3）．ただし，スパイクRNAは1点を規定するものではなく，5種類の枯草菌遺伝子に対するRNA（哺乳類の配列と交叉しない）を適切な公比を持たせて5段階の濃度に割り振ったカクテルとして用いることが特徴である（図4）．これにより，絶対コピー数の指標となると同時に，広い用量範囲について検量線を各サンプルに導入したことになり，mRNA抽出からGeneChipの蛍光測光までの過程で生じるデータ全体の歪みを補正する際に威力を発揮するとともに，すべてのGeneChipの発現値を統一基準下で安定的に絶対量化する効果を有している．サンプルに由来するすべての測定値はHill関数の直線化式により直線化されたGSC検量線に基づいて絶対量に変換される．

$$\mathrm{Log}\,(S/S_{MAX}-S) = \gamma \log C - \gamma \log SC_{50}$$

式中，Sは測定値，S_{MAX}は最大測定値，Cはスパイク RNAの濃度，SC_{50}は50%反応濃度，γはHill係数を示す．なお，高発現側の歪みを気にしない場合には，S_{MAX}を無限大に置いた近似式での代用が可能である．

1例として，スキャナーを取り替えた際のデータの歪みを矯正した事例を紹介する（図5）．複数のサンプルの間での，あるいは複数の実験間でのある遺伝子の発現変動の比較は本システムにより飛躍的に向上することが示されている．例えば，日内変動遺伝子の日内変動が絶対表示により直読可能であり，その発現様態は発現値をも含めて実験間で再現されている．

他方，チップ内での異なる遺伝子の発現量の正確さに関しては，GeneChipのプローブセットの設計に依存する．アフィメトリクスはプローブの設計に際してそれらのtm値を一定に保つアルゴリズムを用いている．これについては，利用者として個々に定量的PCRなどにより検証する必要がある．

V．LBM

1．システムの定量性の検定

LBMは当方の便宜上，肝と脳の組み合わせを用いたが，遺伝子発現プロファイルの異なるペアであればどのような組み合わせでも利用可能である．複数のペアを併用すればさらに精度の良い検定が可能となる．GSCをDNA濃度に応じて添加したLBMセットを測定し，絶対量化した結果は，グラフ化すると直線を描くはずであり（図6），さらに50：50のサンプルで除した場合，理想的にはすべての遺伝子が50：50のところで1の値をとり，100：0あるいは0：100では0から2の間の値をとるところの直線を描くはずである．この結果から，マイクロアレイの定量性が確認される．

2．GeneChipの新旧バージョン間のデータ変換

さらに，このようなLBMサンプルをバージョンアップ前の古いGeneChipと新しいバージョンのGeneChipで測定し

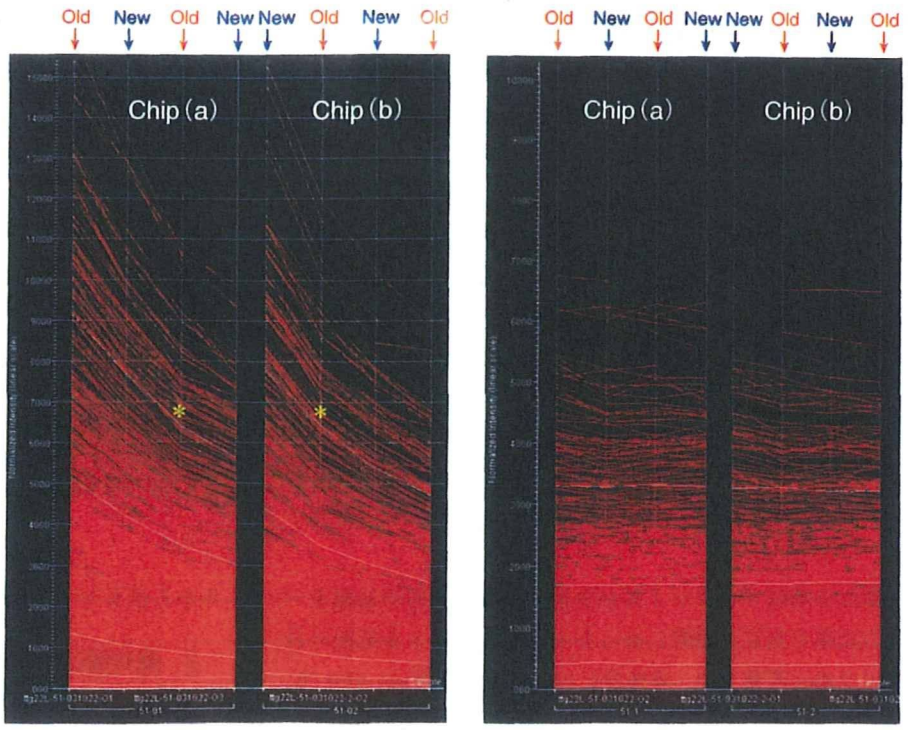

図5．絶対量化の効果の1例
新旧2台のスキャナーの特性比較を行った．1つのサンプルを2つに分け，a，b各2枚のGeneChipにハイブリダイズした．Chip（a）を新スキャナーにて，Chip（b）を旧スキャナーにて測定し，さらにChipを取り替えて再スキャンすることを，3回繰り返した．その結果をGeneSpring（Silicon Genetics社）を用いてグラフ化した（縦軸はゼロを起点とする均等目盛り表示）．スキャンを繰り返すたびに蛍光強度は減衰してしまう．傾斜の角度の違い（図中＊の部分の角度参照）から減衰の度合いは旧スキャナーで若干強いことがわかる．このデータを絶対量化したデータでは，減衰がほぼ完全に補正される（シグナルの高いところでも，値の相対的な振れは1割程度に収まっている）．白線はGSC．

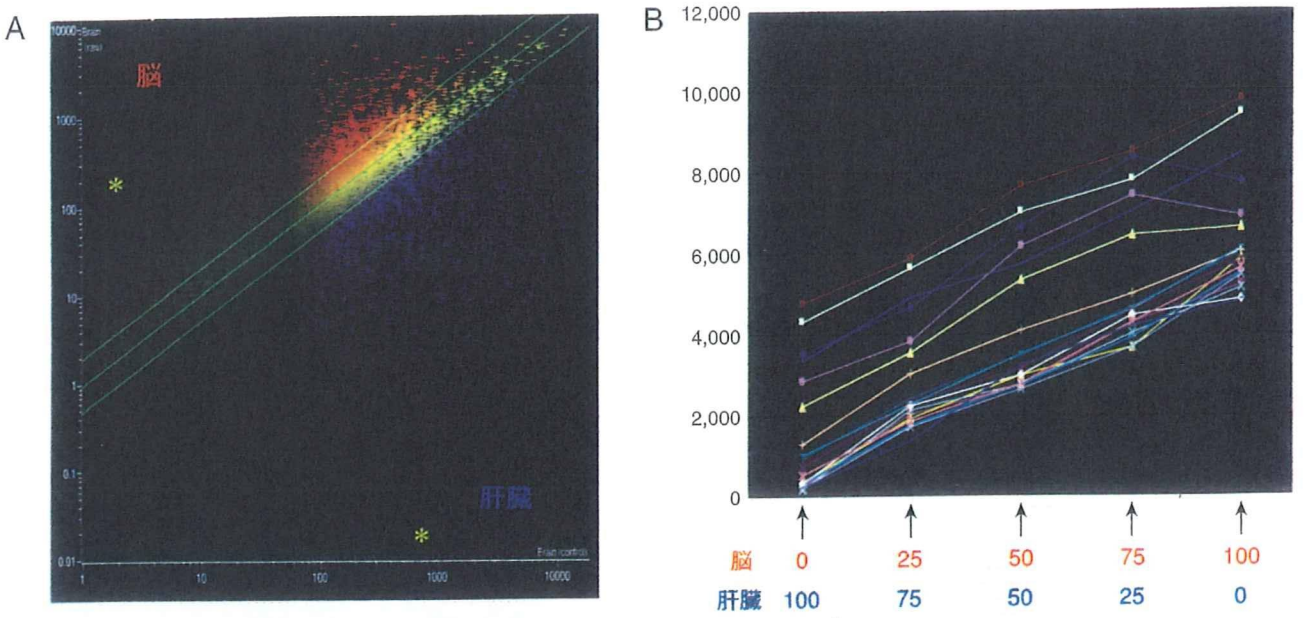

図6. LBM（liver-brain mix）標準サンプルセットによるシステムの定量性の検定

Aのスキャッターグラフが示すように脳と肝臓では発現遺伝子のレパートリーが大きく異なり，X軸およびY軸に沿って遺伝子が線状に並ぶ（＊）ことでわかるとおり，片方の臓器にのみ発現する遺伝子が多い．このような臓器の対からサンプルを調製し，100：0，75：25，50：50，25：75，および0：100の混合比の5サンプルをGeneChip（MG-U74v2A）にて測定するとBのように，ほぼ直線を得る．すべての遺伝子について50：50の値に対する比を求め，同様にグラフを描くと，理想的な性能を有するマイクロアレイではすべての遺伝子が50：50のところで1を通り，100：0におけるy切片が0～2の範囲に収まる直線を描く．

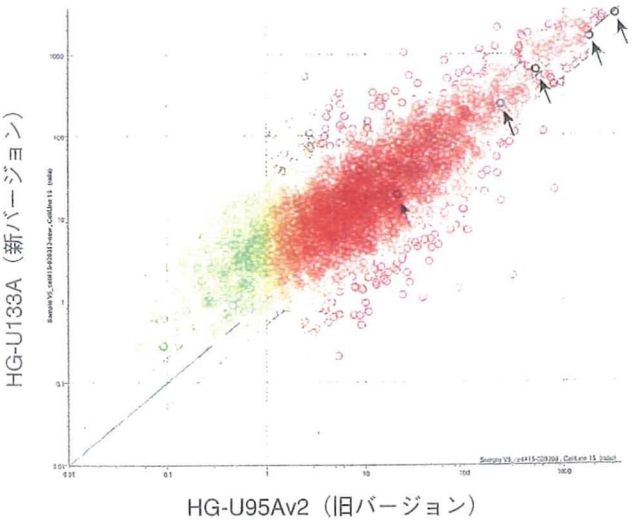

図7. GeneChipの新旧バージョン間のデータの関係

LBMサンプルセットを新旧のバージョンのGeneChipにおいて測定すると，ここにスキャッターグラフで示すような関係が5組得られる．矢印で示す黒丸がGSCである．GSCを基準に新旧のChipでの発現値が標準化され，そのような点が5組のデータから5点得られることから，ここにプロットされた遺伝子（両バージョンに同一または対応するアノテーションが得られ，かつ，LBMサンプルに発現されているもの）については個々について直接変換式が得られる．これは，条件により定量PCRやアフィメトリクス以外のマイクロアレイプラットフォームにも原理的に拡張可能である．

ておくことにより，LBMに含まれるすべての遺伝子について，5点から成る新旧のチップにおける用量相関関数を求めることができる．LBMに他の臓器の組み合わせを用いることで取り扱える遺伝子数を増やすことが可能である（図7）．

3．データ互換性の他システムへの拡張

本システムのGSCを添加したサンプルはスパイクRNAを検出するプライマーセットを用意することでPCRにおいても容易に絶対量化データを得ることができる．詳細は他に譲るが，プライマーペアの増幅効率のばらつきを勘案した絶対化アルゴリズムとともにPercellome定量PCRシステムを構築中である．アフィメトリクスGeneChip以外のプラットフォームとのデータ互換も原理的に可能である．本システムが適応可能なプラットフォームの条件としては，GSCを受け付けるプローブセットが用意されていること，および用量相関性が確保されてることの2点を満たしている必要がある（現在，2社検討開発中）．

Ⅵ．形質非依存型トキシコゲノミクスへの適用

創薬開発促進のためのトキシコゲノミクス・プロジェクトと，化学物質の安全性評価のためのプロジェクトの2つが現在，Percellomeシステムのもとで進行中である（表1）．両プロジェクトではともに，4～5段階の用量について4時点

表1. 厚生労働省におけるトキシコゲノミクス研究

プロジェクト名	「トキシコゲノミクス手法を用いた医薬品安全性評価予測システムの構築とその基盤に関する研究」（厚生労働科学研究費補助金，萌芽的先端医療技術推進研究事業）H14年度～5年計画	「化学物質リスク評価の基盤整備としてのトキシコゲノミクスに関する研究」（厚生労働科学研究費補助金，化学物質リスク研究事業）H15年度～3年計画
組織	創薬関連企業17社参加「産学官連携」プロジェクト〔プロジェクトリーダー：長尾 拓（国立衛研所長）〕	研究班体制の研究プロジェクト 国立衛研・安全性生物試験研究センター内（主任研究者：菅野 純）
目標	創薬過程における安全性の早期予測システムの構築	国が行う既存化学物質の点検を，より迅速，安価かつ正確に実施する毒性予測システムの構築
期待される効果	医薬品による副作用の早期予測による，臨床段階での開発中止の回避，創薬の経費削減と効率化の促進 予期せぬ副作用の低減による国民被害の減少，より安全性の高い医薬品の創製による製薬企業の活性化と国民の健康増進への寄与	日常生活において使用される数万種の化学物質の毒性を，従来の毒性試験よりも，迅速，安価かつ網羅的に予測することによる国民の安全・安心の向上 毒性発現メカニズムに支えられた包括的な毒性評価の体制の整備
検討物質	開発中止，あるいは販売中止となった医薬品・医薬品候補物質を中心としたDrug-likeな化合物（150物質／5年）	日常使用される数万種に及ぶ化学物質を中心とした各種の物質（約90物質／3年）
モデル動物	ラット（医薬品の審査に使用されるSD/IGSラット）	マウス（遺伝子改変マウスの活用を見越しC57BL/6マウス）
検索臓器	肝および腎．ヒトおよびラット肝細胞由来培養細胞	肝および化学物質固有の標的臓器

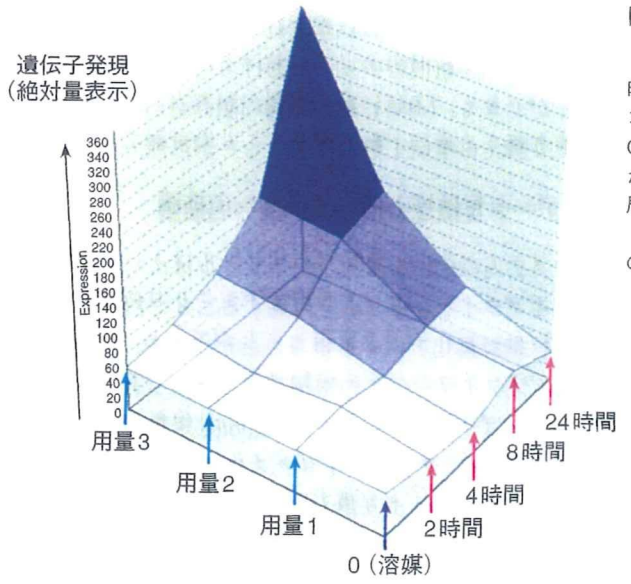

図8. トキシコゲノミクス・プロジェクトにおける単回投与実験の基本構成

時間と用量の組み合わせから成る4×4～4×5のマトリックス構造のプロトコールにてデータを生成中である．各群3匹とし，サンプルはプールせず個別にGeneChip解析を実施している．X軸に時間，Y軸に用量，Z軸に発現量（ゼロからの均等目盛り表示）をプロットすることにより，1つの遺伝子につき1枚の局面を描くことができる．現在使用中のMOE430v2は約45,000のプローブセット情報を生成するため，1つの化合物のトランスクリプトーム情報は45,000枚の局面の集合体（ミルフィーユ・データ）で表される．

での遺伝子発現を観測する16～20群（1群3匹）の構成から成るプロトコールを採用した．1つの化合物について48～60匹の動物からのサンプルを解析しPercellomeデータを生成する．遺伝子の発現値を3次元表示することでその用量・時間依存性が視覚化できる．X軸に時間，Y軸に用量，Z軸に発現量（ゼロからの均等目盛り表示）をプロットすることにより，1つの遺伝子につき16～20格子点（48～60枚のGeneChipからのデータ）から成る1枚の局面を描くことができる（図8）．1つのGeneChipが45,000のプローブセットから成る場合，1つの化合物の用量・時間依存的データ3次元表示では45,000枚の局面の層状集合体から成る（ミルフィーユ・データと名付けた）．このミルフィーユ・データは各格子点が3匹の動物に由来する3つのデータをもとにしており，格子点のデータの信頼性の評価を含めて，アーチファクトの除去や，生物学的な蓋然性のある変化であるか否かの判別に適しているうえに，類似の用量・時間反応を示す遺伝子の選別に威力を発揮する．

Ⅶ. 形質非依存型トキシコゲノミクスにおけるデータ解析法

創薬開発促進のためのトキシコゲノミクス・プロジェク

トでは，特に開発中止となった化合物を含む薬剤関連の化学物質を中心としてラットを用いた実験を進めており，すでに蓄積されている膨大なラット毒性情報との対比に重点を置いた解析を（株）日立製作所とともに進めている．他方，筆者らが進めている化学物質の安全性評価のためのプロジェクトにおいては，一般的な化学物質が対象であるため毒性データが必ずしも豊富でないこともあり，生体反応の分子メカニズム解析（カスケード解析）に重点を置き，遺伝子欠失動物の活用を見込んで，マウスを用いた実験を重ねている．こちらでは遺伝子発現プロファイルを体系化するために形質発現情報に頼らず，完全な教師なしクラスタリングを実施する．ミルフィーユ・データを基礎に，生物学者が視覚的に確認できる変数を利用する方法をNTTコムウェア（株）と共同開発し，Teradata〔日本NCR（株）〕による解析・データベース上に搭載した．このクラスタリング手法は，クラスター数およびクラスター径を指定せず，通常45,000プローブセット（MOE430v2）を小さいものから順に数百クラスターに分類する．今後この方法と，適切な遺伝子欠失マウスによるミルフィーユ・データ生成により，客観的な遺伝子カスケード構築を進める．そのうえで，既知の情報との比較を行い，必要に応じて確認のための小実験を別途追加して実施し，最終的に信頼性の高い遺伝子カスケードデータベースの構築と，これに基づいた効率的で正確な毒性評価・予測技術の開発を目指している．

おわりに

毒性学は毒性という形質発現をもとに成り立ってきているが，その効率化と正確性向上のために分子毒性学的なメカニズム解析の導入を図るに当たり，形質が発現する以前の段階，あるいはフィードバック機構が働くために形質発現が乏しい状態など，形質発現を直接には伴わないところでの遺伝子発現変動を網羅的に捕らえて毒性に関わる遺伝子発現カスケードの全容を解明する必要に迫られている．これに応えるために，筆者らは形質非依存型トキシコゲノミクスの概念の導入と，それに必要な技術であるマイクロアレイから細胞1個当たりのmRNA絶対量情報を生成するPercellomeシステムを開発した．

絶対量化されたPercellomeデータは，そのすべてを生物学者にとってわかりやすい3次元ミルフィーユ・データとして表示することが可能であり，その結果，クラスター化などのデータ解析過程を生物学的蓋然性に基づいて比較的容易に検証することができるようになった．本システムは4×4～4×5マトリックス方式の大型プロジェクトを対象として開発したものであるが，実際には小規模の実験サンプルに対しても有用性が高いことが実証されている．特に変動遺伝子リストの遺伝子数が飛躍的に増大することが多い．それは，変動比率による足切りやハズレ値計算のような統計手法を用いる必要がなく，個々の遺伝子について逐一比較ができるためである．

絶対量化された発現値はコントロール群を含めてGeneChip間あるいは実験間でそのままの形で相互に直接比較が可能であり，例えば日内変動遺伝子がそのままミルフィーユ・データとして表示されている．さらに，異なったバージョン間，定量PCRとの間，さらにはアフィメトリクス以外のPercellomeに対応したプラットフォームとの間でのデータ変換のための変換関数を導き出すことが可能であり，この特徴は複数の研究者や組織がデータを持ち寄るデータベースの構築に貢献する可能性が期待される．

以上，Percellomeシステムはミルフィーユ・データと相まってトランスクリプトームの精度と相互互換性を有意に高めることが示唆されつつある．現在まで創薬プロジェクトも当方の化学物質プロジェクトも4×4ないし4×5マトリックス規模の実験を30～40化合物程度実施し，膨大なデータの蓄積を開始した．今後，この形態のデータの有用性を客観的に評価頂くためのPercellomeコンソーシアムの構築を考えていきたい．

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The OECD Program to Validate the Rat Uterotrophic Bioassay. Phase 2: Dose–Response Studies

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The Organisation for Economic Co-operation and Development has completed phase 2 of an international validation program for the rodent uterotrophic bioassay. The purpose of the validation program was to demonstrate the performance of two versions of the uterotrophic bioassay, the immature female rat and the adult ovariectomized rat, in four standardized protocols. This article reports the dose–response studies of the validation program; the coded single-dose studies are reported in an accompanying paper. The dose–response study design used five selected weak estrogen agonists, bisphenol A, genistein, methoxychlor, nonylphenol, and *o,p'*-DDT. These weak agonists were administered in a prescribed series of doses to measure the performance and reproducibility of the protocols among the participating laboratories. All protocols successfully detected increases in uterine weights when the weak agonists were administered. Within each protocol, there was good agreement and reproducibility of the dose response among laboratories with each substance. Substance-specific variations were observed in the influence of the route of administration on the uterine response, the potency as related to the dose producing the first statistically significant increase in uterine weights, and the maximum increase in uterine weight. Substantive performance differences were not observed between the uterotrophic bioassay versions or among the standardized protocols, and these were judged to be qualitatively equivalent. It is noteworthy that these results were reproducible under a variety of different experimental conditions (e.g., animal strain, diet, housing, bedding, vehicle, animal age), indicating that the bioassay's performance as a screen is robust. In conclusion, both the intact, immature, and adult OVX versions, and all protocols appear to be reproducible and transferable across laboratories and are able to detect weak estrogen agonists. *Key words:* endocrine disruption, estrogen, rat uterus, uterotrophic. *Environ Health Perspect* 111:1530–1549 (2003). doi:10.1289/ehp.5780 available via *http://dx.doi.org/* [Online 23 January 2003]

The Organisation for Economic Co-operation and Development (OECD) initiated a high-priority activity in 1997 to revise existing guidelines and to develop new guidelines for the screening and testing of potential endocrine disrupters (OECD 1998a). This activity is managed by a Validation Management Group (VMG) reporting to the Task Force on Endocrine Disrupters Testing and Assessment as part of the OECD Test Guidelines Programme. One portion of the activity is to validate the rodent uterotrophic bioassay, an *in vivo* screen intended to identify compounds that are suspected agonists or antagonists of estrogen, and to assist the prioritization of positive compounds for further testing. In phase 1 of the validation program, standardized protocols were developed for two versions of the uterotrophic bioassay, the immature rat and the adult ovariectomized (OVX) rat. These protocols have been successfully tested against a high-potency reference estrogen-receptor agonist, 17α-ethinyl estradiol (EE), and a reference estrogen-receptor antagonist, ZM 189,154. All protocols were robust, reproducible, and transferable across laboratories using these reference compounds (Kanno et al. 2001). Therefore, the VMG proceeded with the design and execution of phase 2 of the uterotrophic bioassay's validation program.

A key objective of validation exercises is to demonstrate the reliability of the standardized protocols. Reliability includes a demonstration of the transferability of the protocols among laboratories and the reproducibility of the results from those protocols among laboratories. Such a demonstration is expected to employ test substances that represent the substances of likely concern in regulatory use, for example, in the case of the uterotrophic bioassay, weak estrogen-receptor agonists. This article compares the reproducibility of the dose responses of five weak estrogen agonists using four protocols that include both oral gavage and subcutaneous (sc) routes of administration. An accompanying article demonstrates the reproducibility of the uterotrophic bioassay with prescribed doses selected from this study with blind or coded samples of all five weak agonists, two prescribed EE doses, and a negative test substance (Kanno et al. 2003).

Materials and Methods

Test substances and animals. A centralized chemical repository at TNO, Zeist, the Netherlands, received donated or purchased test substances, weighed and prepared appropriate aliquots in vials for shipment, provided specific instructions for dilution of each substance to prearranged dosages, and arranged the shipment of test substances to the participating laboratories. The test substance sources were Kraemer & Martin (Krefeld, Germany) for EE (CAS no. 57-63-6; purity min. 99%); Bayer AG (Wuppertal, Germany) for bisphenol A (BPA; CAS no. 80-05-7; purity 99.9%); ChemCon GmbH (Freiburg, Germany) for genistein (GN; CAS no. 446-72-0, purity min. 98%; chemically synthesized); Sigma-Aldrich (St. Louis, MO, USA) for methoxychlor (MX; CAS no. 72-43-5; purity 95%); Schenectady International Inc. (Schenectady, NY, USA) for a branched-chain isomers mixture of nonylphenol (NP; CAS no. 25154-52-3, lot 14081-001; purity 95.6%); and Promochem GmbH (Wesel, Germany) for 1,1,1-trichloro-2,2-bis(*o,p'*-chlorophenyl)ethane (*o,p'*-DDT; CAS no. 789-02-6, purity 99.8%). Separate vials of test substance were supplied for the dose–response study and the parallel coded, single-dose study. The laboratories weighed out the required test substance amounts to make up the necessary test solutions in accordance with prepared instructions using their normal standard operating procedures. The instructions were provided to ensure that the doses were comparable across the laboratories for the statistical analyses.

Participating laboratories obtained animals from their normal external or internal sources, including the strain and the animal supply source for the program records.

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These animal studies were performed in accordance with the OECD's guidelines on animal care (OECD 2000) and appropriate national regulations. Animal housing temperature was 22 ± 3C°, the relative humidity was between 30 and 70%, and lighting cycle was 12 hr light and 12 hr dark. If bedding was used, the type and supplier were recorded. Immature animals were group housed with two or three animals per cage, and housing practices for OVX animals were from one to two animals per cage. Feed and tap or filtered drinking water were provided *ad libitum*. The rats were fed the usual rodent diet of the participating laboratory, and the particular diet, the supplier, and the batch or lot number(s) of the diet were recorded. Laboratories did not change the diet during the validation program, and a sample of each lot was frozen and retained for phytoestrogen analyses. In those cases where multiple lots of diet may have been used in a laboratory, the same lot of diet was used for a given protocol. The dietary analyses and the relation of phytoestrogen levels to the uterotrophic bioassay's performance are reported separately (Owens et al. 2003).

Immature, intact animals. Immature animals, if externally supplied, were received either with dams or foster dams on approximately postnatal day (pnd) 14 (date of birth = pnd 0) or as weanlings on pnd 17. Animals were examined for overt signs of ill health and anomalies, and healthy animals were reacclimatized. Animals were allocated into treatment groups of six animals by randomization, ensuring that all groups of animals had a mean weight within ± 5% probability level. Test substance administration could begin at the choice of the participating laboratory on pnd 18, 19, or 20.

Ovariectomized animals. At the time of ovariectomy, the animals were between 42 and 56 days of age. The dorsolateral abdominal wall was opened at the midpoint between the costal inferior border and the iliac crest and a few millimeters lateral to the lateral margin of the lumbar muscle. The ovaries were located, removed from the abdominal cavity, and detached by incision at the junction of the oviduct and each uterine horn. After confirming that no significant bleeding occurred, the abdominal wall was closed by suture, and the skin was closed, for example, by autoclips. The animals were allowed to recover and the uterus weight was allowed to regress for a minimum of 14 days before use.

Protocols. The individual protocols have been described previously (Kanno et al. 2001). Briefly, protocol A used intact, immature female rats as described above with dosing by oral gavage for 3 consecutive days. Protocol B used intact, immature female rats with dosing by sc injection for 3 consecutive days. Protocol C used young adult OVX rats as described above with dosing by sc injection for 3 consecutive days. Protocol D [previously called "protocol C" (Kanno et al. 2001)] also used young adult OVX rats and extended the sc injection dosing to a total of 7 days. As with phase 1, for demonstrating the basic toxicologic attribute of differences in chemical potency due to the route of administration, the VMG decided that only the immature version and the satellite study were adequate to conserve animals and resources. In all protocols, animals were humanely sacrificed 24 hr after the last dose administration.

Vehicle, test substance preparation, and dosing. Test substances were dissolved in a minimal amount of 95% ethanol and diluted to final working concentration in the test vehicle typically used by the participating laboratory (e.g., corn, arachis, sesame, or olive oil). If necessary, the test substance was dissolved with the assistance of gentle heating and vigorous mechanical assistance, for example, homogenized for several minutes in a rotor-stator homogenizer. As the literature indicated, the substances were stable, and most laboratories prepared the test substance weekly. The participating laboratories recorded the nature of the vehicle, the supplier, and lot number, and a sample of the vehicle was retained for analysis, if that became necessary.

Test substance administration was once per day for 3 consecutive days in three protocols (A, B, and C), and once per day for 7 consecutive days in a fourth protocol (D). The amount administered was calculated using the body weight (bw) of the animal recorded on the day of treatment. Treatment on each consecutive day was at approximately the same time and sequence for each animal. For oral gavage (protocol A and a single satellite study using oral gavage with OVX animals for 3 days), the total volume per rat per day did not exceed 5 mL/kg bw/day. For sc injection (protocols B, C, and D), the total amount of sc injection per rat per day did not exceed 4 mL/kg bw/day, and the maximum volume per injection site per rat did not exceed 0.2 mL. The precise method and volumes of administration by the individual participating laboratory were recorded. Animals were observed for clinical signs, the body weights were recorded daily to 0.1 g, any animals observed to be in distress were humanely sacrificed, and any animals found dead were disposed of.

Necropsy, dissection, and uterine weight. Twenty-four hours after the last treatment, the animals were humanely killed by the method routinely used by the participating laboratory in the same sequence as the test substance was administered. The uterus was carefully dissected, the ovaries of immature animals removed, and the uterus trimmed of fascia and fat to avoid loss of luminal contents. The uterus and cervix were removed by incision at the vaginal fornix to preserve the luminal fluid contents. The uterus was transferred to a marked, tared container with care to avoid desiccation. This first uterine weight (wet weight) included the luminal fluid contents and was recorded to the nearest 0.1 mg. Each uterus was then opened by piercing or longitudinal cuts into the uterine wall, and the luminal fluid was expressed with gentle pressure on moistened filter paper. The uterus was then weighed a second time (blotted weight), and the weight was recorded to the nearest 0.1 mg.

Study management and quality control. The study director was on the OECD staff, and each laboratory nominated a principal investigator as recommended by OECD Good Laboratory Practice and Study Management guidelines (OECD 2002). The laboratories were requested to perform these studies under these OECD Good Laboratory Practice guidelines and most, but not all, did so. When data were assembled and an initial statistical analysis performed, all laboratories were requested to audit these raw data and to respond to specific queries on outliers and questionable data. A small number of data corrections were made, and reporting errors on dilutions, samples, and identity of control groups were either corrected or clarified.

Statistics. The raw data uterine weights and body weights from each participating laboratory were recorded on a standardized electronic spreadsheet and submitted to an independent statistician for analysis. The uterine data were evaluated by an analysis of covariance approach with body weight at necropsy as the covariable. A variance-stabilizing logarithmic transformation was carried out on the uterine data prior to the data analysis. The Dunnett and Hsu test was used for making pairwise comparisons of each dosed group to vehicle controls and to calculate the confidence intervals. Studentized residual plots were used to detect possible outliers and to assess homogeneity of variances. The data were analyzed using the PROC GLM in the Statistical Analysis System (version 8; SAS Institute, Cary, NC). In addition to the 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 animal at necropsy was also calculated.

Design of Phase 2 Dose–Response Study

The principal question was whether the standardized protocols would achieve the same degree of reliability and reproducibility,

as demonstrated with the strong agonist EE, when testing selected substances of lower estrogenic potencies. The primary objectives of the phase 2 dose–response studies were to demonstrate that participating laboratories could detect several selected weak estrogen agonists by a statistically significant increase in uterine weights, that the results were reproducible across laboratories, and that the animals would respond in a dose-related manner. The doses producing the first significant increase in uterine weights and the magnitude of the responses of these weak agonists were also to be compared with the potent reference estrogen, EE. Other objectives were to test whether the intact, immature version and the OVX version were generally equivalent in performance and their ability to detect the activity of weak agonists, and to quantify the variability of the dose response among laboratories and among protocols testing the equivalence of the protocols. The statistical analyses of these performance comparisons and determinations required a series of identical, prescribed doses for each test substance. If any laboratory was unable to detect the selected weak agonists, an effort would be made to determine the responsible factors.

Selection of Weak Agonists and Doses

The VMG selected five weak estrogen agonists: BPA, GN, MX, NP, and *o,p'*-DDT. For these substances, *a)* individual binding affinities to the estrogen receptor had been determined in a single laboratory, *b)* evidence from the literature was available for estrogen-mediated activity in other *in vitro* assay systems, *c)* evidence from the literature was available that each weak agonist displayed positive response in the uterotrophic bioassay, and *d)* either subchronic or chronic testing data were available to indicate whether the compounds elicited estrogen-related effects, or such subchronic or chronic testing was in progress. Collectively, such data indicated that the selected substances were weak estrogen receptor agonists *in vitro*, were positive challenge substances for a validation study of the uterotrophic bioassay, and there were sufficient data for estrogen-related effects in higher tiers to assess the predictivity of the uterotrophic bioassay at the end of the validation program. These data are compliant with test substance selection recommendations to demonstrate the characteristics of a bioassay for validation studies and the relationship of a bioassay to other assays in a hierarchical, tiered approach (ICCVAM 1997; OECD 1998b). The chemical identities and estrogen-receptor binding data from Blair et al. (2000) and Branham et al. (2002) are shown in Table 1. The binding affinities of the selected weak agonists relative to 17β-estradiol cover a range of almost three orders of magnitude, for example, log −0.35 to −3.20, and even the two most potent selected agonists, GN and the metabolite of MX, are almost three orders of magnitude weaker than the reference EE agonist. Therefore, the selected weak agonists were judged to represent the range of potency that the uterotrophic bioassay would likely encounter in regulatory applications.

In addition, test substances were selected for expected differences in behavior in pharmacokinetic behavior to represent the variety of test substances likely to be encountered by the uterotrophic bioassay during use and to demonstrate differences between the oral and sc routes of administration observed in several pharmacokinetic studies below. Three test substances—BPA, GN, and NP—are reported to be rapidly eliminated and to undergo significant intestinal and hepatic conjugation, leading to a hypothesis of lower potency by the oral route of administration (Chang et al. 2000; Coldham and Sauer 2000; Fennell et al. 1998; Miyakoda et al. 2000; Müller et al. 1998; Pottenger et al. 2000). MX is reported to undergo hepatic activation, leading to a hypothesis of higher potency by the oral route of administration (Bulger et al. 1978). Finally, *o,p'*-DDT was selected because of the absence of a hydroxyl group necessary for rapid conjugation, and its persistence and bioaccumulation, leading to the hypothesis that it might display unique pharmacokinetic characteristics. Therefore, the selected weak agonists were judged to represent the range of test substance characteristics that the uterotrophic bioassay would likely encounter in regulatory applications.

As part of the overall design, five doses were recommended for each test substance. However, because of possible resource constraints, participating laboratories were required to use only the three intermediate doses. The VMG established a working group to review the scientific literature concerning each of the test substances, to consult researches for unpublished data, and then to select the doses for each substance and route of administration. Unfortunately, much of the background literature information from both published and "gray" sources did not report all necessary protocol details, use defined and closely interspersed doses, or consistently report the data as absolute uterine weight increases. Thus, the literature studies were not strictly comparable or unambiguous for dose-selection purposes. Because of the urgency and the complex logistics of an international validation program, the VMG decided to forego preliminary dose-setting studies. Therefore, the working group was required to rely upon its own expert judgment to recommend the dose levels, and risks were accepted that some laboratories might not achieve a complete dose–response curve.

To conserve animals and resources and to achieve a core of robust data for comparison, the VMG decided that priority in the dose–response work was to compare the results for NP and BPA. If additional laboratory resources were available, the remaining weak agonists, GN, MX and *o,p'*-DDT, would be examined. The doses recommended for the oral gavage studies were as follows: for BPA—60, 200, 375, 600, and 1,000 mg/kg/day; for GN—20, 60, 120, 300, and 500 mg/kg/day; for MX—20, 50, 120, 300, and 500 mg/kg/day; for NP—15, 75, 125, 250, and 350 mg/kg/day; and for *o,p'*-DDT—10, 50, 125, 300, and 600 mg/kg/day. The doses recommended for the sc injection studies were as follows: for BPA—10, 100, 300, 600, and 800 mg/kg/day; for GN—1, 15, 35, 50, and 80 mg/kg/day; for MX—20, 100, 250, 500, and 800 mg/kg/day; for NP—5, 15, 35, 80, and 100 mg/kg/day; and for *o,p'*-DDT—5, 25, 50, 100, and 200 mg/kg/day. All of the above doses were lower than the standard toxicologic limit dose of 1,000 mg/kg/day except for the final oral gavage dose of BPA, which was at the limit dose.

Results of Phase 2 Dose–Response Studies

A total of 86 dose–response studies were performed by 17 laboratories. Four other laboratories, which either participated in phase 1 (Kanno et al. 2001) or the coded single-dose studies in phase 2 (Kanno et al. 2003), did

Table 1. Rat uterine cytosol ERα receptor–binding data.[a,b]

Chemical name	Mean IC_{50} (M) ± SEM	RBA (%)	Log RBA
EE	$4.73 \times 10^{-10} \pm 0.60 \times 10^{-10}$	190.063	2.28
17β-Estradiol	$8.99 \times 10^{-10} \pm 0.27 \times 10^{-10}$	100.000	2.00
GN	$2.00 \times 10^{-7} \pm 0.21 \times 10^{-7}$	0.443	−0.35
HPTE	$3.55 \times 10^{-7} \pm 0.15 \times 10^{-7}$	0.253	−0.60
NP	$3.05 \times 10^{-6} \pm 0.15 \times 10^{-6}$	0.029	−1.53
BPA	$1.17 \times 10^{-5} \pm 0.64 \times 10^{-6}$	0.008	−2.11
o,p'-DDT	$6.43 \times 10^{-5} \pm 0.89 \times 10^{-6}$	0.001	−2.85
MX	$1.44 \times 10^{-4} \pm 0.66 \times 10^{-4}$	0.001	−3.20

Abbreviations: IC_{50}, the concentration of ligand that reduces the binding of native 17β-estradiol by 50%; RBA, relative binding affinity of the ligand to the native 17β-estradiol.
[a]Data modified from tables in Blair et al. (2000) and Branham et al. (2002). [b]The binding curves were generated in a single laboratory on the basis of a single protocol using closely interspersed concentrations and performed in triplicate.

not participate in the dose–response studies. Because the laboratory numbers were kept consistent from 1 through 21 throughout the entire program, laboratories numbers 10, 16, 17, and 19 will not appear in this paper.

Mortalities, decreases in body weight or body weight gain, and clinical signs. Out of a total of 2,652 animals, there were 45 mortalities observed in 10 laboratories: 5 in BPA studies, 6 in MC studies, 19 in NP studies, and 15 in DDT studies. Forty-two of the mortalities were in protocol A treatment studies using oral gavage. A dose-related pattern of modest reductions in body weights and diminished body weight gains was often observed in the immature animal studies and in the extending dosing of the OVX studies. Decreases in body weights at terminal sacrifice approaching or greater than 10%, indicating that the dose exceeded a maximum tolerated dose, were observed at doses of 100 mg BPA/kg/day and higher in both protocol D studies, at doses of 500 mg 4X/kg/day and higher in both protocol D studies, at doses of 75 mg NP/kg/day and higher in 3 of 4 protocol A studies, and at doses of 300 mg DDT/kg/day in all protocol A studies. Clinical signs were reported in conjunction with the mortalities and body weight losses, including piloerection, lethargy and reduced mobility, and labored breathing.

Bisphenol A. A total of 22 dose–response studies were conducted with BPA, including 4 with protocol A, 10 with protocol B, 5 with protocol C, 2 with protocol D, and a satellite study using oral gavage with OVX animals. Twenty of 21 studies were successful in detecting increases in uterine weights at one or more of the prescribed doses. In the case of laboratory 21, the required terminal body weights were not recorded for the dose–response studies. Because the statistical analysis was based upon using terminal body weights as a covariant with the uterine blotted weight data, the body weight–adjusted statistical analysis was not performed on the data from this laboratory. However, the data such as mean wet and blotted uterine weights for laboratory 21 are reported in Table 3 and Figure 1, and these have been statistically compared without body weight adjustment.

Within each protocol, there was overall agreement among different laboratories both in the magnitude of the uterine weight increases and in the BPA doses first producing a statistically significant increase in uterine weight. In protocol A using oral gavage, all four studies detected statistically significant increases in uterine weights at lowest observed effect level (LOEL) doses of 375 mg BPA/kg/day (two studies), 600 mg/kg/day (one study), and 1,000 mg/kg/day (one study) (Table 2). In protocol B, eight studies detected statistically significant increases in uterine weights at doses of 10 mg BPA/kg/day (one study), 100 mg/kg/day (three studies), 300 mg/kg/day (three studies), and 600 mg/kg/day (one study). However, in a ninth study, statistical significance was achieved at doses of 10 and 100 mg BPA/kg/day, no statistical difference was observed at 300 mg/kg/day, and statistically significant decreases in uterine weights were observed at 600 and 800 mg BPA/kg/day. Effectively, the reported dose response in this laboratory was the mirror opposite of the expectations and the results from all other laboratories (Table 3, laboratory 20). In protocol C, all five of the studies detected statistical significance at doses of 100 mg BPA/kg/day (Table 4). In protocol D, both studies detected statistical significance at doses of 100 mg BPA/kg/day (Table 5). The satellite study with OVX animals using oral gavage administration did not detect statistically significant increases in uterine weight at the highest of the three intermediate doses used in that study, 600 mg/kg/day (i.e., the highest 1,000-mg BPA/kg/day dose was not tested in this laboratory with this protocol) (Table 6).

The BPA results, except for the satellite study, are shown graphically in Figure 1. In protocols B, C, and D using sc injection, the ratio of the maximum mean uterine weights of the treated groups relative to the vehicle controls was generally between 3 and 4. The slope appeared to be steeper in the OVX animals, and the extension of the dosing to 7 days appeared to slightly increase the overall response. The maximum increase observed in uterine weights was considerably lower in protocol A, where the ratio of the maximum uterine weight increase to the vehicle controls was approximately 1.5 relative to the controls, and there was greater variability among the

Table 2. Uterine weights, body weights, and ratio of the relative increase in uterine weights for bisphenol A in protocol A.

Laboratory	Measure	Vehicle	Dose 1 (60 mg/kg/day)	Dose 2 (200 mg/kg/day)	Dose 3 (375 mg/kg/day)	Dose 4 (600 mg/kg/day)	Dose 5 (1,000 mg/kg/day)
2	Wet weight (mg, mean ± SD)	26.5 ± 4.20	26.8 ± 3.23	30.1 ± 1.60	30.4 ± 5.82	37.0 ± 5.54	44.1 ± 8.36[a]
	Blotted weight (mg, mean ± SD)	25.4 ± 4.19	25.7 ± 2.94	29.0 ± 1.86	29.4 ± 5.85	35.8 ± 5.68	42.8 ± 8.32
	bw (g, mean ± SD)	46.6 ± 7.14	48.0 ± 5.86	47.7 ± 3.48	42.5 ± 4.59	44.3 ± 4.00	45.3 ± 3.35
	Absolute ratio[b]		1.01	1.14	1.16	1.41	1.69
	bw adjusted ratio[c]		0.99	1.13	1.26*	1.49*	1.73*
	(Lower CL, upper CL)[d]		(0.83, 1.17)	(0.95, 1.34)	(1.06, 1.50)	(1.25, 1.77)	(1.45, 2.07)
	Wet weight (mg, mean ± SD)	30.9 ± 2.95	33.1 ± 3.24	36.0 ± 3.46	37.5 ± 4.35	50.9 ± 18.34	52.0 ± 3.19
	Blotted weight (mg, mean ± SD)	29.5 ± 2.95	32.2 ± 3.13	34.8 ± 3.48	36.1 ± 3.89	49.1 ± 17.77	50.4 ± 2.94
	bw (g, mean ± SD)	56.7 ± 1.74	56.3 ± 3.51	55.0 ± 3.15	54.8 ± 2.75	53.5 ± 3.92	53.5 ± 2.29
	Absolute ratio		0.89	0.96	1.00	1.36	1.40
	bw adjusted ratio		0.89	0.97	1.00	1.31*	1.40*
	(Lower CL, upper CL)		(0.70, 1.13)	(0.76, 1.22)	(0.79, 1.27)	(1.03, 1.66)	(1.10, 1.78)
12	Wet weight (mg, mean ± SD)	24.2 ± 2.48	Not done	29.6 ± 5.75	31.0 ± 1.43	39.1 ± 7.43[e]	Not done
	Blotted weight (mg, mean ± SD)	20.6 ± 1.81		25.8 ± 5.19	26.8 ± 2.44	33.8 ± 6.04	
	bw (g, mean ± SD)	39.7 ± 3.10		38.5 ± 2.17	33.7 ± 3.82	39.5 ± 6.00	
	Absolute ratio			1.26	1.30	1.61	
	bw adjusted ratio			1.25	1.36*	1.63*	
	(Lower CL, upper CL)			(0.9995[f], 1.56)	(1.05, 1.76)	(1.29, 2.06)	
13	Wet weight (mg, mean ± SD)	39.0 ± 6.51	39.7 ± 4.93	49.7 ± 20.39	43.3 ± 5.35	43.0 ± 4.30[e]	59.0 ± 9.27
	Blotted weight (mg, mean ± SD)	31.8 ± 3.66	32.0 ± 3.35	32.6 ± 14.60	32.3 ± 3.98	34.4 ± 2.70	49.0 ± 8.72
	Body weight (g, mean ± SD)	41.5 ± 2.74	42.2 ± 3.66	42.3 ± 19.70	39.5 ± 3.78	31.4 ± 3.36	36.0 ± 3.58
	Absolute ratio		1.01	1.02	1.02	1.08	1.54
	bw adjusted ratio		1.00	1.16	1.03	1.17	1.57*
	(Lower CL, upper CL)		(0.77, 1.31)	(0.88, 1.51)	(0.78, 1.35)	(0.79, 1.72)	(1.18, 2.08)

[a]One animal died in 1,000-mg BPA/kg/day group before necropsy. [b]Ratio of arithmetic means of the treated blotted uterine weights relative to the vehicle control blotted uterine weights. [c]Ratio of geometric means of treated blotted uterine weights relative to the vehicle control blotted uterine weights after adjusting for the body weights at necropsy as a covariable [d]Lower and upper 95% confidence limits (CL) for ratio of blotted uterine weights based on body weights as a covariable. [e]One animal died in 600 mg BPA/kg/day group before necropsy. [f]With the lower 95% confidence limit not > 1.0, the result is not statistically significant. *Level of significance, $p < 0.05$.

studies. Comparing protocols B and C, the dose–response curves among laboratories are somewhat more variable between the intact, immature animals and the OVX animals are not appreciably different, taking into consideration the larger number of laboratories conducting protocol B (Figure 1).

Genistein. A total of 14 dose–response studies were conducted with GN, including 4 with protocol A, 4 with protocol B, 3 with protocol C, 2 with protocol D, and a satellite study using oral gavage with OVX animals. All studies in all protocols were successful in detecting increases in uterine weights at one or more prescribed doses.

Table 3. Uterine weights, body weights, and ratio of the relative increase in uterine weights for bisphenol A in protocol B.

Laboratory	Measure	Vehicle	Dose 1 (10 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (300 mg/kg/day)	Dose 4 (600 mg/kg/day)	Dose 5 (800 mg/kg/day)
2	Wet weight (mg, mean ± SD)	28.1 ± 1.98	31.0 ± 2.42	46.2 ± 6.92	62.1 ± 6.24	98.3 ± 27.58	144.5 ± 53.95
	Blotted weight (mg, mean ± SD)	26.5 ± 1.80	29.4 ± 2.44	44.5 ± 6.40	59.8 ± 5.72	88.0 ± 17.01	105.0 ± 15.13
	bw (g, mean ± SD)	51.5 ± 2.45	49.9 ± 2.88	51.5 ± 3.56	48.8 ± 3.53	50.5 ± 2.23	49.5 ± 3.85
	Absolute ratio		1.11	1.68	2.25	3.32	3.96
	bw adjusted ratio		1.12	1.67*	2.30*	3.30*	4.00*
	(Lower CL, upper CL)[a]		(0.92, 1.36)	(1.37, 2.02)	(1.88, 2.81)	(2.72, 4.01)	(3.28, 4.87)
6	Wet weight (mg, mean ± SD)	61.1 ± 15.24	Not done	72.7 ± 17.73	80.6 ± 16.86	131.7 ± 59.15[b]	Not done
	Blotted weight (mg, mean ± SD)	50.0 ± 14.00		69.1 ± 17.38	76.8 ± 15.96	115.5 ± 39.04	
	bw (g, mean ± SD)	48.9 ± 8.15		49.0 ± 6.92	47.9 ± 7.07	52.6 ± 6.68	
	Absolute ratio			1.19	1.32	1.99	
	bw adjusted ratio			1.18	1.37*	1.75*	
	(Lower CL, upper CL)			(0.90, 1.54)	(1.05, 1.79)	(1.31, 2.33)	
7	Wet weight (mg, mean ± SD)	34.5 ± 4.30	34.0 ± 2.82	44.2 ± 4.32	65.9 ± 10.58	161.6 ± 38.51	209.7 ± 35.88
	Blotted weight (mg, mean ± SD)	32.8 ± 4.26	32.9 ± 2.92	42.8 ± 4.22	64.2 ± 9.86	113.0 ± 10.39	119.0 ± 9.64
	bw (g, mean ± SD)	57.6 ± 4.26	56.6 ± 3.96	57.2 ± 3.70	57.2 ± 3.57	54.9 ± 2.67	54.7 ± 2.69
	Absolute ratio		1.00	1.30	1.95	3.44	3.63
	bw adjusted ratio		1.01	1.31*	1.95*	3.47*	3.66*
	(Lower CL, upper CL)		(0.85, 1.20)	(1.10, 1.56)	(1.64, 2.32)	(2.90, 4.15)	(3.06, 4.37)
8	Wet weight (mg, mean ± SD)	25.2 ± 2.79	29.5 ± 4.42	36.5 ± 5.35	48.1 ± 7.34	53.4 ± 11.59	77.4 ± 16.85
	Blotted weight (mg, mean ± SD)	23.5 ± 2.33	27.8 ± 4.24	34.5 ± 5.07	45.5 ± 7.26	50.7 ± 10.71	70.1 ± 9.13
	bw (g, mean ± SD)	51.9 ± 6.75	52.1 ± 7.57	50.8 ± 1.96	52.6 ± 3.59	51.4 ± 7.00	49.6 ± 5.42
	Absolute ratio		1.18	1.47	1.93	2.15	2.98
	bw adjusted ratio		1.17	1.47*	1.91*	2.13*	3.01*
	(Lower CL, upper CL)		(0.92, 1.50)	(1.15, 1.87)	(1.50, 2.43)	(1.67, 2.71)	(2.36, 3.84)
12	Wet weight (mg, mean ± SD)	26.8 ± 6.97	Not done	34.7 ± 3.59	32.1 ± 6.64	65.2 ± 23.00	Not done
	Blotted weight (mg, mean ± SD)	22.4 ± 6.47		31.4 ± 4.47	28.2 ± 6.64	56.3 ± 17.81	
	bw (g, mean ± SD)	40.4 ± 3.38		38.1 ± 5.62	36.8 ± 5.79	39.7 ± 4.08	
	Absolute ratio			1.40	1.26	2.51	
	bw adjusted ratio			1.47	1.33	2.51*	
	(Lower CL, upper CL)			(0.9925[c], 2.19)	(0.88, 1.99)	(1.70, 3.70)	
13	Wet weight (mg, mean ± SD)	33.4 ± 7.02	37.0 ± 9.27	45.3 ± 6.56	61.5 ± 10.82	112.7 ± 35.60	142.0 ± 25.64
	Blotted weight (mg, mean ± SD)	28.0 ± 3.46	31.2 ± 7.28	38.8 ± 12.95	51.3 ± 15.60	82.2 ± 31.40	104.8 ± 8.80
	bw (g, mean ± SD)	45.2 ± 2.32	44.2 ± 3.60	41.5 ± 4.04	45.8 ± 3.06	43.3 ± 3.56	42.3 ± 2.73
	Absolute ratio		1.11	1.39	1.83	2.93	3.74
	bw adjusted ratio		1.14	1.50	1.72*	2.88*	4.15*
	(Lower CL, upper CL)		(0.71, 1.82)	(0.91, 2.47)	(1.08, 2.76)	(1.78, 4.64)	(2.55, 6.77)
15	Wet weight (mg, mean ± SD)	33.2 ± 5.56	35.3 ± 8.19	36.2 ± 4.26	50.2 ± 6.18	82.8 ± 23.64	132.7 ± 43.37
	Blotted weight (mg, mean ± SD)	28.7 ± 5.47	26.3 ± 4.68	27.3 ± 4.80	36.8 ± 6.91	67.8 ± 13.00	87.5 ± 18.07
	bw (g, mean ± SD)	48.3 ± 3.65	46.3 ± 3.70	46.4 ± 2.38	44.8 ± 3.84	44.3 ± 4.77	46.9 ± 3.16
	Absolute ratio		0.92	0.95	1.28	2.37	3.05
	bw adjusted ratio		0.95	0.98	1.37*	2.54*	3.11*
	(Lower CL, upper CL)		(0.72, 1.25)	(0.75, 1.29)	(1.03, 1.81)	(1.91, 3.37)	(2.37, 4.08)
18	Wet weight (mg, mean ± SD)	25.0 ± 1.66	30.9 ± 3.00	37.7 ± 3.27	51.1 ± 5.50	98.6 ± 16.36	144.9 ± 44.28
	Blotted weight (mg, mean ± SD)	21.3 ± 1.50	28.5 ± 3.62	33.8 ± 3.85	46.6 ± 5.28	72.1 ± 5.41	95.0 ± 10.63
	bw (g, mean ± SD)	52.1 ± 3.70	57.1 ± 4.91	53.0 ± 4.55	55.1 ± 3.76	53.8 ± 3.11	52.6 ± 3.02
	Absolute ratio		1.34	1.59	2.19	3.38	4.46
	bw adjusted ratio		1.28*	1.57*	2.12*	3.33*	4.42*
	(Lower CL, upper CL)		(1.08, 1.51)	(1.34, 1.83)	(1.81, 2.50)	(2.85, 3.91)	(3.78, 5.17)
20	Wet weight (mg, mean ± SD)	57.7 ± 13.08	36.2 ± 7.20	35.8 ± 4.29	53.7 ± 9.83	90.2 ± 18.97	107.3 ± 30.72
	Blotted weight (mg, mean ± SD)	54.3 ± 11.77	27.4 ± 7.56	31.6 ± 4.90	50.8 ± 9.08	81.7 ± 13.65	92.9 ± 15.35
	bw (g, mean ± SD)	50.7 ± 4.01	51.6 ± 1.78	52.8 ± 1.74	51.4 ± 1.87	50.4 ± 3.32	51.4 ± 2.84
	Absolute ratio		1.71	1.50	0.94	0.58	0.51
	bw adjusted ratio		1.75*	1.57*	0.95	0.59	0.50
	(Lower CL, upper CL)		(1.26, 2.43)	(1.12, 2.19)	(0.69, 1.32)	(0.42, 0.81)	(0.36, 0.69)
21	Wet weight (mg, mean ± SD)	58.0 ± 7.84	81.4 ± 9.96	86.8 ± 8.72	107.7 ± 12.03	120.9 ± 15.32	136.1 ± 13.55
	Blotted weight (mg, mean ± SD)	47.3 ± 6.92	67.7 ± 7.79	71.0 ± 9.08	89.0 ± 11.37	93.4 ± 14.04	113.7 ± 10.19
	bw (g, mean ± SD)	—[d]	—[d]	—[d]	—[d]	—[d]	—[d]
	Absolute ratio		1.43*[e]	1.50*[e]	1.89*[e]	1.97*[e]	2.42*[e]
	bw adjusted ratio		—[d]	—[d]	—[d]	—[d]	—[d]
	(Lower CL, upper CL)		—[d]	—[d]	—[d]	—[d]	—[d]

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]One animal died in 600 mg BPA/kg/day group before necropsy. [c]With the lower 95% confidence limit not > 1.0, the result is not statistically significant. [d]Terminal body weights were not recorded by the laboratory. [e]The blotted uterine weights were analyzed without body weight adjustments and were found to be statistically significant. *Level of significance, $p < 0.05$.

Within each protocol, there was overall agreement among different laboratories both in the magnitude of the uterine weight increases and in the GN doses first producing a statistically significant increase in uterine weight. In protocol A using oral gavage, two studies detected statistically significant increases in uterine weights at LOEL doses of 20 mg GN/kg/day and the other two studies at doses of 60 mg/kg/day (Table 7). In protocol B, one study detected statistically significant increases in uterine weights at a dose of 1 mg GN/kg/day and the other three studies at doses of 15 mg/kg/day (Table 8). In protocol C, two of the studies detected statistical significance at doses

Table 4. Uterine weights, body weights, and ratio of the relative increase in uterine weights for bisphenol A in protocol C.

Laboratory	Measure	Vehicle	Dose 1 (10 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (300 mg/kg/day)	Dose 4 (600 mg/kg/day)	Dose 5 (800 mg/kg/day)
2	Wet weight (mg, mean ± SD)	103.9 ± 13.20	116.9 ± 13.00	210.3 ± 62.72	439.1 ± 129.16	588.4 ± 161.90	728.3 ± 201.57
	Blotted weight (mg, mean ± SD)	99.8 ± 10.76	112.5 ± 11.69	188.3 ± 25.51	278.6 ± 35.86	306.7 ± 32.98	301.9 ± 43.25
	bw (g, mean ± SD)	250.9 ± 13.24	251.4 ± 9.97	240.2 ± 12.08	238.0 ± 13.90	236.4 ± 11.03	229.9 ± 17.53
	Absolute ratio		1.13	1.89	2.79	3.07	3.02
	bw adjusted ratio		1.13	1.89[c]	2.79[c]	3.08[c]	3.03[c]
	(Lower CL, upper CL)[a]		(0.93, 1.37)	(1.55, 2.30)	(2.28, 3.41)	(2.52, 3.78)	(2.45, 3.75)
6	Wet weight (mg, mean ± SD)	115.5 ± 19.84	Not done	236.7 ± 43.08	274.1 ± 69.59	728.8 ± 207.15	Not done
	Blotted weight (mg, mean ± SD)	110.7 ± 19.60		219.5 ± 45.59	236.1 ± 53.39	393.7 ± 68.46	
	bw (g, mean ± SD)	299.6 ± 29.76		291.6 ± 12.58	269.6 ± 24.97	277.5 ± 8.91	
	Absolute ratio			1.98	2.13	3.56	
	bw adjusted ratio			2.05*	2.41*	3.92*	
	(Lower CL, upper CL)			(1.58, 2.66)	(1.79, 3.23)	(2.97, 5.18)	
7	Wet weight (mg, mean ± SD)	91.4 ± 13.17	93.9 ± 10.84	150.3 ± 24.55	619.1 ± 157.48[b]	764.9 ± 173.18	825.8 ± 240.53
	Blotted weight (mg, mean ± SD)	88.8 ± 12.90	91.5 ± 10.46	146.7 ± 23.53	294.2 ± 23.44	333.3 ± 32.99	318.5 ± 32.10
	bw (g, mean ± SD)	250.2 ± 12.36	250.6 ± 13.27	243.3 ± 12.50	229.5 ± 11.72	236.5 ± 9.30	237.9 ± 9.74
	Absolute ratio		1.03	1.65	3.31	3.75	3.59
	bw adjusted ratio		1.03	1.67*	3.44*	3.85*	3.67*
	(Lower CL, upper CL)		(0.86, 1.24)	(1.38, 2.01)	(2.76, 4.30)	(3.16, 4.70)	(3.02, 4.47)
8	Wet weight (mg, mean ± SD)	92.5 ± 10.51	90.4 ± 7.82	152.1 ± 29.28	353.9 ± 86.49	355.3 ± 97.07	388.1 ± 113.91
	Blotted weight (mg, mean ± SD)	88.0 ± 9.76	86.0 ± 7.29	139.3 ± 21.94	229.2 ± 35.16	243.4 ± 30.12	239.6 ± 35.55
	bw (g, mean ± SD)	291.0 ± 17.09	291.5 ± 17.04	282.3 ± 11.60	281.2 ± 14.29	276.3 ± 21.93	276.5 ± 19.53
	Absolute ratio		0.98	1.58	2.60	2.77	2.72
	bw adjusted ratio		0.98	1.60*	2.65*	2.85*	2.79*
	(Lower CL, upper CL)		(0.80, 1.20)	(1.31, 1.96)	(2.16, 3.24)	(2.32, 3.51)	(2.27, 3.43)
12	Wet weight (mg, mean ± SD)	106.0 ± 18.84	Not done	225.4 ± 45.83	444.5 ± 89.56	837.0 ± 207.10	Not done
	Blotted weight (mg, mean ± SD)	98.6 ± 22.04		197.4 ± 33.88	266.3 ± 44.60	314.1 ± 60.01	
	bw (g, mean ± SD)	297.2 ± 14.54		291.8 ± 12.26	299.9 ± 10.99	289.3 ± 23.00	
	Absolute ratio			2.00	2.70	3.19	
	bw adjusted ratio			2.03*	2.72*	3.24*	
	(Lower CL, upper CL)			(1.53, 2.70)	(2.05, 3.61)	(2.43, 4.32)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]One animal died in 300 mg BPA/kg/day group before necropsy. [c]Level of significance, $p < 0.05$.

Table 5. Uterine weights, body weights, and ratio of the relative increase in uterine weights for bisphenol A in protocol D.

Laboratory	Measure	Vehicle	Dose 1 (10 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (300 mg/kg/day)	Dose 4 (600 mg/kg/day)	Dose 5 (800 mg/kg/day)
2	Wet weight (mg, mean ± SD)	89.0 ± 13.97	100.8 ± 16.54	214.5 ± 14.44	342.8 ± 42.58	613.0 ± 141.93	484.8 ± 139.04
	Blotted weight (mg, mean ± SD)	86.2 ± 13.56	97.6 ± 16.07	209.7 ± 13.14	306.8 ± 18.43	389.9 ± 57.69	353.9 ± 48.07
	bw (g, mean ± SD)	274.6 ± 15.93	269.2 ± 20.29	246.8 ±9.88	236.2 ± 10.71	242.5 ± 16.54	230.5 ± 24.79
	Absolute ratio		1.13	2.43	3.56	4.52	4.11
	bw adjusted ratio		1.14	2.53*	3.74*	4.69*	4.31*
	(Lower CL, upper CL)[a]		(0.91, 1.41)	(1.99, 3.21)	(2.89, 4.84)	(3.67, 5.99)	(3.28, 5.67)
7	Wet weight (mg, mean ± SD)	82.2 ± 2.94	91.1 ± 7.47	192.7 ± 6.30	358.8 ± 109.44	421.4 ± 72.68	525.8 ± 41.04
	Blotted weight (mg, mean ± SD)	80.4 ± 2.70	88.8 ± 7.70	188.8 ± 4.96	314.1 ± 40.32	346.7 ± 41.94	376.9 ± 27.57
	bw (g, mean ± SD)	283.7 ± 14.51	285.8 ± 14.66	259.1 ± 11.75	245.7 ± 5.74	249.5 ± 7.28	244.5 ± 6.29
	Absolute ratio		1.11	2.35	3.91	4.32	4.69
	bw adjusted ratio		1.10	2.35*	3.90*	4.30*	4.70*
	(Lower CL, upper CL)		(0.97, 1.26)	(2.00, 2.77)	(3.18, 4.78)	(3.56, 5.19)	(3.84, 5.75)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 6. Uterine weights, body weights, and ratio of the relative increase in uterine weights for bisphenol A in satellite OVX protocol by oral gavage.

Laboratory	Measure	Vehicle	Dose 1 (60 mg/kg/day)	Dose 2 (200 mg/kg/day)	Dose 3 (375 mg/kg/day)	Dose 4 (600 mg/kg/day)	Dose 5 (1,000 mg/kg/day)
12	Wet weight (mg, mean ± SD)	101.1 ± 16.93	Not done	120.9 ± 11.63	133.7 ± 38.71	130.9 ± 11.92	Not done
	Blotted weight (mg, mean ± SD)	95.0 ± 16.43		112.4 ± 10.36	125.2 ± 38.35	125.3 ± 10.03	
	bw (g, mean ± SD)	295.5 ± 11.09		281.7 ±14.55	289.7 ± 11.37	278.5 ± 11.92	
	Absolute ratio			1.18	1.32	1.32	
	bw adjusted ratio			1.16	1.27	1.29	
	(Lower CL, upper CL)[a]			(0.86, 1.56)	(0.97, 1.68)	(0.94, 1.75)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable.

of 15 mg GN/kg/day and another at a dose of 35 mg/kg/day (Table 9). In protocol D, both studies detected statistical significance at doses of 15 mg GN/kg/day (Table 10). The satellite study with OVX animals using oral gavage administration detected statistically significant increases in uterine weight at the lowest of the three intermediate doses used in that study, 60 mg/kg/day (i.e., the lowest 20-mg GN/kg/day dose was not tested in this laboratory with this protocol) (Table 11).

The GN results, except for the satellite study, are shown graphically in Figure 2. In protocol A using oral gavage, the ratio of the maximum mean uterine weights of the treated groups to the controls was generally between 2.5 and 3.5. In protocol B with intact, immature animals, the ratio relative to the controls was again 2.5 to nearly 4. In protocol C, the maximum induction was less, with the ratio approaching 2. In protocol D with extended dosing to 7 days, the response in the mature OVX animals reached an equivalent maximum response to the intact immature animals after 3 days of dosing.

Methoxychlor. A total of 14 dose–response studies were conducted with MX, including 4 with protocol A, 4 with protocol B, 3 with protocol C, 2 with protocol D, and a satellite study using oral gavage with OVX animals. All studies in all protocols were successful in detecting increases in uterine weights at one or more prescribed doses.

Within each protocol, there was overall agreement among different laboratories both in the magnitude of the uterine weight increases and in the MX doses first producing a statistically significant increase in uterine weight. In protocol A using oral gavage, three studies detected statistically significant increases in uterine weights at the LOEL dose of 20 mg MX/kg/day. Laboratory 12, however, used only the three intermediate doses and detected statistically significant increases in uterine weights at its lowest dose of 50 mg/kg/day, where the ratio of relative

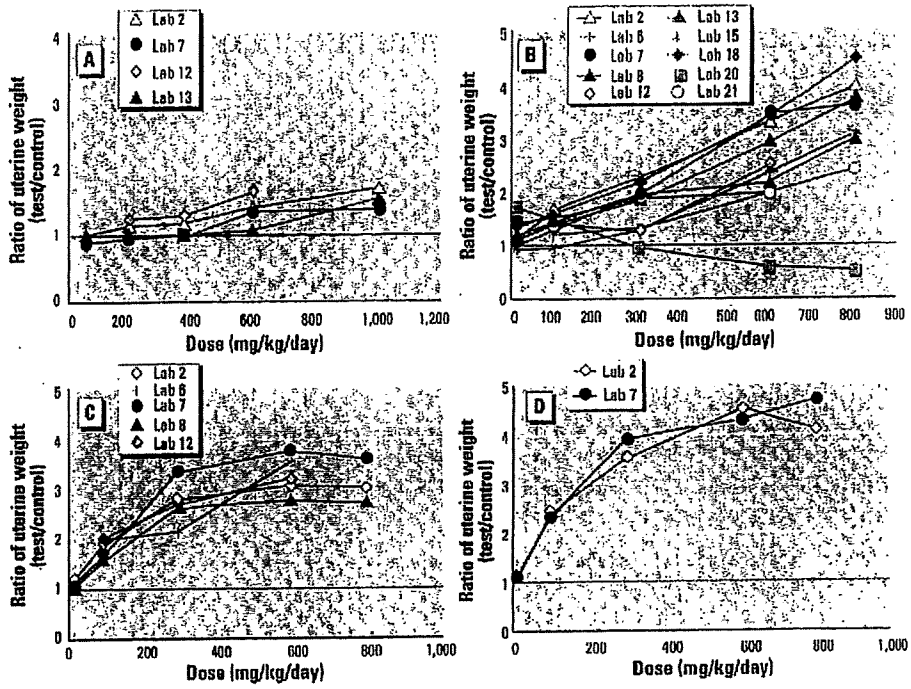

Figure 1. Ratio of the mean blotted uterine weight in response to doses of BPA relative to the vehicle control group. (*A*) Participating laboratory results for protocol A using immature female rats, dosing by oral gavage for 3 consecutive days. (*B*) Participating laboratory results for protocol B using immature female rats, dosing by sc injection for 3 consecutive days. (*C*) Participating laboratory results for protocol C using adult OVX rats, dosing by sc injection for 3 consecutive days. (*D*) Participating laboratory results for protocol C using adult OVX rats and extending sc injection dosing to 7 days. In all cases, animals were humanely sacrificed 24 hr after the last dose administration, the uteri were removed and trimmed, and wet and blotted weights were recorded.

Table 7. Uterine weights, body weights, and ratio of the relative increase in uterine weights for GN in protocol A.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (60 mg/kg/day)	Dose 3 (120 mg/kg/day)	Dose 4 (300 mg/kg/day)	Dose 5 (500 mg/kg/day)
1	Wet weight (mg, mean ± SD)	45.9 ± 6.29	64.2 ± 12.02	80.6 ± 7.40	83.9 ± 8.35	92.4 ± 8.19	112.3 ± 28.77
	Blotted weight (mg, mean ± SD)	39.1 ± 4.10	55.3 ± 11.49	68.3 ± 7.03	74.7 ± 8.69	81.4 ± 7.85	96.8 ± 24.56
	bw (g, mean ± SD)	67.3 ± 2.62	66.3 ± 3.50	66.7 ± 2.81	65.4 ± 5.09	63.6 ± 2.53	63.5 ± 2.30
	Absolute ratio[a]		1.41	1.75	1.91	2.08	2.48
	bw adjusted ratio[b]		1.42*	1.76*	1.97*	2.22*	2.56*
	(Lower CL, upper CL)[c]		(1.08, 1.85)	(1.35, 2.30)	(1.50, 2.58)	(1.67, 2.95)	(1.93, 3.41)
8	Wet weight (mg, mean ± SD)	23.1 ± 3.25	24.8 ± 3.61	36.2 ± 6.11	51.7 ± 3.99	65.5 ± 9.44	69.8 ± 7.82
	Blotted weight (mg, mean ± SD)	21.4 ± 2.56	22.9 ± 3.16	34.1 ± 5.62	49.6 ± 4.09	61.7 ± 8.50	65.7 ± 7.81
	bw (g, mean ± SD)	46.5 ± 5.61	40.8 ± 4.34	44.0 ± 4.49	44.4 ± 4.35	42.9 ± 3.98	42.7 ± 4.73
	Absolute ratio		1.07	1.59	2.32	2.88	3.07
	bw adjusted ratio		1.12	1.61*	2.36*	2.96*	3.16*
	(Lower CL, upper CL)		(0.91, 1.37)	(1.33, 1.96)	(1.94, 2.87)	(2.42, 3.61)	(2.59, 3.86)
9	Wet weight (mg, mean ± SD)	29.7 ± 4.54	39.9 ± 6.49	66.1 ± 13.87	77.0 ± 8.68	74.8 ± 7.25	91.0 ± 15.13
	Blotted weight (mg, mean ± SD)	29.2 ± 4.48	39.4 ± 6.53	65.6 ± 13.93	76.3 ± 8.60	74.1 ± 7.23	89.0 ± 13.52
	bw (g, mean ± SD)	56.7 ± 2.71	58.0 ± 3.90	57.7 ± 3.61	57.2 ± 2.81	58.1 ± 2.12	56.8 ± 3.31
	Absolute ratio		1.35	2.24	2.61	2.53	3.05
	bw adjusted ratio		1.36*	2.23*	2.63*	2.57*	3.04*
	(Lower CL, upper CL)		(1.07, 1.71)	(1.77, 2.82)	(2.08, 3.31)	(2.03, 3.25)	(2.41, 3.84)
12	Wet weight (mg, mean ± SD)	24.2 ± 2.48	Not done	58.5 ± 10.89	72.2 ± 12.41	82.8 ± 11.95	Not done
	Blotted weight (mg, mean ± SD)	20.6 ± 1.81		52.4 ± 9.89	64.5 ± 11.94	74.6 ± 10.43	
	bw (g, mean ± SD)	39.7 ± 3.10		40.7 ± 3.30	41.7 ± 4.90	43.4 ± 4.48	
	Absolute ratio			2.55	3.14	3.63	
	bw adjusted ratio			2.49*	3.03*	3.47*	
	(Lower CL, upper CL)			(1.97, 3.15)	(2.39, 3.85)	(2.71, 4.45)	

[a]Ratio of arithmetic means of the treated blotted uterine weights relative to the vehicle control blotted uterine weight. [b]Ratio of geometric means of treated blotted uterine weights relative to the vehicle control blotted uterine weights after adjusting for body weights at necropsy as a covariable. [c]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 8. Uterine weights, body weights, and ratio of the relative increase in uterine weights for GN in protocol B.

Laboratory	Measure	Vehicle	Dose 1 (1 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (50 mg/kg/day)	Dose 5 (80 mg/kg/day)
1	Wet weight (mg, mean ± SD)	38.2 ± 10.47	44.5 ± 11.46	62.8 ± 6.75	82.9 ± 14.09	105.2 ± 16.99	120.2 ± 20.31
	Blotted weight (mg, mean ± SD)	33.4 ± 9.32	39.6 ± 10.26	58.0 ± 5.64	75.4 ± 11.61	94.2 ± 10.91	105.9 ± 14.33
	bw (g, mean ± SD)	63.1 ± 4.45	62.4 ± 3.10	62.8 ± 3.36	62.0 ± 3.18	62.5 ± 3.50	60.8 ± 3.14
	Absolute ratio		1.19	1.74	2.26	2.82	3.17
	bw adjusted ratio		1.20	1.79*	2.33*	2.91*	3.30*
	(Lower CL, upper CL)[a]		(0.90, 1.59)	(1.35, 2.38)	(1.75, 3.10)	(2.19, 3.86)	(2.47, 4.42)
8	Wet weight (mg, mean ± SD)	22.6 ± 1.40	26.0 ± 2.28	46.5 ± 9.17	58.8 ± 11.38	67.6 ± 10.51	84.3 ± 8.44
	Blotted weight (mg, mean ± SD)	20.9 ± 1.12	24.4 ± 1.87	44.4 ± 8.63	56.4 ± 10.91	64.8 ± 10.08	80.4 ± 7.84
	bw (g, mean ± SD)	52.3 ± 5.95	51.1 ± 5.78	51.9 ± 5.34	51.1 ± 5.10	51.6 ± 4.42	52.6 ± 4.92
	Absolute ratio		1.17	2 12	2.70	3.10	3.85
	bw adjusted ratio		1.18	2.10*	2.69*	3.08*	3.83*
	(Lower CL, upper CL)		(0.96, 1.45)	(1.71, 2.58)	(2.19, 3.30)	(2.51, 3 79)	(3.12, 4.71)
9	Wet weight (mg, mean ± SD)	34.9 ± 3.47	41.3 ± 8.49	65.9 ± 4.95	89.9 ± 4.69	106.7 ± 7.71	145.3 ± 29.46
	Blotted weight (mg, mean ± SD)	34.1 ± 3.67	40.0 ± 8.20	64.7 ± 5.18	88.7 ± 4.61	104.1 ± 7.12	120.0 ± 13.10
	bw (g, mean ± SD)	58.0 ± 2.27	57.1 ± 3.54	57.7 ± 3.30	59.3 ± 3.89	57.2 ± 1.99	58.4 ± 2.84
	Absolute ratio		1.18	1.90	2.61	3.06	3.52
	bw adjusted ratio		1.18*	1.91*	2.57*	3.10*	3.50*
	(Lower CL, upper CL)		(1.0006[b], 1.39)	(1.63, 2.25)	(2.19, 3.02)	(2.63, 3.64)	(2.98, 4.11)
12	Wet weight (mg, mean ± SD)	26.8 ± 6.97	Not done	41.7 ± 8.36	55.9 ± 13.68	66.2 ± 14.75	Not done
	Blotted weight (mg, mean ± SD)	22.4 ± 6.47		35.2 ± 9.19	50.6 ± 10.96	59.4 ± 12.36	
	bw (g, mean ± SD)	40.4 ± 3.38		42.1 ± 5.40	40.6 ± 4.57	40.3 ± 3.58	
	Absolute ratio			1.57	2.26	2.65	
	bw adjusted ratio			1.48*	2.28*	2.70*	
	(Lower CL, upper CL)			(1.10, 1.98)	(1.70, 3.05)	(2.02, 3.61)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]With the lower 95% confidence limit > 1.0, the result is statistically significant. *Level of significance, $p < 0.05$.

Table 9. Uterine weights, body weights, and ratio of the relative increase in uterine weights for GN in protocol C.

Laboratory	Measure	Vehicle	Dose 1 (1 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (50 mg/kg/day)	Dose 5 (80 mg/kg/day)
1	Wet weight (mg, mean ± SD)	93.5 ± 12.30	84.0 ± 7.86	144.7 ± 18.42	162.6 ± 13.97	151.0 ± 14.35	177.6 ± 40.11
	Blotted weight (mg, mean ± SD)	85.9 ± 13.10	77.2 ± 6.44	131.5 ± 15.40	151.6 ± 13.14	142.1 ± 11.98	163.4 ± 33.74
	bw (g, mean ± SD)	272.5 ± 20.75	277.0 ± 13.53	275.5 ± 14.15	270.4 ± 14.70	267.4 ± 10.96	272.4 ± 15.03
	Absolute ratio		0.90	1.53	1.77	1.65	1.90
	bw adjusted ratio		0 90	1.53*	1.78*	1.68*	1.89*
	(Lower CL, upper CL)[a]		(0.74, 1.10)	(1.25, 1.88)	(1.46, 2.18)	(1.37, 2.05)	(1.54, 2.31)
9	Wet weight (mg, mean ± SD)	87.2 ± 11.74	85 9 ± 10 39	136.9 ± 23.21	161.4 ± 7.49	161.0 ± 17.13	172.6 ± 13.57
	Blotted weight (mg, mean ± SD)	86.3 ± 11.88	85.0 ± 10.35	135.0 ± 22.92	160.1 ± 7.33	179.4 ± 16.71	170.6 ± 11.92
	bw (g, mean ± SD)	256.1 ± 8.87	257.9 ± 10.05	258.4 ± 9.90	255.2 ± 11.14	253.3 ± 12.09	253.6 ± 10.56
	Absolute ratio		0.99	1.57	1.86	2.08	1.98
	bw adjusted ratio		0.99	1.57*	1.87*	2.08*	1.98*
	(Lower CL, upper CL)		(0.83, 1.18)	(1.32, 1.88)	(1.56, 2.23)	(1.74, 2.48)	(1.66, 2.37)
12	Wet weight (mg, mean ± SD)	106.0 ± 18.84	Not done	146.1 ± 33.23	162.2 ± 26.36	183.3 ± 57.85	Not done
	Blotted weight (mg, mean ± SD)	98.6 ± 22.04		133.9 ± 30.80	152.0 ± 24.29	168.3 ± 54.04	
	bw (g, mean ± SD)	297.2 ± 14.54		303.6 ± 12.80	297.2 ± 17.70	303.4 ± 17.97	
	Absolute ratio			1.36	1.54	1.71	
	bw adjusted ratio			1.31	1.56*	1.62*	
	(Lower CL, upper CL)			(0.92, 1.87)	(1.09, 2.21)	(1.14, 2.32)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 10. Uterine weights, body weights, and ratio of the relative increase in uterine weights for GN in protocol D.

Laboratory	Measure	Vehicle	Dose 1 (1 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (50 mg/kg/day)	Dose 5 (80 mg/kg/day)
1	Wet weight (mg, mean ± SD)	96.4 ± 17.25	96.9 ± 15.94	161.8 ± 20.55	207.8 ± 29.01	222.0 ± 29.72	394.0 ± 76.24
	Blotted weight (mg, mean ± SD)	87.2 ± 15.17	85.8 ± 11.09	145.8 ± 19.66	189.3 ± 22.50	200.0 ± 25.22	303.6 ± 24.41
	bw (g, mean ± SD)	281.5 ± 19.95	268.8 ± 13.92	270.2 ± 14.68	264.2 ± 14.56	266.4 ± 12.93	265.3 ± 12.31
	Absolute ratio		0.98	1 67	2.17	2.29	3.48
	bw adjusted ratio		0.99	1.67*	2.20*	2.31*	3.55*
	(Lower CL, upper CL)[a]		(0.80, 1.23)	(1.35, 2.07)	(1.78, 2.73)	(1.87, 2.86)	(2.87, 4.40)
9	Wet weight (mg, mean ± SD)	76.8 ± 4.59	90.9 ± 9.17	157.5 ± 21.51	193.5 ± 12.44	209.9 ± 11.29	243.8 ± 76.43
	Blotted weight (mg, mean ± SD)	75.9 ± 4.97	89.7 ± 9.13	156.7 ± 21.76	192.4 ± 11.73	208.7 ± 10.70	215.3 ± 37.35
	bw (g, mean ± SD)	282.1 ± 12.40	282.6 ± 11 77	276.9 ± 12.39	280.2 ± 10.44	277.1 ± 12.44	275.3 ± 12.18
	Absolute ratio		1.18	2.06	2.53	2.75	2.84
	bw adjusted ratio		1.18	2.06*	2.54*	2.75*	2.81*
	(Lower CL, upper CL)		(0 9995[b], 1 39)	(1.74, 2.43)	(2.15, 2.99)	(2.33, 3.25)	(2.38, 3.32)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]With the lower 95% confidence limit not > 1.0, the result is not statistically significant. *Level of significance, $p < 0.05$.

increase in uterine weight was already approaching 4 (Table 12). In protocol B, four studies detected statistically significant increases in uterine weights at the second dose of 100 mg MX/kg/day (Table 13). In protocols C and D, all studies detected statistical significance at the second dose of 100 mg MX/kg/day (Tables 14 and 15). The satellite study with OVX animals using oral gavage administration detected statistically significant increases in uterine weight at the lowest of the three intermediate doses used in that study, 50 mg/kg/day (i.e., the lowest 20-mg MX/kg/day dose was not tested in this laboratory with this protocol) (Table 16).

The MX results, except for the satellite study, are shown graphically in Figure 3. In protocol A, all studies at the lowest dose had ratios of the maximum mean uterine weights of the treated groups to the controls of 2 to 3.5. Thus, the selected doses were unable to indicate a minimal effective dose. In the case of MX, the oral route of administration was more sensitive than sc injection (Table 12). In protocols B, C, and D, the lowest dose producing a statistically significant increase in uterine weights was similar (Tables 13–15). However, protocol B produced a somewhat higher ratio of the maximum mean uterine weights relative to the controls of 2.5 to 3.5. The extended, 7-day dosing in protocol D did not lead to any increase in the maximum increase in uterine weights in the case of MX. With MX, the dose–response curves of protocol B appeared to be more variable than protocols C and D (Figure 3). The satellite study with OVX animals using oral gavage administration detected statistically significant increases in uterine weight at the lowest of the three intermediate doses used in that study, 60 mg/kg/day (i.e., the lowest 20-mg MX/kg/day dose was not tested in this laboratory with this protocol) (Table 16).

Nonylphenol. A total of 22 dose–response studies were conducted with NP, including 4 with protocol A, 10 with protocol B, 5 with protocol C, 2 with protocol D, and a satellite study using oral gavage with OVX animals. Three of the 21 NP studies were unsuccessful in detecting increases in uterine weights at any of the prescribed doses. Again, laboratory 21 did not record the required terminal body weights, and these studies could not be statistically analyzed using body weight adjustment. However, the wet and blotted uterine results are included in Table 18 and Figure 4, and these have been statistically compared without body weight adjustment.

Within each protocol, there was overall agreement among different laboratories both in the magnitude of the uterine weight increases and in the NP doses first producing a statistically significant increase in uterine weight. In protocol A using oral gavage, all four studies detected statistically significant increases in uterine weights at LOEL doses of 75 mg NP/kg/day (Table 17). In protocol B, seven of nine studies detected statistically significant increases in uterine weights at doses of 35 mg NP/kg/day (one study), 80 mg/kg/day (five studies), and 100 mg/kg/day (one study). One of two laboratories that failed to detect a significantly increased uterine weight used only the three intermediate doses and did not use the highest dose (Table 18). In protocol C, four of five studies detected statistical significant increases in uterine weights at doses of 35 mg NP/kg/day (one study), 80 mg/kg/day (one study) and 100 mg/kg/day (two studies) (Table 19). The laboratory that failed to detect a significant increase in uterine weight used only the three intermediate doses and did not use the highest dose. In protocol D, both studies detected statistical significance at a dose of 35 mg NP/kg/day (Table 20). The satellite study with OVX animals using oral gavage administration detected statistically significant increases in uterine weight at the lowest of the three intermediate doses used in that study, 75 mg/kg/day (i.e., the lowest 15-mg NP/kg/day dose was not tested in this laboratory with this protocol) (Table 21).

The NP results, except for the satellite study, are shown graphically in Figure 4. In protocol A using oral gavage, the ratio of the

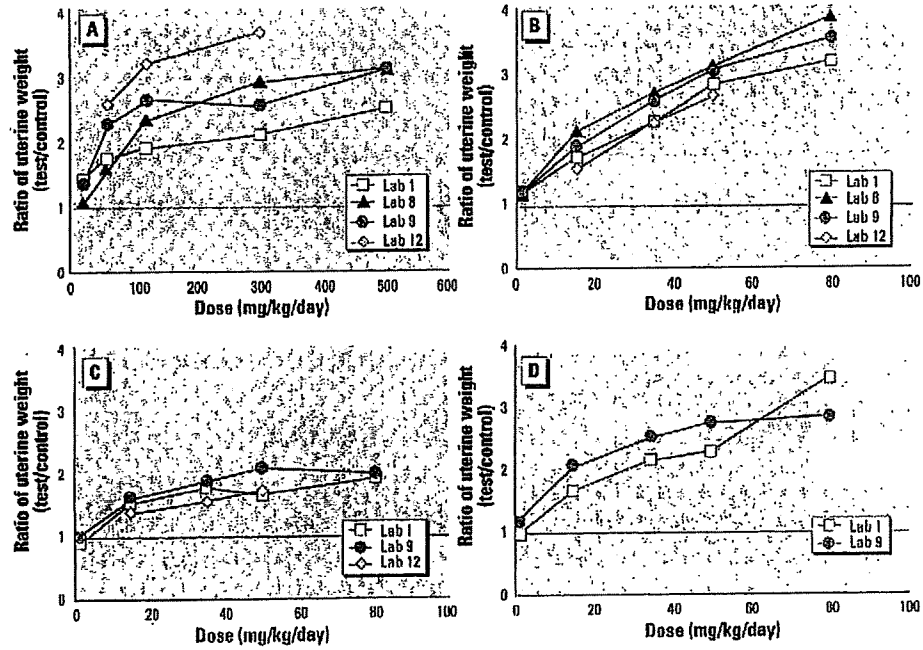

Figure 2. Ratio of the mean absolute blotted uterine weight in response to doses of GN relative to the vehicle control group. (*A*) Participating laboratory results for protocol A using immature female rats, dosing by oral gavage for 3 consecutive days. (*B*) Participating laboratory results for protocol B using immature female rats, dosing by sc injection for 3 consecutive days. (*C*) Participating laboratory results for protocol C using adult OVX rats, dosing by sc injection for 3 consecutive days. (*D*) Participating laboratory results for protocol C using adult OVX rats and extending sc injection dosing to 7 days. In all cases, animals were humanely sacrificed 24 hr after the last dose administration, the uteri were removed and trimmed, and wet and blotted weights were recorded.

Table 11. Uterine weights, body weights, and ratio of the relative increase in uterine weights for GN in satellite OVX protocol by oral gavage.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (60 mg/kg/day)	Dose 3 (120 mg/kg/day)	Dose 4 (300 mg/kg/day)	Dose 5 (500 mg/kg/day)
12	Wet weight (mg, mean ± SD)	101.1 ± 16.93	Not done	194.5 ± 50.41	191.7 ± 43.16	270.9 ± 92.51	Not done
	Blotted weight (mg, mean ± SD)	95.0 ± 16.43		172.6 ± 38.92	178.9 ± 39.60	195.1 ± 20.90	
	bw (g, mean ± SD)	295.5 ± 11.09		291.2 ± 12.85	285.7 ± 6.30	283.4 ± 12.36	
	Absolute ratio			1.82	1.88	2.05	
	bw adjusted ratio			1.83[c]	1.93*	2.16*	
	(Lower CL, upper CL)[a]			(1.34, 2.48)	(1.40, 2.66)	(1.55, 3.00)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

maximum mean uterine weights of the treated groups to the controls was generally between 2 and 3 for the treated groups relative to the controls. The ratio of treated to vehicle groups was 1.5 to 3 in protocol B, a more modest 1.5 in protocol C, and 2 by extending the dosing to 7 days in protocol D. Again, dose–response curves among laboratories in protocol B appeared to be more variable than protocols C and D (Figure 4).

o,p′-DDT. A total of 14 dose–response studies were conducted with *o,p′*-DDT, including 4 with protocol A, 4 with protocol B, 3 with protocol C, 2 with protocol D, and a satellite study using oral gavage with OVX animals. Thirteen of the 14 studies were successful in detecting increases in uterine weights at one or more prescribed doses. Within each protocol, there was overall agreement among different laboratories both in the magnitude of the uterine weight increases and in the *o,p′*-DDT doses first producing a statistically significant increase in uterine weight. In protocol A using oral gavage, one study

Table 12. Uterine weights, body weights, and ratio of the relative increase in uterine weights for MX in protocol A.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (50 mg/kg/day)	Dose 3 (120 mg/kg/day)	Dose 4 (300 mg/kg/day)	Dose 5 (500 mg/kg/day)
1	Wet weight (mg, mean ± SD)	44.3 ± 6.44	88.2 ± 17.18	104.5 ± 13.59	103.4 ± 11.55	112.6 ± 8.44	114.3 ± 6.37
	Blotted weight (mg, mean ± SD)	38.4 ± 6.50	76.4 ± 16.74	87.1 ± 11.13	86.2 ± 7.74	93.1 ± 7.50	98.8 ± 5.14
	bw (g, mean ± SD)	61.6 ± 2.49	61.4 ± 3.78	61.1 ± 1.84	61.1 ± 3.57	59.2 ± 2.21	58.0 ± 2.96
	Absolute ratio[a]		1.99	2.27	2.25	2.43	2.58
	bw adjusted ratio[b]		1.98*	2.31*	2.30*	2.59*	2.83*
	(Lower CL, upper CL)[c]		(1.65, 2.39)	(1.91, 2.79)	(1.90, 2.77)	(2.13, 3.15)	(2.31, 3.46)
3	Wet weight (mg, mean ± SD)	34.6 ± 3.93	74.7 ± 10.74	90.7 ± 10.65	98.5 ± 9.11	108.8 ± 9.62	98.0 ± 16.90
	Blotted weight (mg, mean ± SD)	32.6 ± 4.23	72.2 ± 10.22	90.8 ± 9.67	94.7 ± 8.91	105.0 ± 9.75	94.8 ± 15.37
	bw (g, mean ± SD)	62.9 ± 1.64	62.2 ± 3.38	62.0 ± 3.04	62.1 ± 3.44	58.4 ± 3.31	58.0 ± 4.00
	Absolute ratio		1.82	2.29	2.39	2.65	2.39
	bw adjusted ratio		1.88*	2.37*	2.47*	2.94*	2.65*
	(Lower CL, upper CL)		(1.52, 2.31)	(1.92, 2.93)	(2.00, 3.05)	(2.34, 3.69)	(2.11, 3.35)
12	Wet weight (mg, mean ± SD)	24.2 ± 2.48	Not done	89.3 ± 26.37	88.3 ± 17.06	86.3 ± 10.42	Not done
	Blotted weight (mg, mean ± SD)	20.6 ± 1.81		79.6 ± 24.95	77.9 ± 14.63	78.6 ± 10.02	
	bw (g, mean ± SD)	39.7 ± 3.10		39.8 ± 5.76	39.2 ± 3.38	38.3 ± 3.30	
	Absolute ratio			3.87	3.79	3.83	
	bw adjusted ratio			3.71*	3.88*	3.98*	
	(Lower CL, upper CL)			(2.87, 4.79)	(2.93, 4.90)	(3.07, 5.15)	
14	Wet weight (mg, mean ± SD)	18.4 ± 2.19	58.3 ± 16.27	55.2 ± 8.01	65.4 ± 8.20[d]	63.4 ± 11.41[d]	62.0 ± 4.58[d]
	Blotted weight (mg, mean ± SD)	14.8 ± 2.28	51.0 ± 12.98	45.2 ± 8.13	59.2 ± 9.65	55.6 ± 8.20	51.7 ± 3.79
	bw (g, mean ± SD)	40.3 ± 6.83	45.8 ± 4.26	40.5 ± 4.69	48.2 ± 5.81	46.3 ± 5.47	47.6 ± 4.17
	Absolute ratio		3.45	3.05	4.00	3.76	3.49
	bw adjusted ratio		3.14*	3.03*	3.59*	3.46*	3.19*
	(Lower CL, upper CL)		(2.31, 4.26)	(2.27, 4.05)	(2.57, 5.01)	(2.51, 4.77)	(2.19, 4.63)

[a]Ratio of arithmetic means of the treated blotted uterine weights relative to the vehicle control blotted uterine weights. [b]Ratio of geometric means of treated blotted uterine weights relative to the vehicle control blotted uterine weights after adjusting for body weights at necropsy as a covariable. [c]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [d]One animal died in 120 mg MX/kg/day group before necropsy; one animal died in 300 mg MX/kg/day group before necropsy; three animals died in 500 mg MX/kg/day group before necropsy; and one animal also died in the vehicle control group before necropsy. *Level of significance, $p < 0.05$.

Table 13. Uterine weights, body weights, and ratio of the relative increase in uterine weights for MX in protocol B.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (250 mg/kg/day)	Dose 4 (500 mg/kg/day)	Dose 5 (800 mg/kg/day)
1	Wet weight (mg, mean ± SD)	39.7 ± 7.58	44.9 ± 9.18	52.4 ± 9.38	77.0 ± 13.82	99.3 ± 17.57	103.4 ± 6.86
	Blotted weight (mg, mean ± SD)	35.2 ± 6.34	40.9 ± 8.42	48.0 ± 9.43	68.0 ± 11.28	86.3 ± 10.15	93.5 ± 6.15
	bw (g, mean ± SD)	66.6 ± 4.48	65.0 ± 3.87	65.4 ± 4.20	65.5 ± 3.69	64.7 ± 2.92	64.8 ± 3.84
	Absolute ratio		1.16	1.36	1.93	2.45	2.66
	bw adjusted ratio		1.16	1.36*	1.94*	2.47*	2.69*
	(Lower CL, upper CL)[a]		(0.89, 1.50)	(1.05, 1.76)	(1.50, 2.51)	(1.91, 3.21)	(2.08, 3.49)
3	Wet weight (mg, mean ± SD)	34.2 ± 3.49	47.1 ± 6.83	86.2 ± 19.85	108.9 ± 20.26	121.9 ± 30.68	139.7 ± 10.78
	Blotted weight (mg, mean ± SD)	31.5 ± 3.97	45.1 ± 6.82	81.0 ± 16.97	101.4 ± 16.06	107.4 ± 17.90	132.2 ± 9.09
	bw (g, mean ± SD)	63.5 ± 3.92	63.2 ± 2.13	62.6 ± 1.54	61.5 ± 3.38	62.3 ± 2.59	63.8 ± 1.54
	Absolute ratio		1.20	2.16	2.70	2.86	3.52
	bw adjusted ratio		1.21	2.21*	2.88*	2.98*	3.52*
	(Lower CL, upper CL)		(0.9911[b], 1.49)	(1.80, 2.71)	(2.34, 3.55)	(2.42, 3.65)	(2.87, 4.31)
12	Wet weight (mg, mean ± SD)	26.8 ± 6.97	Not done	71.3 ± 10.13	89.2 ± 8.98	83.2 ± 10.60	Not done
	Blotted weight (mg, mean ± SD)	22.4 ± 6.47		62.3 ± 9.23	76.2 ± 38.60	72.0 ± 9.42	
	bw (g, mean ± SD)	40.4 ± 3.38		39.7 ± 6.98	39.6 ± 1.66	38.7 ± 5.27	
	Absolute ratio			2.78	3.40	3.21	
	bw adjusted ratio			2.86*	3.53*	3.34*	
	(Lower CL, upper CL)			(2.18, 3.77)	(2.68, 4.65)	(2.53, 4.40)	
14	Wet weight (mg, mean ± SD)	18.3 ± 3.61	23.5 ± 4.18	40.8 ± 14.58	58.2 ± 13.33	74.3 ± 18.48	72.6 ± 21.34
	Blotted weight (mg, mean ± SD)	16.3 ± 3.78	17.8 ± 4.54	27.7 ± 6.28	44.3 ± 12.34	61.8 ± 13.12	60.2 ± 7.60
	bw (g, mean ± SD)	44.2 ± 5.25	44.7 ± 4.52	46.4 ± 4.97	40.7 ± 9.15	44.7 ± 5.00	46.8 ± 4.49
	Absolute ratio		1.09	1.69	2.71	3.79	3.69
	bw adjusted ratio		1.07	1.62*	2.89*	3.76*	3.56*
	(Lower CL, upper CL)		(0.79, 1.45)	(1.20, 2.19)	(2.13, 3.93)	(2.78, 5.09)	(2.58, 4.88)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]With the lower 95% confidence limit not > 1.0, the result is not statistically significant. *Level of significance, $p < 0.05$.

detected statistically significant increases in uterine weights at a LOEL dose of 10 mg DDT/kg/day, and three studies at 50 mg/kg/day (Table 22). In protocol B, one study detected statistically significant increases at a dose of 100 mg DDT/kg/day, and the other three laboratories achieved statistical significance at doses of 200 mg/kg/day (Table 23). In protocol C, one study detected statistical significance at a dose of 50 mg DDT/kg/day and one study at a dose of 100 mg/kg/d (Table 24). The laboratory that did not achieve statistical significance used only the three intermediate doses and did not use the high dose of 200 mg/kg/day. In protocol D, one study detected statistical significance at a dose of 50 mg DDT/kg/day and the other at a dose of 100 mg/kg/day (Table 25). The satellite study with OVX animals using oral gavage administration detected statistically significant increases in uterine weight at the lowest of the three intermediate doses used in that study, 50 mg/kg/day (i.e., the lowest 10-mg DDT/kg/day dose was not tested in this laboratory with this protocol) (Table 26).

The *o,p'*-DDT results, except for the satellite study, are shown graphically in Figure 5. In protocol A using oral gavage, the ratio of the maximum mean uterine weights of the treated groups to the controls was generally between 2.5 and 3.5 and plateaued at the second-highest dose of 300 mg/kg/day. In protocols B, C, and D, the ratio in uterine weights was approximately 1.5. Extending the dosing to 7 days did not lead to an apparent increase in the maximum induction in uterine weights. Within the sc protocols, there was no apparent difference in variability of the dose–response curves between the intact, immature, and OVX animals.

Discussion and Conclusions

Reproducibility of the dose response among laboratories within a given protocol was good. It is noteworthy that this reproducibility was

Table 14. Uterine weights, body weights, and ratio of the relative increase in uterine weights for MX in protocol C.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (250 mg/kg/day)	Dose 4 (500 mg/kg/day)	Dose 5 (800 mg/kg/day)
1	Wet weight (mg, mean ± SD)	92.1 ± 8.86	88.5 ± 6.03	118.3 ± 15.22	173.3 ± 34.90	256.7 ± 80.39	282.1 ± 90.49
	Blotterl weight (mg, mean ± SD)	84.9 ± 8.03	81.4 ± 6.04	108.3 ± 13.16	156.5 ± 25.31	197.1 ± 46.05	203.3 ± 34.71
	bw (g, mean ± SD)	255.9 ± 10.19	259.4 ± 16.10	251.7 ± 9.52	249.3 ± 9.53	248.0 ± 11.64	246.6 ± 13.26
	Absolute ratio		0.96	1.28	1 84	2.32	2.39
	bw adjusted ratio		0.95	1.28*	1.85*	2.32*	2.42*
	(Lower CL, upper CL)[a]		(0.77, 1.19)	(1.03, 1.59)	(1.49, 2.31)	(1.86, 2.89)	(1.93, 3.02)
3	Wet weight (mg, mean ± SD)	90.8 ± 8.37.	97.1 ± 8.47	155.7 ± 19.42	184.4 ± 33.95	298.4 ± 126.20	298.9 ± 56.93
	Blotted weight (mg, mean ± SD)	85.5 ± 8.57	92.5 ± 8.93	149.1 ± 18.59	173.6 ± 30.05	212.0 ± 27.48	226.2 ± 23.49
	bw (g, mean ± SD)	278.5 ± 11.15	278.7 ± 10.33	2727.1 ± 15.33	272.4 ± 10.41	264.2 ± 12.98	264.5 ± 12.62
	Absolute ratio		1.08	1.74	2.03	2.49	2.64
	bw adjusted ratio		1.08	1.72*	1.99*	2.42*	2.59*
	(Lower CL, upper CL)		(0.89, 1.32)	(1.41, 2.10)	(1.63, 2.43)	(1.96, 2.98)	(2.10, 3.19)
12	Wet weight (mg, mean ± SD)	106.0 ± 18.84	Not done	170.2 ± 31.93	196.8 ± 48.65	214.6 ± 24.88	Not done
	Blotted weight (mg, mean ± SD)	98 6 ± 22.04		155.8 ± 32.81	171.7 ± 36.49	185.0 ± 22.30	
	bw (g, mean ± SD)	297.2 ± 14.54		287.4 ± 16.77	290.0 ± 12.36	288.7 ± 9.24	
	Absolute ratio			1.58	1.74	1.88	
	bw adjusted ratio			1.63*	1.79*	1.95*	
	(Lower CL, upper CL)			(1.21, 2.19)	(1.33, 2.39)	(1.45, 2.62)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 15. Uterine weights, body weights, and ratio of the relative increase in uterine weights for MX in protocol D.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (100 mg/kg/day)	Dose 3 (250 mg/kg/day)	Dose 4 (500 mg/kg/day)	Dose 5 (800 mg/kg/day)
1	Wet weight (mg, mean ± SD)	98.1 ± 15.43	90.6 ± 11.93	128.1 ± 28.27	167.9 ± 32.11	254.0 ± 61.79	246.9 ± 30.58
	Blotted weight (mg, mean ± SD)	90.8 ± 15.92	84.8 ± 10.43	121.2 ± 26.36	155.9 ± 28.39	211.9 ± 41.21	216.7 ± 27.12
	bw (g, mean ± SD)	272.1 ± 14.66	271.6 ± 16.86	237.2 ± 14.85	251.4 ± 14.90	246.5 ± 17.59	245.8 ± 14.41
	Absolute ratio		0.93	1.34	1.72	2.33	2.39
	bw adjusted ratio		0.94	1.33*	1.75*	2.39*	2.46*
	(Lower CL, upper CL)[a]		(0.72, 1.23)	(1.02, 1.75)	(1.30, 2.34)	(1.76, 3.22)	(1.82, 3.34)
3	Wet weight (mg, mean ± SD)	96.8 ± 6.73	104.3 ± 5.76	151.0 ± 26.12	237.7 ± 29.06	236.8 ± 40.63	252.0 ± 34.97
	Blotted weight (mg, mean ± SD)	92.6 ± 5.87	99.3 ± 4.97	144.6 ± 25.61	221.2 ± 20.14	228.2 ± 38.44	246.9 ± 30.58
	bw (g, mean ± SD)	282.7 ± 19.53	277.6 ± 17.55	265.2 ± 18.00	256.6 ± 14.68	254.7 ± 8.95	250.9 ± 10.80
	Absolute ratio		1.07	1.56	2.39	2.46	2.59
	bw adjusted ratio		1.08	1.55*	2.41*	2.47*	2.61*
	(Lower CL, upper CL)		(0.89, 1.30)	(1.27, 1.90)	(1.94, 2.98)	(1.98, 3.06)	(2.08, 3.26)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 16. Uterine weights, body weights, and ratio of the relative increase in uterine weights for MX in satellite OVX protocol by oral gavage.

Laboratory	Measure	Vehicle	Dose 1 (20 mg/kg/day)	Dose 2 (50 mg/kg/day)	Dose 3 (120 mg/kg/day)	Dose 4 (300 mg/kg/day)	Dose 5 (500 mg/kg/day)
12	Wet weight (mg, mean ± SD)	101.1 ± 16.93	Not done	247.1 ± 20.31	301.8 ± 54.21	388.6 ± 1.8.89	Not done
	Blotted weight (mg, mean ± SD)	95.0 ± 16.43		194.7 ± 33.94	217.1 ± 16.75	231.8 ± 23.71	
	bw (g, mean ± SD)	295.5 ± 11.09		278.7 ± 12.96	275.0 ± 11.90	279.0 ± 9.69	
	Absolute ratio			2.05	2.29	2.44	
	bw adjusted ratio			2.11*	2.36*	2.50*	
	(Lower CL, upper CL)[a]			(1.71, 2.60)	(1.89, 2.94)	(2.03, 3.08)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

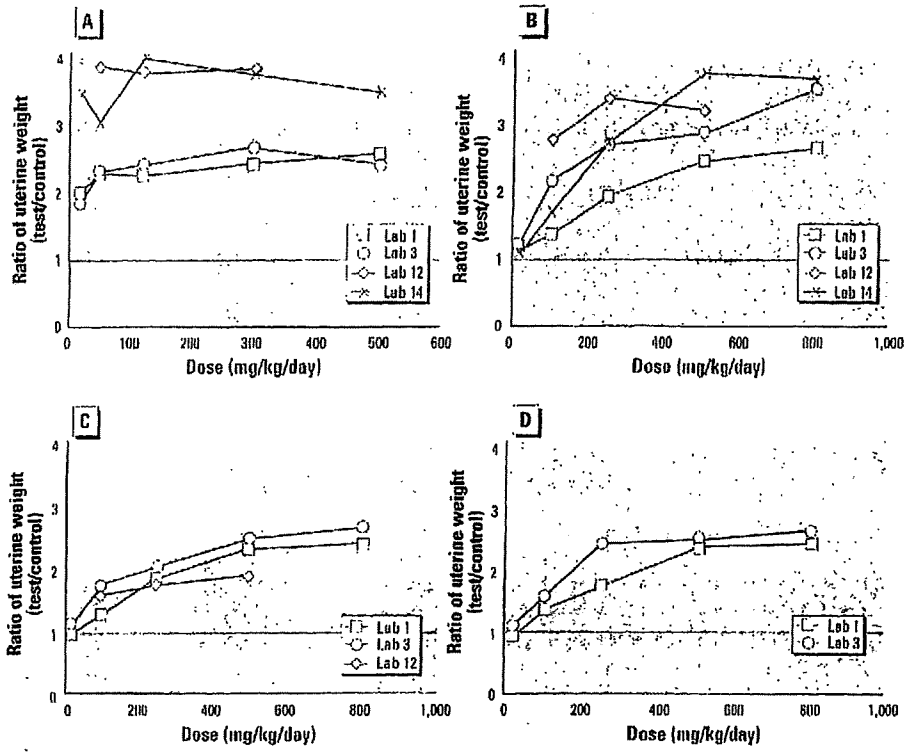

Figure 3. Ratio of the mean absolute blotted uterine weight in response to doses of MX relative to the vehicle control group. (*A*) Participating laboratory results for protocol A using immature female rats, dosing by oral gavage for 3 consecutive days. (*B*) Participating laboratory results for protocol B using immature female rats, dosing by sc injection for 3 consecutive days. (*C*) Participating laboratory results for protocol C using adult OVX rats, dosing by sc injection for 3 consecutive days. (*D*) Participating laboratory results for protocol C using adult OVX rats and extending sc injection dosing to 7 days. In all cases, animals were humanely sacrificed 24 hr after the last dose administration, the uteri were removed and trimmed, and wet and blotted weights were recorded.

achieved under a variety of different study conditions (e.g., strain, diet, housing protocol, bedding, vehicle); modest differences in the age of the immature animals (pnd 18–20), age at ovariectomy, and time of regression after ovariectomy; and a significant range in laboratory experience and proficiency (Table 27). For example, some laboratories have conducted uterotrophic studies for several years, whereas a number of others were conducting the bioassay for only for the first or second time. These variations and possible difference in experience would be expected to contribute to some degree of variability for a given protocol. In this light, the good reproducibility observed suggests that the uterotrophic bioassay itself is robust. This reproducibility is similar to that observed in phase 1 using the potent reference estrogen EE (Kanno et al. 2001). In addition, the uterine increase is observed even under conditions of severe systemic toxicity, as evidenced by mortalities and decreases in body weights sometimes greater than 10% (Tables 2D, 4D, 5A, and 6A). This easily observed response at doses exceeding the maximum tolerated dose further supports that the uterotrophic assay is a robust screen for detecting possible estrogen agonists.

For all protocols, the blotted uterine weights appeared to show less interlaboratory and intragroup variability than uterine wet weight. This suggests that the blotted weight will provide greater power for detecting uterotrophic effects than wet weight.

Table 17. Uterine weights, body weights, and ratio of the relative increase in uterine weights for NP in protocol A.

Laboratory	Measure	Vehicle	Dose 1 (15 mg/kg/day)	Dose 2 (75 mg/kg/day)	Dose 3 (125 mg/kg/day)	Dose 4 (250 mg/kg/day)	Dose 5 (350 mg/kg/day)
4	Wet weight (mg, mean ± SD)	31.3 ± 10.31	30.8 ± 5.42	50.5 ± 10.54	52.5 ± 5.72	62.3 ± 8.02[a]	See note[a]
	Blotted weight (mg, mean ± SD)	29.3 ± 10.91	28.7 ± 4.68	45.2 ± 5.98	49.3 ± 5.89	60.3 ± 6.99	
	bw (g, mean ± SD)	42.7 ± 2.91	38.8 ± 7.12	39.7 ± 3.49	36.3 ± 5.00	28.5 ± 1.55	
	Absolute ratio[b]		0.98	1.54	1.68	2.05	
	Boy weight Adjusted Ratio[c]		1.07	1.68*	1.91*	2.61*	
	(Lower CL, upper CL)[d]		(0.80, 1.43)	(1.26, 2.22)	(1.40, 2.61)	(1.69, 4.04)	
7	Wet weight (mg, mean ± SD)	31.4 ± 2.47	33.0 ± 4.42	44.8 ± 7.36	49.8 ± 4.24	65.3 ± 10.10[e]	69.2 ± 8.66[e]
	Blotted weight (mg, mean ± SD)	30.0 ± 2.30	31.8 ± 4 34	43.5 ± 7.07	48.1 ± 4.13	62.8 ± 9.44	64.6 ± 8.30
	bw (g, mean ± SD)	57.8 ± 3.50	56.6 ± 4.97	54.3 ± 4.61	55.5 ± 4.50	49.8 ± 7.12	43.8 ± 4.57
	Absolute ratio		1.06	1.45	1.60	2.09	2.15
	bw adjusted ratio		1.06	1.46*	1.62*	2.17*	2.32*
	(Lower CL, upper CL)		(0.87, 1.30)	(1.19, 1.80)	(1.32, 1.98)	(1.72, 2.74)	(1.71, 3.12)
9	Wet weight (mg, mean ± SD)	29.7 ± 4.54	36.9 ± 7.82	42.5 ± 5.30	60.2 ± 11.17	58.1 ± 9.28	60.6 ± 4.03[f]
	Blotted weight (mg, mean ± SD)	29.2 ± 4.48	36.4 ± 7.80	42.0 ± 5.36	59.7 ± 11.14	57.6 ± 8.98	60.0 ± 4.22
	bw (g, mean ± SD)	56.7 ± 2.71	59.0 ± 2.29	58.3 ± 3.16	57.0 ± 3.11	47.1 ± 9.55	33.8 ± 3.83
	Absolute ratio		1.24	1.44	2.04	1.97	2.05
	bw adjusted ratio		1 20	1.42*	2.02*	2.17*	2.61*
	(Lower CL, upper CL)		(0.94, 1.54)	(1.11, 1.81)	(1.59, 2.58)	(1.62, 2.90)	(1.61, 4.23)
12	Wet weight (mg, mean ± SD)	24.2 ± 2.48	Not done	50.5 ± 10.39	45.0 ± 9.30	66.9 ± 20.29[g]	Not done
	Blotted weight (mg, mean ± SD)	20.6 ± 1.81		45.5 ± 9.22	42.0 ± 7.85	62.2 ± 18.95	
	bw (g, mean ± SD)	39.7 ± 3.10		44.1 ± 4.91	40.5 ± 3 44	39.9 ± 2.05	
	Absolute ratio			2.21	1.91	3.03	
	bw adjusted ratio			1.96*	1.85*	2.95*	
	(Lower CL, upper CL)			(1.45, 2.64)	(1.41, 2.42)	(2.02, 4.32)	

Two animals died in 250 mg NP/kg/day group before necropsy; all animals died in 350 mg NP/kg/day group before necropsy. [b]Ratio of arithmetic means of the treated blotted uterine weights relative to the vehicle control blotted uterine weights. [c]Ratio of geometric means of treated blotted uterine weights relative to the vehicle control blotted uterine weights after adjusting for body weights at necropsy as a covariable. [d]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [e]One animal died in 250 mg NP/kg/day group before necropsy; three animals died in 350 mg NP/kg/day group before necropsy. [f]Three animals died in 350 mg NP/kg/day group before necropsy. [g]Four animals died in 250 mg NP/kg/day group before necropsy. *Level of significance, $p < 0.05$.

Nevertheless, the wet weight and blotted weights were generally consistent in achieving statistical significance. Again, this outcome is identical to that of phase 1 using the potent reference estrogen EE, where the blotted weights were slightly less variable than the wet weights (Kanno et al. 2001). Additionally, the observation was made that wet and blotted weights in the rat vary by only a few milligrams with both EE and the weak agonists employed here, as long as the relative ratio of the uterine increase is about 2 or less. As the relative ratio exceeds 2, a rapid increase in the quantity of imbibed intraluminal fluid occurs. This was true with EE

Table 18. Uterine weights, body weights, and ratio of the relative increase in uterine weights for NP in protocol B.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (80 mg/kg/day)	Dose 5 (100 mg/kg/day)
4	Wet weight (mg, mean ± SD)	34.7 ± 3.47	34.3 ± 7.89	29.7 ± 2.88	37.2 ± 2.40	64.5 ± 18.51	53.5 ± 13.49
	Blotted weight (mg, mean ± SD)	34.1 ± 3.67	31.3 ± 7.53	28.2 ± 3.76	34.0 ± 4.00	61.5 ± 17.41	51.2 ± 13.23
	bw (g, mean ± SD)	45.6 ± 3.93	45.3 ± 3.88	44.1 ± 3.88	44.8 ± 2.73	44.4 ± 3.26	44.0 ± 2.91
	Absolute ratio		1.02	0.91	1.10	1.99	1.66
	bw adjusted ratio		1.04	0.97	1.16	2.05[a]	1.72*
	(Lower CL, upper CL)[a]		(0.73, 1.48)	(0.68, 1.38)	(0.81, 1.65)	(1.44, 2.92)	(1.21, 2.46)
6	Wet weight (mg, mean ± SD)	61.1 ± 15.24	Not done	52.7 ± 17.14	66.7 ± 12.35	79.5 ± 37.03	Not done
	Blotted weight (mg, mean ± SD)	58.0 ± 14.00		50.5 ± 16.83	62.4 ± 12.38	75.5 ± 33.32	
	bw (g, mean ± SD)	48.9 ± 8.15		49.2 ± 11.85	50.8 ± 6.52	48.8 ± 8.97	
	Absolute ratio			0.87	1.00	1.30	
	bw adjusted ratio			0.84	1.03	1.24	
	(Lower CL, upper CL)			(0.62, 1.13)	(0.76, 1.40)	(0.91, 1.68)	
7	Wet weight (mg, mean ± SD)	30.7 ± 4.18	32.2 ± 3.35	37.1 ± 5.53	34.9 ± 7.49	52.5 ± 13.23	68.1 ± 7.85
	Blotted weight (mg, mean ± SD)	29.6 ± 3.85	31.0 ± 3.29	35.8 ± 5.36	33.8 ± 7.27	50.9 ± 12.73	66.3 ± 7.69
	bw (g, mean ± SD)	57.2 ± 4.01	57.0 ± 3.13	56.8 ± 4.30	57.4 ± 4.17	57.4 ± 4.20	57.0 ± 3.02
	Absolute ratio		1.05	1.21	1.14	1.72	2.24
	bw adjusted ratio		1.05	1.22	1.12	1.68*	2.25*
	(Lower CL, upper CL)		(0.85, 1.30)	(0.99, 1.50)	(0.91, 1.39)	(1.36, 2.08)	(1.83, 2.78)
8	Wet weight (mg, mean ± SD)	26.0 ± 3.06	23.9 ± 2.69	26.1 ± 2.13	29.2 ± 1.59	37.0 ± 9.72	38.8 ± 5.26
	Blotted weight (mg, mean ± SD)	24.2 ± 2.77	22.3 ± 2.50	24.5 ± 1.83	27.5 ± 1.45	35.2 ± 9.30	36.9 ± 4.80
	bw (g, mean ± SD)	52.9 ± 6.02	52.0 ± 4.96	51.0 ± 5.77	51.4 ± 4.34	50.3 ± 4.84	50.4 ± 4.42
	Absolute ratio		0.92	1.01	1.14	1.45	1.52
	bw adjusted ratio		0.93	1.02	1.15	1.44*	1.54*
	(Lower CL, upper CL)		(0.74, 1.16)	(0.82, 1.28)	(0.92, 1.44)	(1.15, 1.80)	(1.23, 1.93)
9	Wet weight (mg, mean ± SD)	34.9 ± 3.47	37.1 ± 6.88	38.8 ± 6.28	46.3 ± 5.79	65.1 ± 9.37	82.8 ± 13.92
	Blotted weight (mg, mean ± SD)	34.1 ± 3.67	36.4 ± 6.67	38.1 ± 6.32	45.2 ± 5.89	63.9 ± 9.31	80.5 ± 13.10
	bw (g, mean ± SD)	58.0 ± 2.27	58.1 ± 3.84	58.2 ± 3.50	57.1 ± 1.85	58.3 ± 2.75	56.6 ± 2.30
	Absolute ratio		1.07	1.12	1.33	1.88	2.36
	bw adjusted ratio		1.06	1.11	1.33*	1.86*	2.38*
	(Lower CL, upper CL)		(0.83, 1.34)	(0.88, 1.41)	(1.05, 1.70)	(1.47, 2.36)	(1.87, 3.03)
12	Wet weight (mg, mean ± SD)	26.8 ± 6.97	Not done	32.4 ± 5.92	33.3 ± 14.40	49.1 ± 8.42	Not done
	Blotted weight (mg, mean ± SD)	22.4 ± 6.47		27.4 ± 5.34	27.8 ± 15.41	42.5 ± 5.94	
	bw (g, mean ± SD)	40.4 ± 3.38		43.6 ± 2.47	37.1 ± 7.50	40.2 ± 2.46	
	Absolute ratio			1.22	1.24	1.95	
	bw adjusted ratio			1.05	1.31	2.02*	
	(Lower CL, upper CL)			(0.76, 1.44)	(0.95, 1.80)	(1.49, 2.75)	
15	Wet weight (mg, mean ± SD)	33.2 ± 5.56	31.3 ± 5.43	33.5 ± 2.43	30.3 ± 4.50	42.8 ± 9.77	57.0 ± 17.52
	Blotted weight (mg, mean ± SD)	28.7 ± 5.47	27.0 ± 3.35	23.5 ± 3.56	24.7 ± 4.08	35.2 ± 7.76	43.8 ± 13.82
	bw (g, mean ± SD)	48.3 ± 3.65	49.1 ± 2.49	48.9 ± 2.50	47.3 ± 5.28	48.1 ± 3.89	47.0 ± 2.59
	Absolute ratio		0.94	0.82	0.86	1.23	1.53
	bw adjusted ratio		0.94	0.82	0.87	1.22	1.52*
	(Lower CL, upper CL)		(0.70, 1.26)	(0.61, 1.10)	(0.65, 1.17)	(0.91, 1.65)	(1.13, 2.05)
18	Wet weight (mg, mean ± SD)	25.0 ± 1.66	20.7 ± 2.82	23.1 ± 2.97	28.2 ± 4.57	52.4 ± 5.42	72.9 ± 6.30
	Blotted weight (mg, mean ± SD)	21.3 ± 1.50	19.0 ± 2.42	19.8 ± 3.35	24.9 ± 5.27	41.9 ± 4.00	64.4 ± 6.70
	bw (g, mean ± SD)	52.1 ± 3.70	54.4 ± 4.81	56.5 ± 3.86	57.2 ± 4.23	55.2 ± 3.20	58.1 ± 2.54
	Absolute ratio		0.89	0.93	1.17	1.97	3.02
	bw adjusted ratio		0.88	0.90	1.12	1.93*	2.92*
	(Lower CL, upper CL)		(0.71, 1.08)	(0.72, 1.12)	(0.89, 1.40)	(1.56, 2.39)	(2.32, 3.69)
20	Wet weight (mg, mean ± SD)	57.7 ± 13.08	41.9 ± 20.16	43.8 ± 8.70	45.3 ± 10.93	49.4 ± 12.65	50.5 ± 10.88
	Blotted weight (mg, mean ± SD)	54.3 ± 11.77	37.1 ± 8.88	33.7 ± 7.91	37.7 ± 11.77	41.3 ± 10.96	39.0 ± 10.01
	bw (g, mean ± SD)	50.7 ± 4.01	51.8 ± 3.00	50.1 ± 3.12	52.2 ± 5.09	51.2 ± 3.16	50.4 ± 1.95
	Absolute ratio		0.68	0.62	0.69	0.76	0.72
	bw adjusted ratio		0.68	0.62*[b]	0.68	0.75	0.71
	(Lower CL, upper CL)		(0.46, 1.0035)[c]	(0.42, 0.91)	(0.46, 1.01)	(0.51, 1.11)	(0.48, 1.05)
21	Wet weight (mg, mean ± SD)	58.0 ± 7.84	84.8 ± 17.77	75.4 ± 15.29	83.2 ± 11.67	82.9 ± 15.24	78.3 ± 20.80
	Blotted weight (mg, mean ± SD)	47.3 ± 6.92	66.6 ± 15.54	64.2 ± 16.29	68.2 ± 10.19	75.2 ± 15.77	60.0 ± 15.00
	bw (g, mean ± SD)	—[d]	—[d]	—[d]	—[d]	—[d]	—[d]
	Absolute ratio		1.39*[e]	1.34	1.44*[e]	1.58*[e]	1.25
	bw adjusted ratio		—[d]	—[d]	—[d]	—[d]	—[d]
	(Lower CL, upper CL)		—[d]	—[d]	—[d]	—[d]	—[d]

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]The recorded decrease in uterine weights from the control vehicle group was statistically significant. [c]With the upper 95% confidence limit not < 1.0, the result is not statistically significant. [d]Terminal body weights were not recorded by the laboratory. [e]The blotted uterine weights were analyzed without body weight adjustments and were found to be statistically significant. *Level of significance, $p < 0.05$.

in both phases 1 and 2 and, in most cases, with the weak agonists in phase 2 (data not shown). Combining the observations of lower variability and the intermittent limited increase in uterine weights with weak estrogen agonists, the blotted weight appears to be the metric of choice.

Despite the excellent overall agreement among laboratories within protocols, there was some variability concerning the actual doses at which statistical significance was first achieved. This variability in the dose first achieving statistical significance was greatest for BPA in protocols A and B. Here, the dose range was about 3-fold for protocol A

Table 19. Uterine weights, body weights, and ratio of the relative increase in uterine weights for NP in protocol C.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (80 mg/kg/day)	Dose 5 (100 mg/kg/day)
6	Wet weight (mg, mean ± SD)	115.5 ± 19.84	Not done	123.6 ± 18.60	130.8 ± 8.90	136.7 ± 33.99	Not done
	Blotted weight (mg, mean ± SD)	110.7 ± 19.60		112.5 ± 16.77	123.8 ± 12.21	131.2 ± 33.16	
	bw (g, mean ± SD)	299.6 ± 29.76		300.3 ± 17.75	297.2 ± 14.12	302.6 ± 22.87	
	Absolute ratio			1.02	1.12	1.19	
	bw adjusted ratio			1.02	1.14	1.16	
	(Lower CL, upper CL)[a]			(0.79, 1.30)	(0.89, 1.46)	(0.90, 1.48)	
7	Wet weight (mg, mean ± SD)	75.7 ± 6.68	92.0 ± 12.94	84.4 ± 11.52	96.5 ± 9.82	124.5 ± 20.06	115.5 ± 22.27
	Blotted weight (mg, mean ± SD)	73.9 ± 6.58	89.9 ± 12.43	83.0 ± 11.56	94.2 ± 9.67	122.0 ± 18.56	113.5 ± 21.29
	bw (g, mean ± SD)	251.5 ± 10.11	252.4 ± 10.26	251.0 ± 15.54	253.0 ± 11.48	252.9 ± 8.20	249.9 ± 10.75
	Absolute ratio		1.22	1.12	1.27	1.65	1.54
	bw adjusted ratio		1.21	1.12	1.27*	1.64*	1.52*
	(Lower CL, upper CL)		(0.97, 1.50)	(0.90, 1.39)	(1.02, 1.58)	(1.32, 2.03)	(1.22, 1.89)
8	Wet weight (mg, mean ± SD)	86.1 ± 9.20	84.7 ± 10.07	84.8 ± 8.03	99.5 ± 12.82	101.1 ± 7.13	122.2 ± 15.96
	Blotted weight (mg, mean ± SD)	82.2 ± 8.89	80.0 ± 9.91	81.2 ± 8.20	94.7 ± 12.02	96.5 ± 7.50	117.0 ± 14.52
	bw (g, mean ± SD)	286.7 ± 21.81	291.2 ± 17.60	289.3 ± 18.64	289.3 ± 17.75	290.8 ± 13.88	287.8 ± 15.55
	Absolute ratio		0.97	0.99	1.15	1.17	1.42
	bw adjusted ratio		0.97	0.98	1.15	1.17	1.42*
	(Lower CL, upper CL)		(0.81, 1.15)	(0.83, 1.17)	(0.96, 1.36)	(0.98, 1.39)	(1.19, 1.67)
9	Wet weight (mg, mean ± SD)	87.2 ± 11.74	86.3 ± 5.17	89.0 ± 14.35	94.5 ± 20.04	106.9 ± 14.18	140.0 ± 22.49
	Blotted weight (mg, mean ± SD)	86.3 ± 11.88	85.6 ± 4.91	87.5 ± 14.47	93.4 ± 19.85	105.8 ± 13.74	137.7 ± 21.83
	bw (g, mean ± SD)	256.1 ± 8.87	255.3 ± 8.93	260.1 ± 9.96	254.0 ± 11.51	256.1 ± 11.48	254.4 ± 12.33
	Absolute ratio		0.99	1.01	1.08	1.22	1.60
	bw adjusted ratio		1.00	0.99	1.08	1.23	1.61*
	(Lower CL, upper CL)		(0.81, 1.24)	(0.80, 1.23)	(0.88, 1.34)	(0.99, 1.52)	(1.30, 1.97)
12	Wet weight (mg, mean ± SD)	106.0 ± 18.84	Not done	117.6 ± 18.15	118.1 ± 18.76	146.7 ± 26.86	Not done
	Blotted weight (mg, mean ± SD)	98.6 ± 22.04		105.3 ± 17.70	105.1 ± 15.22	129.6 ± 20.50	
	bw (g, mean ± SD)	297.2 ± 14.54		301.4 ± 15.13	299.1 ± 16.72	297.1 ± 16.15	
	Absolute ratio			1.07	1.07	1.31	
	bw adjusted ratio			1.09	1.08	1.33*	
	(Lower CL, upper CL)			(0.83, 1.41)	(0.83, 1.41)	(1.02, 1.73)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

Table 20. Uterine weights, body weights, and ratio of the relative increase in uterine weights for NP in protocol D.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (15 mg/kg/day)	Dose 3 (35 mg/kg/day)	Dose 4 (80 mg/kg/day)	Dose 5 (100 mg/kg/day)
7	Wet weight (mg, mean ± SD)	81.5 ± 12.75	84.9 ± 9.93	88.1 ± 4.53	106.8 ± 11.92	171.8 ± 17.90	158.9 ± 29.05
	Blotted weight (mg, mean ± SD)	83.6 ± 13.41	83.0 ± 9.98	85.7 ± 4.94	104.7 ± 12.12	166.9 ± 17.97	154.4 ± 29.48
	bw (g, mean ± SD)	272.8 ± 14.89	273.1 ± 15.08	268.9 ± 12.51	267.8 ± 11.72	265.5 ± 11.47	261.3 ± 15.27
	Absolute ratio		1.02	1.05	1.29	2.05	1.90
	bw adjusted ratio		1.02	1.08	1.31*	2.11*	1.96*
	(Lower CL, upper CL)[a]		(0.84, 1.24)	(0.88, 1.31)	(1.08, 1.60)	(1.73, 2.58)	(1.60, 2.40)
9	Wet weight (mg, mean ± SD)	76.8 ± 4.59	80.4 ± 9.84	88.0 ± 10.30	106.3 ± 16.24	140.2 ± 14.09	160.6 ± 18.76
	Blotted weight (mg, mean ± SD)	75.9 ± 4.97	79.5 ± 10.04	87.3 ± 10.09	105.4 ± 16.29	139.0 ± 13.58	158.0 ± 17.42
	bw (g, mean ± SD)	282.1 ± 12.40	282.3 ± 14.46	278.9 ± 13.94	274.9 ± 13.89	271.6 ± 19.50	271.2 ± 12.52
	Absolute ratio		1.05	1.15	1.39	1.83	2.08
	bw adjusted ratio		1.04	1.15	1.38*	1.83*	2.08*
	(Lower CL, upper CL)		(0.87, 1.26)	(0.95, 1.38)	(1.14, 1.66)	(1.51, 2.21)	(1.71, 2.51)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance $p < 0.05$.

Table 21. Uterine weights, body weights, and ratio of the relative increase in uterine weights for NP in satellite OVX protocol by oral gavage.

Laboratory	Measure	Vehicle	Dose 1 (15 mg/kg/day)	Dose 2 (75 mg/kg/day)	Dose 3 (125 mg/kg/day)	Dose 4 (250 mg/kg/day)	Dose 5 (350 mg/kg/day)
12	Wet weight (mg, mean ± SD)	101.1 ± 16.93	Not done	163.5 ± 24.57	179.0 ± 30.90	179.2 ± 15.50	Not done
	Blotted weight (mg, mean ± SD)	95.0 ± 16.43		153.8 ± 23.74	168.7 ± 28.77	168.9 ± 16.16	
	bw (g, mean ± SD)	295.5 ± 11.09		284.5 ± 10.86	283.7 ± 13.93	280.4 ± 11.60	
	Absolute ratio			1.62	1.77	1.78	
	bw adjusted ratio			1.60*	1.74*	1.76*	
	(Lower CL, upper CL)[a]			(1.23, 2.09)	(1.34, 2.27)	(1.34, 2.32)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.

(375–1,000 mg BPA/kg/day) and 60-fold for protocol B (10–600 mg BPA/kg/day), whereas protocols C and D first achieved statistical significance at the dose of 100 mg BPA/kg/day in all studies.

The doses at which the weak estrogen agonists first reach statistical significance were far in excess of those determined for the potent reference estrogen EE in phase 1. By oral gavage (protocol A), 16 laboratories achieved statistical significance in phase 1 at either 0.3 or 1 μg EE/kg/day (Kanno et al. 2001). By contrast in phase 2, doses of the weak agonists ranged from 1,000- to 10,000-fold higher as estimated for MX and shown for GN, respectively, to over 300,000-fold higher for BPA. Similar disparities are observed with sc injection, as statistical significance with EE was achieved at 0.1 or 0.3 μg EE/kg/day in phase 1 (Kanno et al. 2001) and from 5 to 200 mg/kg/day with the weak agonists in phase 2, including with extended dosing in protocol D.

As expected for weak estrogen agonists, the maximum increase in the uterine weights was also generally less than that observed for EE in phase 1 of the validation program. The maximum relative ratio responses of EE-treated were 4 to 5 in protocol A, 4.5 to 6 in protocol B, 3.25 to 5 in protocol C, and approximately 4 in protocol D (Kanno et al. 2003). The maximum uterine weights reached by the weak estrogen agonists in these phase 2 studies were route, protocol, and test substance dependent, as is apparent by comparing the data in Figures 1–5.

Differences were found between the routes of administration in study responsiveness, i.e., the dose producing the first statistically significant increase in uterine weight, from test substance to test substance. Although many parties might choose to use the term "sensitivity" rather than "responsiveness," validation experts have used the term sensitivity for a measure of assay performance: the proportion of all positive chemicals that are correctly

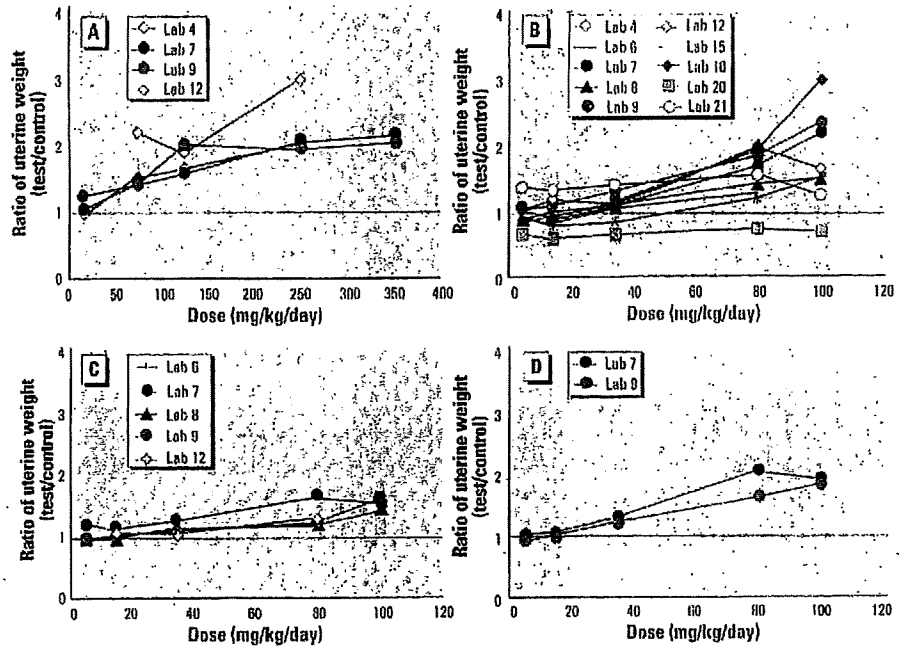

Figure 4. Ratio of the mean absolute blotted uterine weight in response to doses of NP relative to the vehicle control group. (*A*) Participating laboratory results for protocol A using immature female rats, dosing by oral gavage for 3 consecutive days. (*B*) Participating laboratory results for protocol B using immature female rats, dosing by sc injection for 3 consecutive days. (*C*) Participating laboratory results for protocol C using adult OVX rats, dosing by sc injection for 3 consecutive days. (*D*) Participating laboratory results for protocol C using adult OVX rats and extending sc injection dosing to 7 days. In all cases, animals were humanely sacrificed 24 hr after the last dose administration, the uteri were removed and trimmed, and wet and blotted weights were recorded.

Table 22. Uterine weights, body weights, and ratio of the relative increase in uterine weights for *o,p'*-DDT in protocol A.

Laboratory	Measure	Vehicle	Dose 1 (10 mg/kg/day)	Dose 2 (50 mg/kg/day)	Dose 3 (125 mg/kg/day)	Dose 4 (300 mg/kg/day)	Dose 5 (600 mg/kg/day)
3	Wet weight (mg, mean ± SD)	34.6 ± 3.93	44.6 ± 7.09	64.4 ± 4.90	81.3 ± 12.90	101.6 ± 11.55	229.6 ± 65.83[a]
	Blotted weight (mg, mean ± SD)	32.6 ± 4.23	42.5 ± 7.03	62.0 ± 5.06	78.3 ± 12.46	97.3 ± 11.28	104.1 ± 13.22
	bw (g, mean ± SD)	63.2 ± 3.07	62.7 ± 2.21	62.7 ± 1.77	60.6 ± 2.96	54.3 ± 9.56	36.1 ± 2.69
	Absolute ratio[b]		1.07	1.57	1.98	2.46	2.63
	bw adjusted ratio[c]		1.09	1.60*	2.04*	2.67*	3.30*
	(Lower CL, upper CL)[d]		(0.84, 1.40)	(1.24, 2.05)	(1.58, 2.63)	(1.99, 3.59)	(1.80, 6.04)
5	Wet weight (mg, mean ± SD)	45.5 ± 7.68	54.3 ± 11.72	67.3 ± 6.11	87.3 ± 17.05	123.4 ± 40.55[e]	181.2 ± 9.83[e]
	Blotted weight (mg, mean ± SD)	43.3 ± 7.91	52.5 ± 11.61	65.1 ± 5.43	83.4 ± 15.78	100.3 ± 20.64	123.7 ± 21.57
	bw (g, mean ± SD)	58.6 ± 5.07	60.5 ± 2.19	58.2 ± 4.38	55.0 ± 6.53	44.3 ± 11.26	35.0 ± 1.27
	Absolute ratio		1.21	1.50	1.93	2.32	2.86
	bw adjusted ratio		1.19	1.53*	2.01*	2.71*	3.72*
	(Lower CL, upper CL)		(0.91, 1.54)	(1.18, 1.99)	(1.54, 2.63)	(1.92, 2.24)	(2.24, 6.18)
11	Wet weight (mg, mean ± SD)	29.5 ± 4.35	34.2 ± 3.71	63.7 ± 5.55	67.9 ± 4.38	103.8 ± 31.81	210.3 ± 127.63[f]
	Blotted weight (mg, mean ± SD)	25.3 ± 3.75	31.3 ± 3.87	58.7 ± 5.24	64.2 ± 4.20	87.0 ± 3.90	95.2 ± 5.73
	bw (g, mean ± SD)	38.6 ± 3.91	40.6 ± 2.40	41.3 ± 4.01	37.4 ± 2.57	39.5 ± 3.56	28.3 ± 2.30
	Absolute ratio		1.24	2.32	2.54	3.44	3.76
	bw adjusted ratio		1.21*	2.25*	2.60*	3.43*	4.33*
	(Lower CL, upper CL)		(1.04, 1.41)	(1.94, 2.63)	(2.24, 3.01)	(2.96, 3.98)	(3.35, 5.59)
12	Wet weight (mg, mean ± SD)	24.2 ± 2.48	Not done	61.4 ± 9.24	74.0 ± 14.28	129.2 ± 58.42[g]	Not done
	Blotted weight (mg, mean ± SD)	20.6 ± 1.81		54.6 ± 9.03	67.5 ± 14.06	67.4 ± 16.63	
	bw (g, mean ± SD)	39.7 ± 3.10		40.5 ± 2.54	41.3 ± 5.85	33.0 ± 8.16	
	Absolute ratio			2.66	3.28	3.28	
	bw adjusted ratio			2.61*	3.18*	3.45*	
	(Lower CL, upper CL)			(2.01, 3.40)	(2.43, 4.15)	(2.41, 4.94)	

[a]Four animals died in 600 mg DDT/kg/day group before necropsy. [b]Ratio of arithmetic means of the treated blotted uterine weights relative to the vehicle control blotted uterine weights. [c]Ratio of geometric means of treated blotted uterine weights relative to the vehicle control blotted uterine weights after adjusting for body weights at necropsy as a covariable. [d]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [e]One animal died in 300 mg DDT/kg/day group before necropsy; four animals died in 600 mg DDT/kg/d group before necropsy. [f]Three animals died in 600 mg DDT/kg/day group before necropsy. [g]Three animals died in 300 mg DDT/kg/day group before necropsy. *Level of significance, $p < 0.05$.

Table 23. Uterine weights, body weights, and ratio of the relative increase in uterine weights for *o,p'*-DDT in protocol B.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (25 mg/kg/day)	Dose 3 (50 mg/kg/day)	Dose 4 (100 mg/kg/day)	Dose 5 (200 mg/kg/day)
3	Wet weight (mg, mean ± SD)	34.2 ± 3.49	35.0 ± 3.74	40.8 ± 7.04	40.2 ± 2.19	40.0 ± 3.62	51.3 ± 8.10
	Blotted weight (mg, mean ± SD)	31.5 ± 3.97	33.2 ± 3.33	39.0 ± 6.85	38.4 ± 1.82	37.8 ± 3.49	49.5 ± 7.97
	bw (g, mean ± SD)	65.4 ± 3.13	65.1 ± 4.32	64.3 ± 3.60	65.2 ± 4.67	64.6 ± 1.70	65.0 ± 2.97
	Absolute ratio		1.05	1.24	1.22	1.20	1.57
	bw adjusted ratio		0.88	1.03	1.02	1.01	1.31*
	(Lower CL, upper CL)[a]		(0.72, 1.08)	(0.85, 1.26)	(0.84, 1.25)	(0.83, 1.23)	(1.07, 1.59)
5	Wet weight (mg, mean ± SD)	39.0 ± 10.51	44.2 ± 9.83	41.5 ± 15.56	37.7 ± 8.94	44.3 ± 9.77	53.4 ± 8.45
	Blotted weight (mg, mean ± SD)	36.1 ± 10.07	40.5 ± 9.34	38.1 ± 14.74	33.7 ± 8.68	39.5 ± 8.87	48.5 ± 8.89
	bw (g, mean ± SD)	57.5 ± 5.69	57.3 ± 4.97	56.7 ± 5.27	56.5 ± 4.62	55.9 ± 6.36	56.7 ± 4.54
	Absolute ratio		1.12	1.06	0.93	1.09	1.34
	bw adjusted ratio		1.15	1.06	0.97	1.18	1.41*
	(Lower CL, upper CL)		(0.88, 1.49)	(0.82, 1.38)	(0.75, 1.27)	(0.91, 1.54)	(1.09, 1.84)
11	Wet weight (mg, mean ± SD)	27.1 ± 4.15	27.5 ± 3.55	30.6 ± 4.84	27.1 ± 2.09	28.4 ± 2.82	35.8 ± 8.34
	Blotted weight (mg, mean ± SD)	23.6 ± 3.87	24.7 ± 3.31	25.7 ± 3.47	24.1 ± 2.18	25.1 ± 2.79	32.9 ± 7.99
	bw (g, mean ± SD)	38.6 ± 4.11	38.3 ± 3.34	40.0 ± 3.87	38.1 ± 3.21	38.3 ± 3.50	39.3 ± 3.95
	Absolute ratio		1.05	1.09	1.02	1.06	1.39
	bw adjusted ratio		1.06	1.06	1.04	1.08	1.36*
	(Lower CL, upper CL)		(0.85, 1.31)	(0.86, 1.32)	(0.84, 1.29)	(0.87, 1.34)	(1.10, 1.68)
12	Wet weight (mg, mean ± SD)	26.8 ± 6.97	Not done	30.8 ± 10.00	37.2 ± 6.82	37.6 ± 10.86	Not done
	Blotted weight (mg, mean ± SD)	22.4 ± 6.47		26.3 ± 9.16	31.8 ± 6.26	33.9 ± 10.49	
	bw (g, mean ± SD)	40.4 ± 3.38		37.6 ± 6.10	42.7 ± 3.54	41.0 ± 5.01	
	Absolute ratio			1.17	1.42	1.51	
	bw adjusted ratio			1.33	1.30	1.47*	
	(Lower CL, upper CL)			(0.997[b], 1.76)	(0.98, 1.72)	(1.11, 1.94)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]With the lower 95% confidence limit not > 1.0, the result is not considered statistically significant. *Level of significance, $p < 0.05$.

Table 24. Uterine weights, body weights, and ratio of the relative increase in uterine weights for *o,p'*-DDT in protocol C.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (25 mg/kg/day)	Dose 3 (50 mg/kg/day)	Dose 4 (100 mg/kg/day)	Dose 5 (200 mg/kg/day)
3	Wet weight (mg, mean ± SD)	90.8 ± 8.37	101.2 ± 10.13	105.0 ± 6.55	116.6 ± 5.31	126.7 ± 10.92	170.9 ± 37.58
	Blotted weight (mg, mean ± SD)	85.5 ± 8.57	96.6 ± 9.84	99.6 ± 6.43	111.1 ± 4.86	122.2 ± 10.32	169.6 ± 27.57
	bw (g, mean ± SD)	268.9 ± 8.50	267.3 ± 7.50	266.8 ± 14.01	266.5 ± 12.36	264.3 ± 10.76	256.6 ± 17.51
	Absolute ratio		1.12	1.15	1.28	1.41	1.84
	bw adjusted ratio		1.13	1.17	1.30*	1.43*	1.86*
	(Lower CL, upper CL)[a]		(0.96, 1.33)	(0.9899[b], 1.37)	(1.11, 1.53)	(1.21, 1.69)	(1.55, 2.21)
11	Wet weight (mg, mean ± SD)	88.9 ± 6.60	87.2 ± 7.21	98.5 ± 8.16	104.9 ± 12.79	103.6 ± 28.85	112.1 ± 18.41
	Blotted weight (mg, mean ± SD)	78.5 ± 8.38	75.8 ± 7.10	90.5 ± 7.63	97.1 ± 12.07	99.6 ± 28.59	103.4 ± 15.93
	bw (g, mean ± SD)	217.0 ± 5.55	216.2 ± 8.56	217.8 ± 8.86	214.4 ± 9.70	214.6 ± 9.22	212.7 ± 6.96
	Absolute ratio		0.97	1.15	1.24	1.27	1.32
	bw adjusted ratio		0.97	1.15	1.25	1.25	1.34*
	(Lower CL, upper CL)		(0.76, 1.23)	(0.90, 1.46)	(0.98, 1.59)	(0.98, 1.59)	(1.05, 1.71)
12	Wet weight (mg, mean ± SD)	106.0 ± 18.84	Not done	111.8 ± 22.24	116.6 ± 13.98	136.6 ± 37.63	Not done
	Blotted weight (mg, mean ± SD)	98.6 ± 22.04		104.1 ± 20.63	107.9 ± 16.45	128.3 ± 36.21	
	bw (g, mean ± SD)	297.2 ± 14.54		300.1 ± 21.01	294.3 ± 22.76	301.3 ± 15.35	
	Absolute ratio			1.06	1.09	1.30	
	bw adjusted ratio			1.07	1.10	1.31	
	(Lower CL, upper CL)			(0.79, 1.45)	(0.81, 1.49)	(0.96, 1.78)	

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. [b]With the lower 95% confidence limit not > 1.0, the result is not considered statistically significant. *Level of significance, $p < 0.05$.

Table 25. Uterine weights, body weights, and ratio of the relative increase in uterine weights for *o,p'*-DDT in protocol D.

Laboratory	Measure	Vehicle	Dose 1 (5 mg/kg/day)	Dose 2 (25 mg/kg/day)	Dose 3 (50 mg/kg/day)	Dose 4 (100 mg/kg/day)	Dose 5 (200 mg/kg/day)
3	Wet weight (mg, mean ± SD)	89.9 ± 4.86	91.4 ± 10.00	98.0 ± 11.77	104.2 ± 6.70	113.4 ± 9.35	147.7 ± 13.13
	Blotted weight (mg, mean ± SD)	86.3 ± 4.86	87.5 ± 9.40	93.8 ± 10.54	100.8 ± 6.21	109.7 ± 9.24	142.5 ± 12.25
	bw (g, mean ± SD)	290.6 ± 19.95	289.0 ± 11.66	291.3 ± 18.44	282.5 ± 10.96	282.6 ± 6.80	273.8 ± 14.48
	Absolute ratio		1.01	1.09	1.17	1.27	1.65
	bw adjusted ratio		0.94	1.01	1.09	1.18*	1.54*
	(Lower CL, upper CL)[a]		(0.82, 1.08)	(0.88, 1.16)	(0.95, 1.25)	(1.03, 1.36)	(1.34, 1.78)
11	Wet weight (mg, mean ± SD)	84.9 ± 14.40	80.5 ± 7.12	83.6 ± 8.98	102.0 ± 7.79	104.8 ± 5.54	125.0 ± 35.69
	Blotted weight (mg, mean ± SD)	71.5 ± 14.84	73.9 ± 6.44	76.7 ± 8.14	93.9 ± 8.43	98.8 ± 5.91	117.6 ± 33.48
	bw (g, mean ± SD)	235.0 ± 8.47	233.8 ± 11.51	235.3 ± 12.16	232.9 ± 12.09	224.1 ± 9.60	221.8 ± 11.09
	Absolute ratio		1.03	1.07	1.31	1.38	1.64
	bw adjusted ratio		1.05	1.08	1.34*	1.48*	1.73*
	(Lower CL, upper CL)		(0.84, 1.32)	(0.87, 1.35)	(1.07, 1.68)	(1.17, 1.87)	(1.36, 2.20)

[a]Lower and upper 95% confidence limits for ratio of blotted uterine weights based on body weights as a covariable. *Level of significance, $p < 0.05$.