資料５－３

別添 1

厚生労働科学研究費補助金

化学物質リスク研究事業

内分泌かく乱性確定試験法及び内分泌かく乱性試験評価包括ガイドラインの開発に関する総合研究
(H16-化学一一般-001)

平成18年度　総括・分担研究報告書

主任研究者　小野　宏

平成19（2007）年 3月

別添 4

厚生労働科学研究費補助金（化学物質リスク研究事業）
Ⅱ. 分担研究報告書

1. 総括補佐及び一生涯試験、OECD バリデーション関連総括（ラットを用いた BPA 及び DES の経胎盤・経母乳暴露による晩発影響についての検討試験；委託研究）

分担研究者 菅野 純
国立医薬品食品衛生研究所 安全性生物試験研究センター 毒性部 部長

研究要旨

従来の多世代繁殖毒性試験の限界を認識し、その改良を含む試験法開発を進めている。具体的には一生涯（発生、発達、成熟、老化）の全ての段階に於いて内分泌かく乱作用により懸念される毒性指標（神経・行動、免疫毒性等、高次生命系及びその成熟に対する障害に焦点を当てた、従来の多世代繁殖試験の指標に限定されない一連の指標）を網羅的に確認する「齧歯類一生涯試験法」の開発を行う。この詳細試験は、厚生労働省の内分泌かく乱化学物質・試験スキームに則り、内分泌かく乱性を検討する必要がある数十万種の対象化合物について、ホルモン活性に焦点を置いたスクリーニング手法の開発と確立と詳細試験に資する優先リストの作成を進めることと並行して実施するものである。この一環として Bisphenol A(BPA) に引き続き Diethylstilbestrol(DES)の低用量試験を実施した。

平成 16 年度の研究において、BPA の大量投与時のみならず、低濃度の妊娠期･授乳期投与によっても pre-middle age における性周期異常が誘導される可能性が示唆された。平成 17 年度の研究では、得られた出生仔の遅発性性周期異常の誘導に関する再現性の有無を検討するため、0、0.5、5、50 μg/kg/day の用量で BPA をラットの妊娠期から授乳期にかけて投与し、得られた雌出生仔の性周期を 12 ヶ月齢まで継続して検査した。性周期検査において、50 μg/kg 群で 6 ヶ月齢時に、3/30 例が persistent estrus を呈し、7 ヶ月齢時には 6/30 例が constant estrus を呈した。以降、膣スメアが持続した発情を示した動物数が 8 ヶ月齢では 10/18 例、9 ヶ月齢では 6/18 例、10 ヶ月齢では 11/18 例、11 ヶ月齢では 12/18 例、12 ヶ月齢では 11/18 例で観察され、持続発情を呈する動物の出現頻度は 7、8、10、12 ヶ月齢時で有意に増加した。

本年度は、前 2 年度のデータを詳細に見直し、その有意性を検討した。その結果、少なくとも 50 μg/kg 群では平成 16 年度と 17 年度において実施した実験結果と合致する結果が得られたことから、低濃度の BPA の妊娠期･授乳期投与によって誘導される遅発性の性周期異常は再現性のある変化であることが確認された。また、その一般化を図る目的で、エストロゲン活性にして相当量の DES について検討を実施した。

更に、経済開発協力機構（OECD)の 第 9 回内分泌かく乱化学物質の試験及び評価に関する EDTA タスクフォース会合、及び第 5 回第 6 回ヴァリデーションマネジメントグループ／動物試験会合に参加し、国際協調の場に於いて、リードカントリーとして内分泌かく乱化学物質に関する試験法の開発のガイドライン作成に取り組んでいる。

A. 研究目的

厚生労働省の内分泌かく乱化学物質の健康影響に関する検討会に於いて策定された試験スキーム（スクリーニング試験系及び詳細試験）に則り、ホルモン様生体影響を及ぼす化学物質と、その延長線上で生体障害

性を発揮する化学物質（内分泌かく乱化学物質；EDCs）のスクリーニング試験等による優先順位設定を経て、最終的にそれらのEDCs としての生体障害性に対する検出確度の高い確定試験法を開発する。

その一環として、未だ確立されていない低用量域の EDCs の生体影響を評価する検出指標、試験方法のひとつの可能性として、EDCs のラット経胎盤・経母乳暴露によって誘導される雌出生仔の遅発性の性周期異常に焦点を当て、これが低用量影響を検出する検査項目になり得るかを検討する。

更に、グローバルに推進する OECD のバリデーション試験について、ガイドライン化に参加し、日本からの独自の情報の発信や提案を行い、国際協調体制を確保する。

B．研究方法

EDCs による生体影響の検討は、従来、生殖毒性にその焦点が置かれてきた。しかし、低用量問題や遅発影響の検討が進むにつれ、EDCs の有害作用は、個体の発生、成長、成熟、老化に関わる広範なものであることが示唆されることとなった。

ホルモン様生体影響を及ぼす化学物質と、その延長線上で生体障害性を発揮する化学物質のスクリーニング試験等による優先順位設定を経て、最終的にそれらの EDCs としての生体障害性に対する検出確度の高い方法体系を開発することに重点を置き、内分泌かく乱性確定試験開発詳細試験（確定試験）としての「齧歯類一生涯試験法」を提案した。

「試験スキーム」のスクリーニングに於いて形成された化学物質優先順位リストの上位化合物について実施される「確定試験」には、従来型の多世代試験が必ずしも最適でないことが内外の研究により示されてきたため、本申請班の前身となる第 2 期研究班において種々の調査研究を実施してきた。その結果、ここでは既存の多世代試験法の改良のみに止まらず、一個体の受精、発生、発達、成熟、及び老化に亘る一生涯を標的とした有害性評価試験系「齧歯類一生涯試験」を構築することを目指す。これは、OECD の Conceptual Frame Work Level 5に対応し、「齧歯類一生涯試験」試験開発研究及び支援基盤研究の２要素から成る。

本分担研究者は、（1）齧歯類一生涯試験の構築に必要と考えられる各項目を調整する際の取り纏めを行うと共に、実際の試験への応用の可能性を明らかにする為に、

（2）BPA あるいは DES を雌性動物の新生児期に低用量暴露した際の性ホルモン系に対する低用量影響に関する動物実験を実施した（委託研究：委託先：(財) 化学物質評価研究機構、(財) 食品農医薬品安全性評価センター)。さらに、（3）OECD/EDTA における国際協調の場で本研究の紹介を行ってきた経緯に基づいて、その対応を検討した。

（1）齧歯類一生涯試験取り纏め事項

神経・行動

・BPA 及び DES 妊娠期・授乳期暴露による行動影響の評価と機序

・マウス・オペラント条件付けによる神経系高次機能影響の評価

・脳の性分化への影響

免疫

・免疫反応や免疫異常状態に及ぼす影響

内分泌（生殖器発達・老化等）

・雄性生殖器発達及びその機能に及ぼす影響

・胎生期・新生児期暴露が雌性生殖器に与える影響

・生殖器系の老化に至る過程に対する影響

内分泌かく乱性確定試験開発・支援基礎研究

・確定試験に関わる発がん性検討：乳腺上皮系

・確定試験に関わる核内受容体転写活性等迅速確認系構築

・多分化能修飾メカニズムとしての ES 細胞分化増殖影響解析

・生殖器制御メカニズムに基づいた内分泌かく乱作用点解析

・神経系形成・発達メカニズムに基づいた内分泌かく乱作用点

詳細試験（確定試験）

・「齧歯類一生涯試験法」の開発の継続と完成

（2）ラットを用いた BPA 及び DES の経胎盤・経母乳暴露による晩発影響についての検討試験（委託研究：委託先：（財）食品農医薬品安全性評価センター）

平成 16、17 年度に行われた実験の結果を詳細に解析する。実験は、以下のプロトコールに従った。即ち、10 週齢の雌 Crl: CD (SD) BR ラット(日本チャールス・リバー株式会社、日野飼育センター)を 60 匹、雄を 30 匹購入した。5 日間の検疫、馴化期間終了後、健常である事が確認された雌を雄ラットと共に夕刻に交配用ケージに移し、1 対 1 の割合で一晩同居させた。翌朝膣栓及び膣垢中の精子の存在をもって交尾が成立したものとみなし、その日を妊娠 0 日と定めた。交尾確認日ごとに確認順に各群に振り分けた。BPA の 0、0.5、5、50 μg/kg/day の用量を、各群 10、10、10 及び 10 匹の交尾が確認された雌に対し妊娠 6 日から分娩後 20 日(離乳前日)まで毎日強制経口投与した。投与量は最新の体重値を基に算出した。

母動物の全例について、妊娠 0 日から哺育状態も含めて分娩後 21 日(出生仔の離乳日)まで毎日 1 回以上観察した。体重は妊娠 0、6、14、17 及び 20 日、分娩後 0、4、7、14 及び 21 日(出生仔の離乳日)に測定した。妊娠動物の全例を自然分娩させ妊娠期間、出産数を求めた。分娩日を分娩 0 日とした。交尾が確認された全動物は分娩21 日(離乳日)にエーテル麻酔下で安楽致死させた後、器官・組織の肉眼的観察を行った。子宮は切開して硫酸アンモニウムに浸漬後着床痕数を算出した。

出生仔については、出生日(0 日齢)に産仔数、死産仔数、出産死亡仔数、出産生仔数、出産生仔性比、出産生仔外表(口腔内を含む)の検査を行った。一般状態については剖検日まで毎日 1 回以上観察した。体重は 0、4、7、14 及び 21 日齢(出生仔の離乳日)、以降週 1 回に加え、包皮分離日、膣開口日及び剖検日に測定した。4 日齢に同腹出生仔数を雌 6 匹、雄 2 匹になるよう無作為抽出法を用いて調整した。哺育期間中を通して生仔数、死亡仔数の検査を行った。

生存する雌離乳仔全例について、21 日齢から膣開口を観察した。また、生存する雄離乳仔全例について、35 日齢から陰茎包皮分離検査を実施した。

生存する雌出生仔については、12 ヶ月齢まで継続して性周期の観察を行う。膣スメアは AM 9:00 から 11:00 の間に採取する。14 日間連続採取後、2 週間休止のサイクルを 12 ヶ月齢まで繰り返し行い、ギムザ染色後性

周期を観察、発情前期、発情期、発情後期、休止期の 4 区分に分類後、さらに normal、persistent diestrus (休止期が 5-9 日継続)、constant diestrus(休止期が 10 日以上継続)、persistent estrus (発情期が 3-7 日継続)、constant estrus(発情期が 8 日以上継続)に分類した。また、いずれの分類区分にもあてはまらなかったものを、common variable とした。

個体数調整後の同腹出生児のうち、全雄出生仔は 12 ヶ月齢剖検群に振り分けた。

生存する雌出生仔のうち、2 ヶ月例時に測定した体重を基に 2 匹を体重送別無作為抽出法により抽出し、3 ヶ月齢剖検群に振り分け、生存する雌仔のうち 6 ヶ月例時に測定した体重を基に 2 匹を体重送別無作為抽出法により抽出し、7 ヶ月齢剖検群に振り分け、残る 2 匹は 12 ヶ月齢剖検群に振り分けた。但し、同腹雌出生仔が 6 匹に満たない場合、先ず 12 ヶ月齢解剖群に 2 匹、次に 3 ヶ月齢解剖群に 2 匹を優先して振り分けた。発情前期を呈する動物を CO_2+O_2 麻酔下で放血し安楽致死させた後、器官・組織の肉眼的観察を行った。剖検時に子宮、卵巣、肝臓、副腎、腎臓、甲状腺、下垂体を採取し、湿重量を測定した。甲状腺、下垂体については 10%中性緩衝ホルマリン液で固定、約 24 時間後に測定を行った。

本年度は、BPA と同様な晩発影響が陽性対照物質 Diethylstilbestrol(DES)の暴露によってもみられるかを確認する試験を実施する。受容体結合試験やその他の内分泌かく乱作用に関する情報から DES は BPA の約 2,500～5,000 倍の作用を持つと考えられる。従って、本試験では、20ng/kg/day を高用量とし、以下 2 及び 20ng/kg/kg/day を中用量及び低用量に設定した。

10 週齢の雌 Crl: CD (SD) IGSBR ラット(日本チャールス・リバー株式会社)を 70 匹、雄を 30 匹購入した。5 日間の検疫・馴化機関終了後、健常であることが確認された雌を雄ラットと共に夕刻に交配ケージに移し、1 対 1 の割合で一晩同居させた。翌朝膣栓及び膣垢中の精子の存在をもって交尾が成立したものと見なし、その日を妊娠 0 日と定めた。交尾確認日ごとに確認順に各群に振り分けた。

DES の 0、0.2、20ng/kg/day の用量、投与容量 5mL/kg を、各群 10、10、10 及び 10 匹の交尾が確認された雌に対して妊娠 6 日～分娩後 20 日 (離乳前日) まで毎日強制経口投与する。

以下、BPA と同等の試験を行う。

(3) OECD/EDTA/VMG-Mammalian

国際協調下における試験法開発、ガイドライン化等について、当該参加各国との調整、検討を行う。下記は、参加日程。

① EDTA／第5回VMG－mammalian (ヴァリデーションマネジメントグループ／動物試験) 会合 (2006 年 4 月 4-5 日　於 ワシントン DC、米国) ;議題として、子宮肥大試験のガイドライン化、Hershberger 試験をサポートするデータの提示、反復投与試験・改良 TG407 のガイドライン化案、甲状腺ホルモンかく乱物質アッセイ法等、について討議する。

②第 9 回内分泌かく乱化学物質の試験及び評価に関する EDTA タスクフォース会合(2006 年 4 月 26-27 日　於：スウェーデン) ;議題として、陰性物質に対する子宮肥大試験、去勢動物あるいは未成熟動物を用いた Hershberger 試験の検討、改良 TG407、確定

試験(Level 5 studies)、甲状腺ホルモンかく乱物質アッセイ法、カエル変態試験の有用性、魚類を用いた内分泌かく乱試験法、無脊椎動物を用いた試験の有用性、ER 親和性レポーター遺伝子アッセイ等を討論する。

③ EDTA／第6回VMG－mammalian（ヴァリデーションマネジメントグループ／動物試験）会合（2007 年 1 月 17-18 日　於：スロベニア、リュブリャナ）；議題として、子宮肥大試験のガイドライン化への追加データ（マウスの有用性等）、飼料中及び床敷きの植物性エストロゲンが子宮肥大に及ぼす影響、去勢あるいは未成熟動物を用いた Hershberger 試験の有用性、確定試験、改良 TG407 の病理組織学的検査法等について議論する。

C．研究結果

（1）齧歯類一生涯試験取り纏め事項

神経・行動に関しては、BPA 妊娠期・授乳期暴露をモデルとし、dopamine 及び serotonin (5-HT)神経系に着目した行動影響の評価と機序、マウス・オペラント条件付けによる神経系高次機能影響の評価及び脳の性分化への影響解析を実施することになった。

免疫系に関しては、自己免疫発症に関わるモデルの改良、Local Lymph Node Assay を用いた免疫機能の修飾影響の解析を実施することとなった。

内分泌系に関しては、従前の生殖毒性に限定せず、中枢を含む性分化への影響、生殖関連臓器の形成、発達、機能、及びその加齢変化に対する影響を視野に入れた研究を実施することとなった。

詳細試験については、神経・免疫・内分泌ネットワークの発生・発達・成熟・老化を考慮した「齧歯類一生涯試験法」の開発を推進することとなった。

（2）ラットを用いた BPA 及び DES の経胎盤・経母乳暴露による晩発影響についての検討試験（委託研究：委託先：(財) 食品農医薬品安全性評価センター）

平成 17 年度に設定した実験について結果を検討する。：妊娠期間中、母動物の一般状態、体重(Fig. 1)に異常はみられなかった。

分娩日の検査において、0.5 μg/kg 群の一例(母動物 No. 13)が妊娠 22 日目に分娩開始を確認したが、雄出生仔 2 匹、雌出生仔 6 匹を娩出後、死亡、一例(母動物 No. 17)が妊娠 22 日目に死亡及び一例(母動物 No. 20)が妊娠 22 日目に正常分娩したものの、仔の胎盤処理、哺育を行わず、分娩後 2 日に全腹仔の死亡が確認されたた。5 μg/kg 群では一例(母動物 No. 23)が妊娠 22 日目に正常分娩したものの、分娩後一般状態の悪化がみられ仔の哺育を行わず、分娩後 3 日に死亡したため、当該動物の腹仔は殺処分した。また、同群の一例(母動物 No. 27)が妊娠 22 日目に正常分娩したものの、仔の胎盤処理、哺育を行わず、分娩後 4 日に全腹仔の死亡が確認された。50 μg/kg 群の一例(母動物 No. 35)が妊娠22日目に正常分娩したものの、仔の胎盤処理、哺育を行わず、分娩後 4 日に全腹仔の死亡が確認された。

その他の母動物では分娩時及び哺育期間中一般状態、哺育状態に異常はみられず、体重も媒体対照群とほぼ同様な推移を示した。

出生仔

哺育期間中、一般状態に異常はみられず、いずれの BPA 投与群においても媒体対照群

とほぼ同様な体重推移を示した。

生後21日に、媒体対照群、0.5、5、50 μg/kg群で各々46、34、35、46匹の雌離乳仔と、20、12、14、18匹の雄離乳仔を得て、後の検査に供した。離乳以降、媒体対照群の雄1例が33日齢で死亡した。本動物の一般状態に異常はみられなかった。また、媒体対照群の雌1例が自発運動低下、呼吸数減少、体温低下、流涙、紅涙、鼻出血、糞量減少、摂餌不良、白濁尿、横臥位を伴い、323日齢で死亡した。この他、異常はみられなかった。また、体重推移に異常はみられなかった。

膣開口検査において、媒体対照群、0.5、5、50 μg/kg群の平均膣開口日齢は各々34.2±2.2、33.2±2.3、35.0±2.1、34.3±2.5、平均膣開口日の体重(g)は各々132.5±15.5、124.3±16.5、140.3±15.6、133.2±22.8であり、いずれのBPA投与群においても媒体対照群との間に差はみられなかった(Table 1)。

包皮分離検査において、媒体対照群、0.5、5、50 μg/kg群の平均包皮分離日齢は各々40.5±1.1、40.2±1.3、40.7±0.6、41.4±1.6、平均包皮分離日体重(g)は各々222.0±20.4、212.1±22.8、224.4±18.2、218.1±19.2であり、いずれのBPA投与群においても媒体対照群との間に差はみられなかった(Table 2)。

雌出生仔の性周期検査 (Table 3)

膣開口直後から開始した性周期検査では、異常周期を示す動物はみられなかった。

3ヶ月齢時の性周期検査では、50 μg/kg群の1/30例でpersistent estrusがみられた。その他の動物に、異常はみられなかった。

4ヶ月齢時の性周期検査では、媒体対照群では異常周期を示す動物はみられなかった。0.5 μg/kg群の1/23例でconstant estrus、5 μg/kg群の2/22例でconstant diestrusがみられた。50 μg/kg群では異常周期を示す動物はみられなかった。

5ヶ月齢時の性周期検査では、媒体対照群の1/31例でpersistent diestrus、2/31例でconstant diestrus、0.5 μg/kg群の1/23例でpersistent diestrus、1/23例でconstant diestrus、1/23例でpersistent estrus、5 μg/kg群の1/22例でpersistent diestrus、50 μg/kg群の1/30例でpersistent diestrusがみられた。

6ヶ月齢時の性周期検査では、媒体対照群では異常周期を示す動物はみられなかった。0.5 μg/kg群の1/23例でpersistent diestrus、1/23例でpersistent estrus、5 μg/kg群の4/22例でpersistent diestrus、1/22例でconstant diestrus、1/22例でpersistent estrus、1/22例でconstant estrus、50 μg/kg群の3/30例でpersistent estrusがみられた。

7ヶ月齢時の性周期検査では、媒体対照群の4/31例でpersistent diestrus、1/31例でconstant diestrus、1/31例でpersistent estrus、0.5 μg/kg群の1/23例でpersistent diestrus、1/23例でpersistent estrus及び4/23例でconstant estrus、5 μg/kg群の1/22例でpersistent diestrus、2/22例でconstant diestrus、1/22例でpersistent estrus、2/22例でconstant estrus、50 μg/kg群の1/30例でconstant diestrus、6/30例でconstant estrusがみられた。

8ヶ月齢時の性周期検査では、媒体対照群の4/19例でpersistent diestrus、2/19例

でconstant diestrus、1/19例でpersistent estrus、1/19例でconstant estrus、0.5 μg/kg群の1/23例でpersistent diestrus、2/23例でpersistent estrus、7/23例でconstant estrus、5 μg/kg群の1/22例でpersistent diestrus、5/22例でconstant diestrus、2/22例でconstant estrus、50 μg/kg群の1/18例でpersistent diestrus、1/18例でconstant diestrus、2/18例でpersistent estrus、8/18例でconstant estrusがみられた。

9ヶ月齢時の性周期検査では、媒体対照群の3/19例でpersistent diestrus、5/19例でconstant diestrus、1/19例でpersistent estrus、0.5 μg/kg群の1/23例でconstant diestrus、5/23例でpersistent estrus、8/23例でconstant estrus、5 μg/kg群の1/22例でpersistent diestrus、2/22例でconstant diestrus、5/22例でpersistent estrus、2/22例でconstant estrus、50 μg/kg群の5/18例でpersistent diestrus、1/18例でconstant diestrus、2/18例でpersistent estrus、4/18例でconstant estrusがみられた。

10ヶ月齢時の性周期検査では、媒体対照群の1/19例でpersistent diestrus、4/19例でconstant diestrus、1/19例でpersistent estrus、0.5 μg/kg群の5/23例でpersistent diestrus、1/23例でconstant diestrus、10/23例でpersistent estrus、5 μg/kg群の1/22例でpersistent diestrus、5/22例でconstant diestrus、1/22例でpersistent estrus、3/22例でconstant estrus、50 μg/kg群の1/18例でpersistent diestrus、1/18例でconstant diestrus、2/18例でpersistent estrus、9/18例でconstant estrusがみられた。

11ヶ月齢時の性周期検査では、媒体対照群の1/18例でpersistent diestrus、4/18例でconstant diestrus、1/18例でpersistent estrus、2/18例でconstant estrus、0.5 μg/kg群の2/23例でpersistent diestrus、3/23例でconstant diestrus、2/23例でpersistent estrus、8/23例でconstant estrus、5 μg/kg群の2/22例でpersistent diestrus、6/22例でconstant diestrus、2/22例でpersistent estrus、7/22例でconstant estrus、50 μg/kg群の2/18例でconstant diestrus、6/18例でpersistent estrus、6/18例でconstant estrusがみられた。

12ヶ月齢時の性周期検査では、媒体対照群の1/18例でpersistent diestrus、3/18例でconstant diestrus、1/18例でpersistent estrus、1/18例でconstant estrus、0.5 μg/kg群の6/23例でpersistent diestrus、1/23例でconstant diestrus、7/23例でpersistent estrus、5/23例でconstant estrus、5 μg/kg群の7/22例でpersistent diestrus、2/22例でconstant diestrus、3/22例でpersistent estrus、5/22例でconstant estrus、1/22例は分類不能、50 μg/kg群の2/18例でpersistent diestrus、3/18例でpersistent estrus、9/18例でconstant estrusがみられ、1/18例は分類不能であった。

統計学的検討では、0.5 μg/kg群で、9ヶ月齢時のconstant estrus、10ヶ月齢時の総異常周期動物数、persistent estrus、12ヶ月齢時の総異常周期動物数が統計学的に有意な発生頻度を示した。5 μg/kg群で、6ヶ月齢時の総異常周期動物数、12ヶ月齢時の総異常周期動物数が統計学的に有意な発

生頻度を示した。50 μg/kg 群で、7 及び 8 ヶ月齢時の constant estrus、10 ヶ月齢時の総異常周期動物数、constant estrus、12 ヶ月齢時の総異常周期動物数、constant estrus が統計学的に有意な発生頻度を示した。

器官重量 (Table 4)

3 ヶ月齢時の器官重量(雌のみ、媒体対照群 13 例、0.5 μg/kg 群 11 例、5 μg/kg 群 13 例、50 μg/kg 群 16 例)では、50 μg/kg 群で甲状腺の相対重量低値がみられた。

7 ヶ月齢時の器官重量(雌のみ、媒体対照群 12 例、50 μg/kg 群 12 例)では、50 μg/kg 群で脳の絶対重量低値、肝臓及び腎臓の相対重量低値がみられた。

12 ヶ月齢時の器官重量(雄、媒体対照群 19 例、0.5 μg/kg 群 12 例、5 μg/kg 群 14 例、50 μg/kg 群 18 例、雌、媒体対照群 18 例、0.5 μg/kg 群 23 例、5 μg/kg 群 22 例、50 μg/kg 群 18 例)では、雄 5 μg/kg 群で精巣の絶対重量高値、0.5 μg/kg 群で腎臓の絶対重量の低値がみられた。50 μg/kg 群の絶対重量及び全ての BPA 投与群の相対重量で媒体対照群との間に差はみられなかった。雌では、いずれの BPA 投与群においても絶対及び相対重量とも媒体対照群との間に差はみられなかった。

剖検 (Table 5)

3 ヶ月齢時の剖検(雌のみ、媒体対照群 13 例、0.5 μg/kg 群 11 例、5 μg/kg 群 13 例、50 μg/kg 群 16 例)では、50 μg/kg 群で腎臓の腫大(1/16)、腎盂拡張(1/16)、尿管の拡張(1/16)、媒体対照群で腎臓の腫大(1/15)、子宮の結節(1/15)がみられた。5、0.5 μg/kg 群で異常はみられなかった。

7 ヶ月齢時の剖検(雌のみ、媒体対照群 12 例、50 μg/kg 群 12 例)では、50 μg/kg 群で卵巣の小型化(3/12)、胸骨の変形(1/12)、媒体対照群で皮下組織の腫瘤(1/12)がみられた。

12 ヶ月齢時の剖検(雄、媒体対照群 19 例、0.5 μg/kg 群 12 例、5 μg/kg 群 14 例、50 μg/kg 群 18 例、雌、媒体対照群 18 例、0.5 μg/kg 群 23 例、5 μg/kg 群 22 例、50 μg/kg 群 18 例)では、雄において、50 μg/kg 群で腎臓の結石(2/18)、嚢胞(1/18)、腎盂拡張(1/18)、精巣上体の白色部(1/18)、大脳の皮質の部分欠損(1/18)、副腎の暗赤色部(1/18)、腫大(1/18)、後肢のべんち(1/18)、5 μg/kg 群で腎臓の嚢胞(1/14)、腎盂拡張(2/14)、後肢のべんち(1/14)、媒体対照群で腎臓の結石(1/19)、腎盂拡張(1/19)、精巣の腫大(1/19)、精巣上体の白色部(1/19)、外耳の肥厚(2/19)、後肢のべんち(2/19)がみられた。0.5 μg/kg 群で異常はみられなかった。

雌において、50 μg/kg 群で卵巣の小型化(4/18)、下垂体の黒色部(1/18)、腫大(1/18)、後肢のべんち(2/18)、皮下組織の腫瘤(1/18)、乳腺の乳汁分泌(5/18)、5 μg/kg 群で肺の暗赤色部(1/22)、前胃の壁の水腫様変化(1/22)、粘膜陥凹部(1/22)、卵巣の嚢胞(1/22)、小型化(3/22)、子宮のポリープ(1/22)、下垂体の腫大(2/22)、結節(4/22)、後肢のべんち(2/22)、皮下組織の腫瘤(1/22)、乳腺の乳汁分泌(2/22)、発達(6/22)、0.5 μg/kg 群で、肺の右肺中葉の無気肺(1/23)、卵巣の嚢胞(2/23)、小型化(6/23)、子宮の嚢胞(1/23)、下垂体の黒色部(1/23)、暗赤色部(1/23)、腫大(2/23)、結節(2/23)、甲状腺の腫大(1/23)、後肢のべんち(2/23)、乳腺の乳汁分泌(14/23)、発達(1/23)、媒体対照群で卵巣の嚢胞(1/18)、小型化(4/18)、子宮のポリープ(1/18)、膣の嚢胞(1/18)、下

垂体の黒色部(1/18)、腫大(2/18)、後肢のべんち(2/18)、乳腺の乳汁分泌(4/18)、発達(3/18)がみられた。

死亡例(媒体対照群: 雌1例、雄1例)では、雌で下垂体の結節、乳腺の発達、雄で脾臓の小型化がみられた。

(3) OECD/EDTA/ VMG-Mammalian

① EDTA／第5回VMG－mammalian（ヴァリデーションマネジメントグループ／動物試験）会合;

●この会議の Agenda Item 10: Level 5 Studies でプレゼンターションを行った。

「確定試験（詳細試験)」に関わる基礎的な検討を進め、「げっ歯類一生涯試験」の構想を打ち出した。従来の毒性評価法に沿ったこれまでの 1 世代、多世代生殖毒性試験を中心とする大規模なバイオアッセイでは、多くの内分泌かく乱陽性候補物質が陰性の結果に終始することが予想される。しかも、これら従来の試験法では、提起されてきている低用量問題への対応が実質的に困難であるとの判断に達検討してきた。「げっ歯類一生涯試験」における低用量問題については、雌マウスの早期持続発情の発現等、エンドポイント明確絞り込むことにより明らかとする。

●TG407 は Phase 1、2 の結果が公表され、強いエストロゲン物質、アンドロゲン物質、甲状腺作用物質の影響が検出され改良 TG407 の方向に変える結論となった。

②第9回内分泌かく乱化学物質の試験及び評価に関する EDTA タスクフォース会合; Hershberger 試験は、Phase3 の結果が公表され、各試験機関の相関性、検出結果は良好であった。

③EDTA／第6回VMG－mammalian（ヴァリデーションマネジメントグループ／動物試験）会合;

●厚生労働省による「最新版・内分泌かく乱化学物質のスクリーニング及び試験法案のレポート」を菅野が OECD に提出した。

●子宮肥大試験は、ピアレビューが終了し、ガイドライン案作成終了段階に達し、追加データ等の検討を行った。

D. 考察

(1) 齧歯類一生涯試験取り纏め事項

神経障害性に関しては、必ずしも明確な器質的障害は誘導されないことが想定される。本研究班では、高次行動異常を当面の焦点に、胎生期・新生児期暴露が認知機能、場面適応性や報酬効果に及ぼす影響の確認実験系の導入のための基礎的事項が特に低用量域を含めて得られた。免疫系に関しては、有害性指標として自己免疫疾患モデル（人に於いて性差が著しいことで知られるシェーグレン病のモデル)、あるいは IV 型免疫応答のモデルである Local Lymph Node Assay の改良形を用いて、化学物質による自己免疫及び獲得免疫機能の修飾の影響を当面の対象として解析した。内分泌系に関しては、生殖機能に関わる従来の指標に加えて、早発閉経等のモデルの一つとしての成熟後の機能異常の発生を中心とした解析を行った。本研究により、一生涯（発生、発達、成熟、老化）の全ての段階に於ける内分泌かく乱作用を考慮する必要が改めて示されたと考える。

以上、懸念される毒性指標として、神経・行動、免疫毒性等、従来の多世代繁殖試験

の指標に限定されない一連の指標を設定し、引き続き網羅的に確認しつつ、受容体原性毒性の立場を踏まえた試験スキームに則り、クロストーク問題、低用量問題等に的確に対応可能な体制を確立すべく、研究開発を進めてきた。これらは、内分泌かく乱性の試験評価に関する包括的なガイドラインの開発に有用な結果をもたらしたと考える。

(2)ラットを用いたBPA及びDESの経胎盤・経母乳暴露による晩発影響についての検討試験（委託研究：委託先：(財) 食品農医薬品安全性評価センター)

H16～17年度に行ったBPAの雌性動物・胎生期/新生児期・低用量暴露による性ホルモン系低用量影響に関する試験により、少なくとも50 μg/kg群においては再現性のある変化であることが確認され、低濃度のBPAの妊娠期・授乳期投与によってpre-middle ageにおける性周期異常が誘導されることが明らかとなった。BPAではER以外の核内受容体との相互作用により、低用量影響を現す系が存在することが報告されている。ここでのBPAによってみられた遅発性の性周期異常も、エストロゲン受容体を介さないメカニズムが関与している可能性は否定されない。この様な作用はBPA固有のものであるか、エストロゲン作用を有する化学物質共通のものであるかを検討する必要があると考え、H18年度は、陽性対象物質DESを用いて検討を行った。

E．結論

これまでリスク評価のための試験法として考えられてきた多世代繁殖試験や、EPAによって提案されている *in utero* through lactational exposure案には、遅発性の機能異常を評価するための観察項目は設定されていないが、委託研究により、EDCs暴露においては長期間に亘る神経、免疫及び内分泌機能への影響に関する評価が必要であることが示唆された。OECD試験法ガイドライン化を視野に入れ、ここで提案した「齧歯類一生涯試験」の更なる開発、バリデーションを進展させる必要がある。

G．研究発表

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菅野 純、低用量問題の研究の現状と課題、第 17 回環境ホルモン学会講演会、2007 年 2 月 28 日、東京

H. 知的財産所有権の出願・登録状況（予定も含む）

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   国内特許申請中（特願 2003-317031、特願 2004-219285）

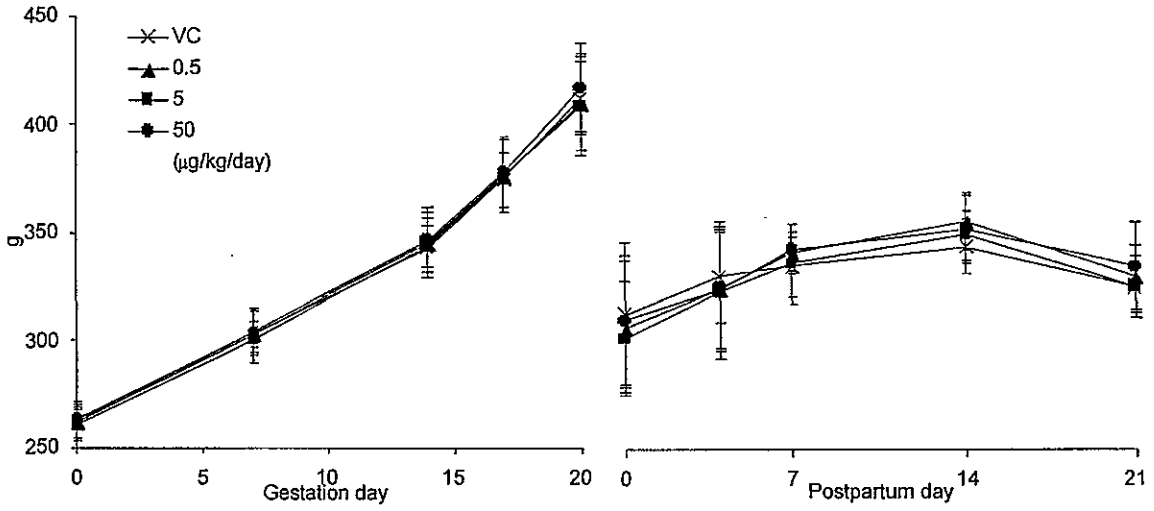


Fig. 1. Mean body weight of dams.

Not significant.

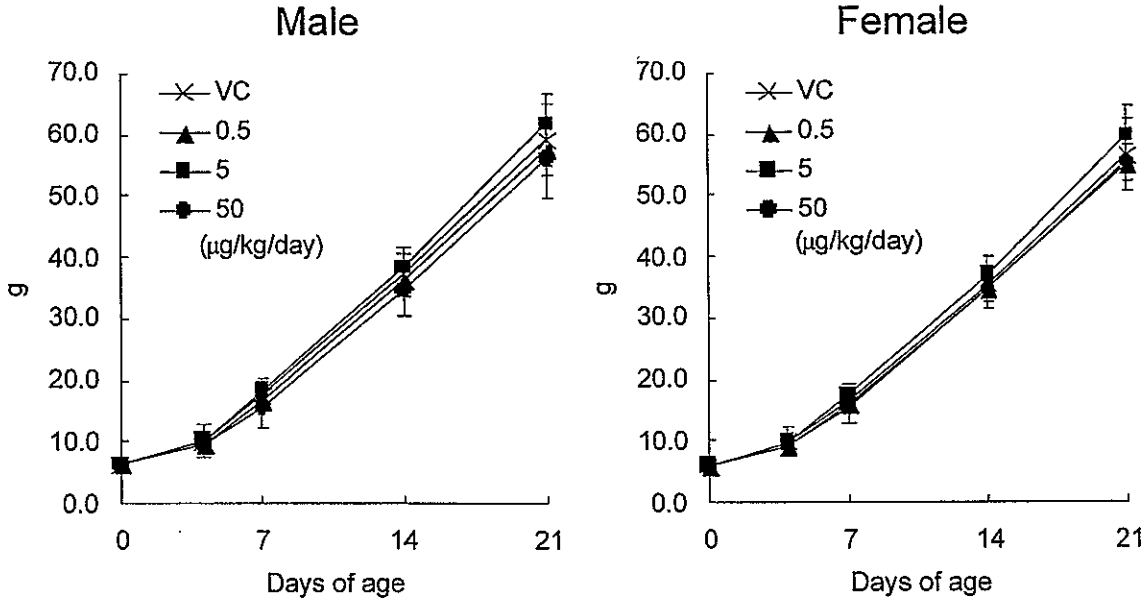


Fig. 2. Mean body weight of offspring (birth to weaning).

Not significant.

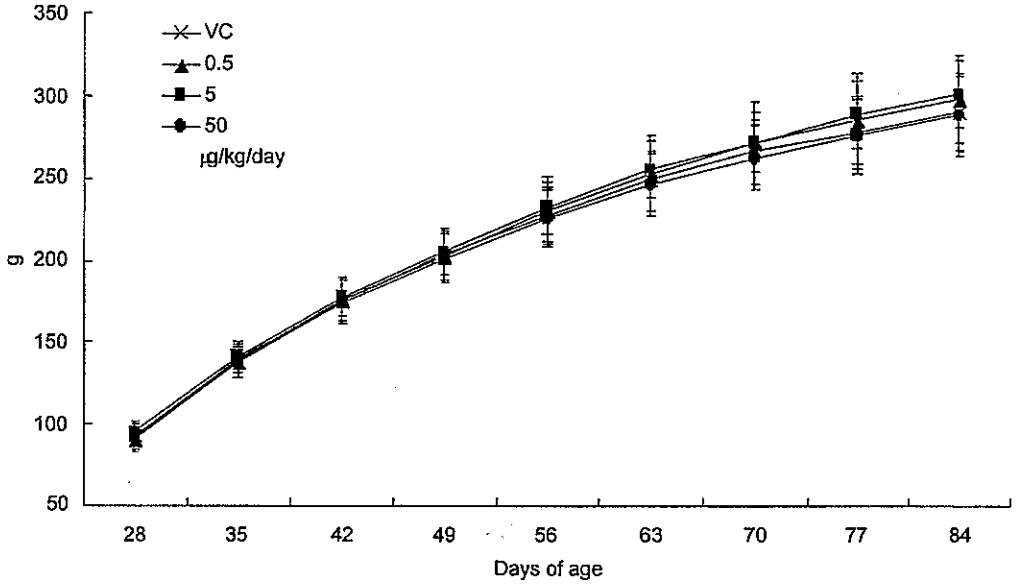


Fig. 4-1. Mean body weight of female offspring.

Not significant.

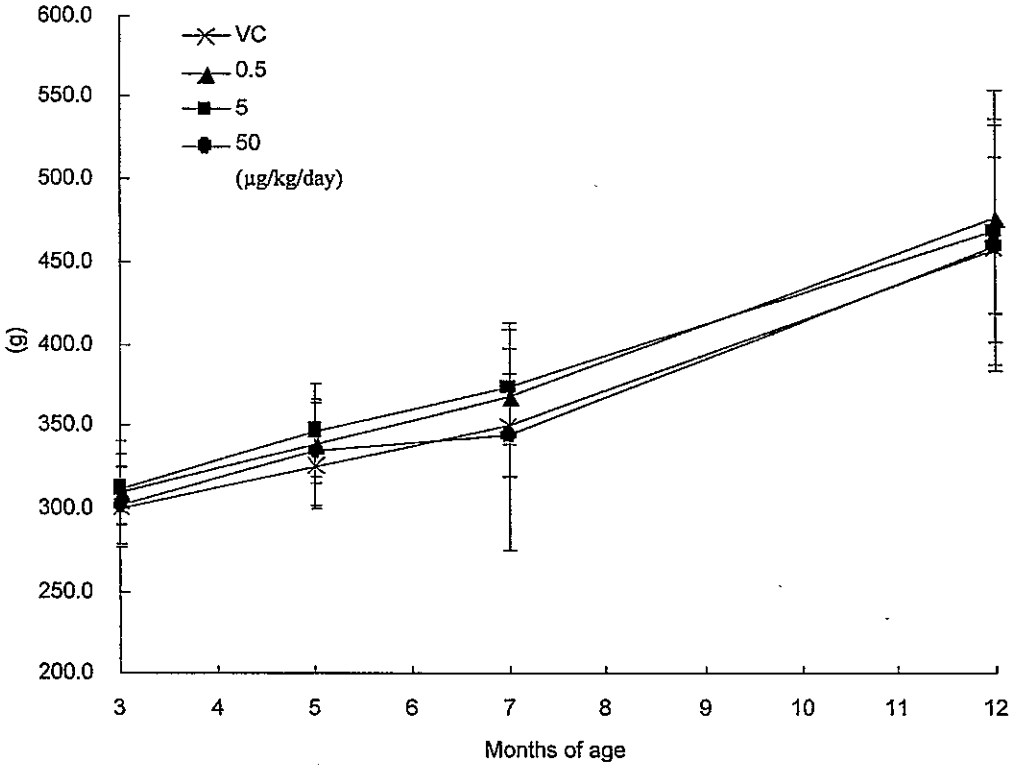


Fig. 4-2. Mean body weight of female offspring.

Not significant.

Table 1. Vaginal opening of offspring.

Maternal dose (μg/kg/day)	age at vaginal opening (days)	body weight at vaginal opening (g)
0	34.2 ±2.2	132.5 ±15.5
0.5	33.2 ±2.3	124.3 ±16.5
5	35.0 ±2.1	140.3 ±15.6
50	34.3 ±2.5	133.2 ±22.8

Not significant.

Table 2. Preputial separation of offspring.

Maternal dose (μg/kg/day)	age at complete separation (days)	body weight at complete separation (g)
0	40.5 ±1.1	222.0 ±20.4
0.5	40.2 ±1.3	212.1 ±22.8
5	40.7 ±0.6	224.4 ±18.2
50	41.4 ±1.6	218.1 ±19.2

Not significant.

Table 3-1. Summary of estrus cycle.

	Dose of BPA (μg/kg/day)			
	0	0.5	5	50
3 months of age				
Normal cycle	31/31 (100.0)	23/23 (100.0)	22/22 (100.0)	29/30 (96.7)
Abnormal cycle	-	-	-	1/30 (3.3)
Persistent diestrus	-	-	-	-
Constant diestrus	-	-	-	-
Persistent estrus	-	-	-	1/30 (3.3)
Constant estrus	-	-	-	-
4 months of age				
Normal cycle	31/31 (100.0)	22/23 (95.6)	20/22 (90.9)	30/30 (100.0)
Abnormal cycle	-	1/23 (4.3)	2/22 (9.1)	-
Persistent diestrus	-	-	-	-
Constant diestrus	-	-	2/22 (9.1)	-
Persistent estrus	-	-	-	-
Constant estrus	-	1/23 (4.3)	-	-
5 months of age				
Normal cycle	28/31 (90.3)	20/23 (87.0)	21/22 (95.5)	29/30 (96.7)
Abnormal cycle	3/31 (9.7)	3/23 (13.0)	1/22 (4.5)	1/30 (3.3)
Persistent diestrus	1/31 (3.2)	1/23 (4.3)	1/22 (4.5)	1/30 (3.3)
Constant diestrus	2/31 (6.5)	1/23 (4.3)	-	-
Persistent estrus	-	1/23 (4.3)	-	-
Constant estrus	-	-	-	-
6 months of age				
Normal cycle	31/31 (100.0)	21/23 (91.3)	15/22 (68.2)	27/30 (90.0)
Abnormal cycle	-	2/23 (8.7)	7/22 (31.8)**	3/30 (10.9)
Persistent diestrus	-	1/23 (4.3)	4/22 (18.2)	-
Constant diestrus	-	-	1/22 (4.5)	-
Persistent estrus	-	1/23 (4.3)	1/22 (4.5)	3/30 (10.0)
Constant estrus	-	-	1/22 (4.5)	-
7 months of age				
Normal cycle	26/31 (83.9)	17/23 (73.9)	16/22 (72.7)	23/30 (76.7)
Abnormal cycle	5/31 (16.1)	6/23 (26.1)	6/22 (27.3)	7/30 (23.3)
Persistent diestrus	4/31 (12.9)	1/23 (4.3)	1/22 (4.5)	-
Constant diestrus	1/31 (3.2)	-	2/22 (9.1)	1/30 (3.3)
Persistent estrus	1/31 (3.2)	1/23 (4.3)	1/22 (4.5)	-
Constant estrus	-	4/23 (17.4)	2/22 (9.1)	6/30 (20.0)*

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
*p <0.05, **p<0.01 (Chi- square test)

Table 3-1 修正

Table . Summary of vaginal cytology.

	Dose of BPA (μg/kg/day)			
	0	0.5	5	50
3 months of age				
Normal cycle	31/31 (100.0)	23/23 (100.0)	22/22 (100.0)	29/30 (96.7)
Abnormal cycle	-	-	-	1/30 (3.3)
Persistent diestrus	-	-	-	1/30 (3.3)
Constant diestrus	-	-	-	-
Persistent estrus	-	-	-	-
Constant estrus	-	-	-	-
4 months of age				
Normal cycle	31/31 (100.0)	22/23 (95.6)	20/22 (90.9)	30/30 (100.0)
Abnormal cycle	-	1/23 (4.3)	2/22 (9.1)	-
Persistent diestrus	-	-	-	-
Constant diestrus	-	-	2/22 (9.1)	-
Persistent estrus	-	-	-	-
Constant estrus	-	1/23 (4.3)	-	-
5 months of age				
Normal cycle	28/31 (90.3)	20/23 (87.0)	21/22 (95.5)	29/30 (96.7)
Abnormal cycle	3/31 (9.7)	3/23 (13.0)	1/22 (4.5)	1/30 (3.3)
Persistent diestrus	1/31 (3.2)	1/23 (4.3)	1/22 (4.5)	1/30 (3.3)
Constant diestrus	2/31 (6.5)	1/23 (4.3)	-	-
Persistent estrus	-	1/23 (4.3)	-	-
Constant estrus	-	-	-	-
6 months of age				
Normal cycle	31/31 (100.0)	21/23 (91.3)	16/22 (68.2)	27/30 (90.0)
Abnormal cycle	-	2/23 (8.7)	6/22 (27.3)**	3/30 (10.9)
Persistent diestrus	-	1/23 (4.3)	3/22 (13.6)	-
Constant diestrus	-	-	1/22 (4.5)	-
Persistent estrus	-	-	2/22 (9.1)	3/30 (10.0)
Constant estrus	-	1/23 (4.3)	-	-
7 months of age				
Normal cycle	26/31 (83.9)	17/23 (73.9)	17/22 (77.3)	23/30 (76.7)
Abnormal cycle	3/31 (9.7)	6/23 (26.1)	5/22 (27.3)	7/30 (23.3)
Persistent diestrus	1/31 (3.2)	1/23 (4.3)	-	-
Constant diestrus	1/31 (3.2)	-	2/22 (9.1)	1/30 (3.3)
Persistent estrus	1/31 (3.2)	1/23 (4.3)	2/22 (9.1)	-
Constant estrus	-	4/23 (17.4)	1/22 (4.5)	6/30 (20.0)*

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
*p <0.05, **p <0.01 (Chi- square test)

Table 3-2. Summary of estrus cycle.

	Dose of BPA (μg/kg/day)			
	0	0.5	5	50
8 months of age				
Normal cycle	11/19 (57.9)	13/23 (56.5)	14/22 (63.6)	6/18 (33.3)
Abnormal cycle	8/19 (42.1)	10/23 (43.5)	8/22 (36.4)	12/18 (66.7)
Persistent diestrus	4/19 (21.1)	1/23 (4.3)	1/22 (4.5)	1/18 (5.6)
Constant diestrus	2/19 (10.5)	-	5/22 (22.7)	1/18 (5.6)
Persistent estrus	1/19 (5.3)	2/23 (8.7)	-	2/18 (11.1)
Constant estrus	1/19 (5.3)	7/23 (30.4)	2/22 (9.1)	8/18 (44.4)*
9 months of age				
Normal cycle	10/19 (52.6)	9/23 (39.1)	12/22 (54.5)	6/18 (33.3)
Abnormal cycle	9/19 (47.4)	14/23 (60.9)	10/22 (45.5)	12/18 (66.7)
Persistent diestrus	3/19 (15.8)	-	1/22 (4.5)	5/18 (27.8)
Constant diestrus	5/19 (26.3)	1/23 (4.3)	2/22 (9.1)	1/18 (5.6)
Persistent estrus	1/19 (5.3)	5/23 (21.7)	5/22 (22.7)	2/18 (11.1)
Constant estrus	-	8/23 (34.8)*	2/22 (9.1)	4/18 (22.2)
10 months of age				
Normal cycle	13/19 (68.4)	7/23 (30.4)	12/22 (54.5)	5/18 (27.8)
Abnormal cycle	6/19 (31.6)	16/23 (69.6)*	10/22 (45.5)	13/18 (72.2)*
Persistent diestrus	1/19 (5.3)	5/23 (21.7)	1/22 (4.5)	1/18 (5.6)
Constant diestrus	4/19 (21.1)	1/23 (4.3)	5/22 (22.7)	1/18 (5.6)
Persistent estrus	1/19 (5.3)	10/23 (43.5)*	1/22 (4.5)	2/18 (11.1)
Constant estrus	-	-	3/22 (13.6)	9/18 (50.0)**
11 months of age				
Normal cycle	10/18 (55.6)	8/23 (34.8)	5/22 (27.3)	4/18 (22.2)
Abnormal cycle	8/18 (44.4)	15/23 (65.2)	17/22 (77.3)	14/18 (77.8)
Persistent diestrus	1/18 (5.6)	2/23 (8.7)	2/22 (9.1)	-
Constant diestrus	4/18 (22.2)	3/23 (13.0)	6/22 (27.3)	2/18 (11.1)
Persistent estrus	1/18 (5.6)	2/23 (8.7)	2/22 (9.1)	6/18 (33.3)
Constant estrus	2/18 (11.1)	8/23 (34.8)	7/22 (31.8)	6/18 (33.3)
12 months of age				
Normal cycle	12/18 (66.6)	4/23 (17.4)	3/22 (13.6)	3/18 (16.7)
Abnormal cycle	6/18 (33.3)	19/23 (82.6)**	19/22 (86.4)**	15/18 (83.3)**
Persistent diestrus	1/18 (5.6)	6/23 (26.1)	7/22 (31.8)	2/18 (11.1)
Constant diestrus	3/18 (16.7)	1/23 (4.3)	2/22 (9.1)	-
Persistent estrus	1/18 (5.6)	7/23 (30.4)	3/22 (13.6)	3/18 (16.7)
Constant estrus	1/18 (5.6)	5/23 (21.7)	5/22 (22.7)	9/18 (50.0)**
Common variable	-	-	1/22 (4.5)	1/18 (5.6)

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
*p <0.05, **p<0.01 (Chi- square test)

Table 3-2 修正

Table . Summary of vaginal cytology.

	Dose of BPA (μg/kg/day)			
	0	0.5	5	50
8 months of age				
Normal cycle	11/19 (57.9)	14/23 (60.9)	14/22 (63.6)	6/18 (33.3)
Abnormal cycle	8/19 (42.1)	9/23 (39.1)	8/22 (36.4)	12/18 (66.7)
Persistent diestrus	4/19 (21.1)	1/23 (4.3)	1/22 (4.5)	1/18 (5.6)
Constant diestrus	2/19 (10.5)	-	5/22 (22.7)	1/18 (5.6)
Persistent estrus	-	1/23 (4.3)	-	2/18 (11.1)
Constant estrus	2/19 (5.3)	7/23 (30.4)	2/22 (9.1)	8/18 (44.4)*
9 months of age				
Normal cycle	12/19 (52.6)	9/23 (39.1)	12/22 (54.5)	6/18 (33.3)
Abnormal cycle	7/19 (47.4)	11/23 (47.8)	10/22 (45.5)	12/18 (66.7)
Persistent diestrus	3/19 (15.8)	-	1/22 (4.5)	5/18 (27.8)
Constant diestrus	5/19 (26.3)	1/23 (4.3)	2/22 (9.1)	1/18 (5.6)
Persistent estrus	1/19 (5.3)	5/23 (21.7)	5/22 (22.7)	2/18 (11.1)
Constant estrus	-	5/23 (21.7)	2/22 (9.1)	4/18 (22.2)
10 months of age				
Normal cycle	13/19 (68.4)	7/23 (30.4)	12/22 (54.5)	5/18 (27.8)
Abnormal cycle	6/19 (31.6)	16/23 (69.6)*	10/22 (45.5)	13/18 (72.2)*
Persistent diestrus	1/19 (5.3)	5/23 (21.7)	3/22 (13.6)	1/18 (5.6)
Constant diestrus	4/19 (21.1)	1/23 (4.3)	5/22 (22.7)	1/18 (5.6)
Persistent estrus	1/19 (5.3)	10/23 (43.5)*	-	3/18 (16.7)
Constant estrus	-	-	2/22 (9.1)	8/18 (44.4)**
11 months of age				
Normal cycle	10/19 (55.6)	8/23 (34.8)	6/22 (27.3)	4/18 (22.2)
Abnormal cycle	8/19 (44.4)	15/23 (65.2)	16/22 (72.7)	14/18 (77.8)
Persistent diestrus	1/19 (5.3)	2/23 (8.7)	1/22 (4.5)	-
Constant diestrus	4/18 (21.1)	3/23 (13.0)	6/22 (27.3)	2/18 (11.1)
Persistent estrus	1/19 (5.3)	2/23 (8.7)	2/22 (9.1)	5/18 (27.8)
Constant estrus	3/19 (15.8)	8/23 (34.8)	6/22 (27.3)	7/18 (38.9)
12 months of age				
Normal cycle	13/18 (72.2)	4/23 (17.4)	3/22 (13.6)	3/18 (16.7)
Abnormal cycle	5/18 (27.8)	17/23 (73.9)**	19/22 (86.4)**	15/18 (83.3)**
Persistent diestrus	1/18 (5.6)	4/23 (17.4)	6/22 (27.3)	2/18 (11.1)
Constant diestrus	3/18 (16.7)	1/23 (4.3)	4/22 (18.2)	-
Persistent estrus	1/18 (5.6)	6/23 26.1)	1/22 (4.5)	3/18 (16.7)
Constant estrus	1/18 (5.6)	6/23 26.1)	7/22 (31.8)	10/18 (55.6)**
Common variable	-	-	1/22 (4.5)	1/18 (5.6)

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
*p <0.05, **p <0.01 (Chi- square test)

Table 3-3. Summary of estrus cycle.

Vehicle control

Rat ID	Months of age									
	3	4	5	6	7	8	9	10	11	12
1	N	N	N	N	CD	N	CD	CD	CD	CD
2	N	N	N	N	N	-	-	-	-	-
3	N	N	CD	N	PE	CE	N	PE	PE	N
4	N	N	N	N	N	N	N	CD	N	N
5	N	N	N	N	N	-	-	-	-	-
6	N	N	CD	N	N	N	N	N	N	N
7	N	N	N	N	N	-	-	-	-	-
8	N	N	N	N	N	N	PE	N	N	N
9	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	-	-	-	-	-
12	N	N	N	N	N	CD	N	N	PE	N
13	N	N	N	N	N	PD	CD	N	N	CD
14	N	N	N	N	N	-	-	-	-	-
15	N	N	N	N	N	-	-	-	-	-
16	N	N	N	N	N	-	-	-	-	-
17	N	N	N	N	N	CE	N	N	CE	CE
18	N	N	N	N	PD	PD	PD	N	N	N
19	N	N	N	N	N	-	-	-	-	-
20	N	N	PD	N	N	-	-	-	-	-
21	N	N	N	N	N	N	CD	N	N	N
22	N	N	N	N	N	CD	CD	PD	CD	PD
23	N	N	N	N	N	N	N	N	N	N
24	N	N	N	N	N	-	-	-	-	-
25	N	N	N	N	N	-	-	-	-	-
26	N	N	N	N	N	N	N	N	PD	N
27	N	N	N	N	N	-	-	-	-	-
28	N	N	N	N	N	PD	N	CD	Dead	Dead
29	N	N	N	N	N	N	N	N	CD	N
30	N	N	N	N	N	PD	CD	CD	CD	CD
31	N	N	N	N	N	N	N	N	CE	N

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)

Table 3-4. Summary of estrus cycle.

BPA 0.5 μg/kg/day

Rat ID	Months of age									
	3	4	5	6	7	8	9	10	11	12
1	N	N	N	N	N	N	N	PD	N	N
2	N	N	N	N	CE	PE	PE	CE	CE	CE
3	N	N	N	N	N	N	N	PD	CD	PD
4	N	N	N	N	N	CE	N	PE	PD	PE
5	N	N	PD	N	CE	CE	CE	N	CE	PE
6	N	N	N	N	N	PE	PE	CE	CE	N
7	N	N	N	N	N	N	N	PD	PD	PD
8	N	N	N	N	PD	N	N	N	CD	PD
9	N	N	N	N	PE	CE	CE	CE	CE	PE
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	CD	N	CD	N
12	N	N	N	N	N	CE	CE	CE	CE	CE
13	N	N	N	N	N	N	N	N	N	CD
14	N	N	N	N	N	N	CE	CE	CE	CE
15	N	N	N	N	N	N	PE	CE	PE	PE
16	N	CE	N	CE	CE	CE	PE	PD	PE	PD
17	N	N	PE	PD	CE	CE	PE	CE	CE	CE
18	N	N	CD	N	N	CE	N	CE	N	PE
19	N	N	N	N	N	PD	PE	N	PD	PE
20	N	N	N	N	N	N	N	N	N	N
21	N	N	N	N	N	N	CE	CE	CE	CE
22	N	N	N	N	N	N	N	PD	N	N
23	N	N	N	N	N	N	N	CE	N	CE

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)

Table 3-5. Summary of estrus cycle.

BPA 5 μg/kg/day

Rat ID	Months of age									
	3	4	5	6	7	8	9	10	11	12
1	N	N	N	N	N	N	N	N	CD	PD
2	N	N	N	PD	N	N	N	N	PD	N
3	N	N	N	PD	N	N	PE	CD	PD	CD
4	N	N	N	PD	CD	CD	CD	CD	CD	PD
5	N	N	N	N	N	N	N	CE	CE	CE
6	N	N	N	N	N	N	N	N	CD	PD
7	N	N	N	N	N	N	CE	PE	CE	PE
8	N	N	N	N	N	CD	PD	N	PE	PE
9	N	N	N	CE	CE	PD	PE	N	PE	CV
10	N	N	N	N	N	CD	N	N	N	N
11	N	CD	N	N	N	CD	N	N	N	CD
12	N	N	N	N	N	N	N	N	N	N
13	N	N	N	N	N	N	N	CD	CD	PD
14	N	N	N	N	N	N	PE	N	CE	PD
15	N	N	N	N	N	N	N	N	CE	CE
16	N	N	N	N	N	N	PE	N	CE	PE
17	N	N	N	N	PE	CE	CE	CE	CE	CE
18	N	CD	N	CD	CD	CD	CD	CD	CD	PD
19	N	N	N	PE	CE	CE	PE	CE	CE	CE
20	N	N	N	N	N	N	N	N	N	CE
21	N	N	N	N	N	N	N	PD	N	CD
22	N	N	PD	PD	PD	N	N	CD	CD	PD

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
CV, common valiable

Table 3-6. Summary of estrus cycle.

BPA 50 μg/kg/day

Rat ID	Months of age									
	3	4	5	6	7	8	9	10	11	12
1	N	N	N	CE	CE	CE	PD	CE	CE	CE
2	N	N	N	N	CE	-	-	-	-	-
3	N	N	N	N	N	-	-	-	-	-
4	N	N	N	N	N	PE	N	CE	CE	CE
5	N	N	N	CE	CE	CE	CE	CE	CE	PE
6	N	N	N	CE	CE	CE	CE	CE	CE	CE
7	N	N	N	N	N	-	-	-	-	-
8	N	N	N	N	N	-	-	-	-	-
9	N	N	N	N	N	CE	PE	PE	PE	CE
10	N	N	PD	N	CD	PE	N	CD	PE	PE
11	N	N	N	N	N	-	-	-	-	-
12	N	N	N	N	N	-	-	-	-	-
13	N	N	N	N	N	N	N	N	CD	N
14	N	N	N	N	N	CE	PE	CE	CE	CE
15	N	N	N	N	N	CE	CE	CE	CE	CE
16	N	N	N	N	N	-	-	-	-	-
17	N	N	N	N	CE	-	-	-	-	-
18	N	N	N	N	N	N	N	N	N	N
19	N	N	N	N	N	N	PD	N	PE	CE
20	N	N	N	N	N	PD	N	N	PE	PD
21	N	N	N	N	N	-	-	-	-	-
22	N	N	N	N	N	CE	PD	PE	N	CV
23	N	N	N	N	N	N	N	CE	CE	CE
24	N	N	N	N	N	-	-	-	-	-
25	N	N	N	N	N	-	-	-	-	-
26	N	N	N	N	N	CE	CE	CE	PE	CE
27	N	N	N	N	N	PD	PD	PD	CD	PD
28	N	N	N	N	CE	-	-	-	-	-
29	N	N	N	N	N	N	CD	N	N	CE
30	N	N	N	N	N	N	N	CE	N	N

PD, persistent diestrus (prolonged diestrus periods lasting 5-9 days)
CD, constant diestrus (prolonged diestrus periods lasting 10 days or more)
PE, persistent estrus (prolonged estrus periods lasting 3-7 days)
CE, constant estrus (prolonged estrus periods lasting 8 days or more)
CV, common valiable

Table 4-1 Summary of absolute organ weights : F1 (3 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g)	Kidney (g)	Ovary (mg)	Uterus (g)	Brain (g)	Pituitary gland (mg)	Thyroid (mg)	Adrenal (mg)	Body weight a) (g)
Female	Vehicle control	15	10.06 ± 0.94	2.01 ± 0.18	85.3 ± 15.2	0.61 ± 0.09	1.99 ± 0.06	18.5 ± 2.5	21.1 ± 4.2	70.7 ± 8.1	293.6 ± 21.6
	0.5	11	10.29 ± 1.01	1.94 ± 0.26	78.4 ± 11.4	0.59 ± 0.09	1.98 ± 0.05	18.0 ± 2.6	22.3 ± 6.0	69.5 ± 9.6	303.6 ± 31.6
	5	13	10.45 ± 1.15	2.01 ± 0.12	89.7 ± 10.1	0.57 ± 0.10	1.96 ± 0.08	18.7 ± 1.9	21.6 ± 3.6	76.5 ± 11.5	312.6 ± 24.4
	50	16	9.64 ± 1.22	1.98 ± 0.33	79.0 ± 9.9	0.59 ± 0.10	1.95 ± 0.07	18.4 ± 2.2	17.7 ± 3.3	72.4 ± 6.3	293.6 ± 28.5

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-2 Summary of absolute organ weights : F1 (7 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g)	Kidney (g)	Ovary (mg)	Uterus (g)	Brain (g)	Pituitary gland (mg)	Thyroid (mg)	Adrenal (mg)	Body weight a) (g)
Female	Vehicle control	12	11.13 ± 1.51	2.28 ± 0.25	80.3 ± 16.5	0.77 ± 0.11	2.08 ± 0.05	24.6 ± 5.4	24.3 ± 3.7	70.9 ± 10.2	360.8 ± 41.0
	0.5	0	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
	5	0	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
	50	12	10.83 ± 2.27	2.14 ± 0.28	72.1 ± 15.3	0.77 ± 0.15	2.01 * ± 0.08	24.9 ± 4.6	25.3 ± 6.6	66.7 ± 6.6	371.8 ± 60.3

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-3 Summary of absolute organ weights : F1 (12 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g)	Kidney (g)	Testis (g)	Epididymis (g)	Ventral prostate (g)	Seminal vesicle (g)	La-bc muscles (mg)	Brain (g)	Pituitary gland (mg)	Thyroid (mg)	Adrenal (mg)	Body weight a) (g)
Male	Vehicle control	19	23.82 ± 3.34	4.35 ± 0.46	3.98 ± 0.36	1.50 ± 0.19	0.64 ± 0.15	2.21 ± 0.30	1407.4 ±168.8	2.34 ± 0.08	17.5 ± 1.7	39.1 ± 5.8	53.7 ± 8.1	784.1 ± 83.7
	0.5	12	23.63 ± 3.64	3.91 * ± 0.51	3.84 ± 0.24	1.54 ± 0.15	0.61 ± 0.26	2.06 ± 0.42	1331.7 ±232.6	2.30 ± 0.09	16.5 ± 2.5	40.5 ± 12.0	55.6 ± 10.2	764.5 ±104.3
	5	14	24.16 ± 3.80	4.32 ± 0.50	4.29 * ± 0.40	1.61 ± 0.15	0.53 ± 0.18	1.98 ± 0.41	1409.3 ±246.0	2.33 ± 0.09	16.1 ± 1.7	41.8 ± 7.5	56.3 ± 6.8	792.7 ± 87.4
	50	18	23.20 ± 3.78	4.13 ± 0.35	3.86 ± 0.37	1.48 ± 0.19	0.62 ± 0.21	2.15 ± 0.39	1365.6 ±256.4	2.34 ± 0.10	18.2 ± 2.9	41.9 ± 9.6	62.1 ± 22.9	795.1 ± 78.8

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-4 Summary of absolute organ weights : F1 (12 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g)	Kidney (g)	Ovary (mg)	Uterus (g)	Brain (g)	Pituitary gland (mg)	Thyroid (mg)	Adrenal (mg)	Body weight a) (g)
Female	Vehicle control	18	13.10 ± 1.94	2.61 ± 0.29	79.3 ± 34.4	0.83 ± 0.21	2.06 ± 0.06	34.7 ± 11.4	29.7 ± 6.9	84.9 ± 18.6	454.5 ± 54.5
	0.5	23	13.13 ± 2.21	2.55 ± 0.23	65.4 ± 23.9	0.86 ± 0.15	2.05 ± 0.08	50.1 ± 43.5	31.4 ± 9.6	82.4 ± 15.3	473.8 ± 60.3
	5	22	12.76 ± 2.58	2.54 ± 0.30	83.1 ± 26.6	0.82 ± 0.22	2.08 ± 0.08	61.5 ±107.1	32.1 ± 7.3	88.1 ± 13.7	466.2 ± 85.6
	50	18	13.04 ± 1.53	2.57 ± 0.21	69.1 ± 27.3	0.88 ± 0.16	2.09 ± 0.12	34.9 ± 8.6	28.2 ± 5.4	86.0 ± 16.6	459.1 ± 72.6

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-5 In utero-through lactational exposure to Bishpenol A

Summary of relative organ weights : F_1 (3 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g/100g)	Kidney (g/100g)	Ovary (mg/100g)	Uterus (g/100g)	Brain (g/100g)	Pituitary gland (mg/100g)	Thyroid (mg/100g)	Adrenal (mg/100g)	Body weight a) (g)
Female	Vehicle control	15	3.43 ± 0.30	0.69 ± 0.06	29.2 ± 6.0	0.21 ± 0.04	0.68 ± 0.05	6.3 ± 0.9	7.2 ± 1.6	24.3 ± 3.9	293.6 ± 21.6
	0.5	11	3.39 ± 0.17	0.64 ± 0.03	25.9 ± 3.1	0.19 ± 0.03	0.66 ± 0.06	5.9 ± 0.7	7.3 ± 1.4	23.0 ± 3.4	303.6 ± 31.6
	5	13	3.34 ± 0.22	0.64 ± 0.04	28.8 ± 3.6	0.18 ± 0.04	0.63 ± 0.04	6.0 ± 0.4	6.9 ± 0.8	24.6 ± 4.4	312.6 ± 24.4
	50	16	3.28 ± 0.23	0.67 ± 0.08	27.1 ± 3.7	0.20 ± 0.04	0.67 ± 0.06	6.3 ± 0.7	6.0 * ± 1.0	24.8 ± 2.5	293.6 ± 28.5

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-6 Summary of relative organ weights : F1 (7 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g/100g)	Kidney (g/100g)	Ovary (mg/100g)	Uterus (g/100g)	Brain (g/100g)	Pituitary gland (mg/100g)	Thyroid (mg/100g)	Adrenal (mg/100g)	Body weight a) (g)
Female	Vehicle control	12	3.08 ± 0.20	0.64 ± 0.07	22.4 ± 4.6	0.21 ± 0.03	0.58 ± 0.07	6.8 ± 1.3	6.8 ± 1.0	19.8 ± 2.5	360.8 ± 41.0
	0.5	0	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
	5	0	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
	50	12	2.90 * ± 0.22	0.58 * ± 0.05	19.8 ± 4.4	0.21 ± 0.03	0.55 ± 0.08	6.8 ± 1.1	6.8 ± 1.1	18.3 ± 2.8	371.8 ± 60.3

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 4-7 Summary of relative organ weights : F1 (12 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g/100g)	Kidney (g/100g)	Testis (g/100g)	Epididymis (g/100g)	Ventral prostate (g/100g)	Seminal vesicle (g/100g)	La-bc muscles (mg/100g)	Brain (g/100g)	Pituitary gland (mg/100g)	Thyroid (mg/100g)	Adrenal (mg/100g)	Body weight a) (g)
Male	Vehicle control	19	3.05 ± 0.39	0.56 ± 0.06	0.51 ± 0.06	0.19 ± 0.02	0.08 ± 0.02	0.28 ± 0.05	181.5 ± 28.4	0.30 ± 0.03	2.2 ± 0.2	5.0 ± 0.8	6.9 ± 1.2	784.1 ± 83.7
	0.5	12	3.09 ± 0.17	0.51 ± 0.04	0.51 ± 0.06	0.20 ± 0.02	0.08 ± 0.03	0.27 ± 0.06	179.0 ± 48.0	0.31 ± 0.03	2.2 ± 0.3	5.3 ± 1.4	7.3 ± 1.2	764.5 ±104.3
	5	14	3.04 ± 0.27	0.55 ± 0.05	0.55 ± 0.09	0.21 ± 0.03	0.07 ± 0.03	0.25 ± 0.06	179.8 ± 37.8	0.30 ± 0.04	2.0 ± 0.3	5.3 ± 1.0	7.2 ± 1.0	792.7 ± 87.4
	50	18	2.91 ± 0.32	0.52 ± 0.05	0.49 ± 0.06	0.19 ± 0.03	0.08 ± 0.03	0.28 ± 0.06	173.1 ± 34.6	0.30 ± 0.04	2.3 ± 0.3	5.3 ± 1.2	7.8 ± 2.7	795.1 ± 78.8

Mean ± S.D.

a) Statistical analysis was not applied.

* *Significantly different from vehicle control at P<0.05.*

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

Table 4-8 Summary of relative organ weights : F1 (12 months after birth)

Sex	Exp.group (?g/kg/day)	Number of animals	Liver (g/100g)	Kidney (g/100g)	Ovary (mg/100g)	Uterus (g/100g)	Brain (g/100g)	Pituitary gland (mg/100g)	Thyroid (mg/100g)	Adrenal (mg/100g)	Body weight a) (g)
Female	Vehicle control	46	1.13 ± 1.43	0.23 ± 0.29	6.8 ± 9.6	0.07 ± 0.10	0.18 ± 0.23	3.0 ± 4.1	2.6 ± 3.4	7.4 ± 9.7	177.8 ±226.7
	0.5	34	1.87 ± 1.33	0.37 ± 0.26	9.5 ± 7.9	0.13 ± 0.09	0.30 ± 0.21	7.3 ± 9.3	4.5 ± 3.5	11.9 ± 9.0	320.5 ±230.3
	5	35	1.72 ± 1.35	0.35 ± 0.28	11.3 ± 9.7	0.11 ± 0.10	0.29 ± 0.24	11.3 ± 36.3	4.4 ± 3.6	12.2 ± 10.2	293.0 ±238.2
	50	46	1.12 ± 1.43	0.22 ± 0.29	6.0 ± 8.6	0.08 ± 0.10	0.18 ± 0.23	3.1 ± 4.1	2.4 ± 3.1	7.5 ± 9.9	179.6 ±230.9

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

差し替え

Table 4-8 Summary of relative organ weights : F1 (12 months after birth)

Sex	Exp.group (μ g/kg/day)	Number of animals	Liver (g/100g)	Kidney (g/100g)	Ovary (mg/100g)	Uterus (g/100g)	Brain (g/100g)	Pituitary gland (mg/100g)	Thyroid (mg/100g)	Adrenal (mg/100g)	Body weight a) (g)
Female	Vehicle control	18	2.89 ± 0.28	0.58 ± 0.07	17.4 ± 7.0	0.19 ± 0.05	0.46 ± 0.06	7.7 ± 2.6	6.6 ± 1.4	18.9 ± 4.6	454.4 ± 54.5
	0.5	23	2.77 ± 0.24	0.54 ± 0.05	14.0 ± 5.2	0.19 ± 0.04	0.44 ± 0.05	10.7 ± 9.6	6.6 ± 2.0	17.7 ± 4.0	473.8 ± 60.3
	5	22	2.74 ± 0.21	0.56 ± 0.08	18.0 ± 5.3	0.18 ± 0.05	0.46 ± 0.10	18.0 ± 44.8	7.0 ± 1.5	19.4 ± 4.7	466.2 ± 85.6
	50	18	2.87 ± 0.30	0.57 ± 0.08	15.4 ± 6.6	0.20 ± 0.05	0.46 ± 0.07	7.8 ± 2.3	6.2 ± 1.0	19.2 ± 4.7	459.1 ± 72.6

Mean ± S.D.

a) Statistical analysis was not applied.

* Significantly different from vehicle control at P<0.05.

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

Table 5-1 Summary of macroscopic examinations : F1 (3 months after birth)

Findings	Female				
	Vehicle control	0.5	5	50	(μg/kg/day)
	ta	ta	ta	ta	
	15[a)]	11	13	16	
No abnormalities detected	13	11	13	15	
Kidney					
Enlargement	0	0	0	1	
Pelvic dilatation	1	0	0	1	
Ureter					
Dilatation	0	0	0	1	
Uterus					
Nodule	1	0	0	0	

ta, terminal autopsy.

a) Number of animals examined.

Table 5-2 Summary of macroscopic examinations : F1 (7 months after birth)

Findings	*Female*		
	Vehicle control	50	(μg/kg/day)
	ta	ta	
	12[a)]	12	
No abnormalities detected	11	8	
Ovary			
Small	0	3	
Sternum			
Deformity	0	1	
Subcutis			
Mass	1	0	

ta, terminal autopsy.

a) Number of animals examined.

Table 5-3 Summary of macroscopic examinations : F1 (12 months after birth)

Findings	Male					Female					
	Vehicle control		0.5	5	50	Vehicle control		0.5	5	50	(μg/kg/day)
	ta	fd	ta	ta	ta	ta	fd	ta	ta	ta	
	19[a)]	1	12	14	18	18	1	23	22	18	
No abnormalities detected	15	0	12	10	11	8	0	4	9	8	
Lung											
Atelectasis of middle lobe of right lung	0	0	0	0	0	0	0	1	0	0	
Dark reddish region	0	0	0	0	0	0	0	0	1	0	
Forestomach											
Edematous change of wall	0	0	0	0	0	0	0	0	1	0	
Recessed region of mucosa	0	1	0	0	0	0	0	0	1	0	
Glandular stomach											
Blackish region of mucosa	0	1	0	0	0	0	0	0	0	0	
Kidney											
Calculi	1	0	0	0	2	0	0	0	0	0	
Cyst	0	0	0	1	1	0	0	0	0	0	
Pelvic dilatation	1	0	0	2	1	0	0	0	0	0	
Testis											
Dark reddish change	0	1	0	0	0						
Enlargement	1	0	0	0	0						
Epididymis											
Whitish region	1	0	0	0	1						
Ovary											
Cyst						1	0	2	1	0	
Small						4	0	6	3	4	
Uterus											
Cyst						0	0	1	0	0	
Polyp						1	0	0	1	0	
Vagina											
Cyst						1	0	0	0	0	

ta, terminal autopsy; fd, found dead.

a) Number of animals examined.

Table 5-4 Summary of macroscopic examinations : F1 (12 months after birth)

Findings	Male					Female					
	Vehicle control		0.5	5	50	Vehicle control		0.5	5	50	(μg/kg/day)
	ta	fd	ta	ta	ta	ta	fd	ta	ta	ta	
	19[a)]	1	12	14	18	18	1	23	22	18	
Cerebrum											
Hydrocepharus	0	1	0	0	0	0	0	0	0	0	
Partial defect of cortex	0	0	0	0	1	0	0	0	0	0	
Spleen											
Small	0	1	0	0	0	0	0	0	0	0	
Pituitary gland											
Blackish region	0	0	0	0	0	1	0	1	0	1	
Dark reddish region	0	0	0	0	0	0	0	1	0	0	
Enlargement	0	0	0	0	0	2	0	2	2	1	
Nodule	0	0	0	0	0	0	1	2	4	0	
Thyroid											
Enlargement	0	0	0	0	0	0	0	1	0	0	
Adrenal											
Dark reddish region	0	0	0	0	1	0	0	0	0	0	
Enlargement	0	0	0	0	1	0	0	0	0	0	
Auricle											
Thickening	2	0	0	0	0	0	0	0	0	0	
Hindlimb											
Tylosis	2	0	0	1	1	2	0	2	2	2	
Subcutis											
Mass	0	0	0	0	0	0	0	0	1	1	
Mammary glamd											
Secretion	0	0	0	0	0	4	0	14	2	5	
Well-developed	0	0	0	0	0	3	1	1	6	0	

ta, terminal autopsy; fd, found dead.

a) Number of animals examined.

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

23-May-2006

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/TG/EDTA/M(2006)2
For Official Use

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

FINAL SUMMARY REPORT OF THE MEETING OF THE VALIDATION MANAGEMENT GROUP FOR MAMMALIAN TESTING

4-5 April 2006, Washington, DC, USA

Ms. Laurence MUSSET
Tel: +33 (0)1 45 24 16 76; Fax: +33 (0)1 45 24 16 75; Email: laurence.musset@oecd.org

JT03209404

English - Or. English

Introduction

1. This meeting was held three years after the fourth VMG-mammalian meeting (April 2003). The main objective of the meeting was to review the status of the work with respect to projects included in the rolling work plan of the Test Guidelines Programme, and to agree on the appropriate next steps.

Agenda Item 1: Opening of the meeting, Introduction of participant

2. Mike Wade (Canada), Chair of the VMG-mammalian, invited all participants to introduce themselves (see Annex 1 for the Participants List).

Agenda Item 2: Approval of the Draft Agenda

3. The agenda [document ENV/JM/TG/EDTA/A(2006)1] was approved with the addition of a discussion on chemical repository under "Other issues".

Agenda Item 3: Need for further work in Parallel with the development of the Test Guideline for the Uterotrophic Bioassay

4. Peter Gelbke, consultant for the Secretariat, summarized the peer review panel report, as well as the peer review panel general agreements and major concerns. The peer review panel was especially concerned that only one oestrogen antagonist, one negative substance and one substance with enhanced activity due to metabolism were tested.

5. Peter Gelbke then presented a proposal for a simple retrospective validation with respect to one or more negative substances. On the basis of negative results in a 2-generation test, an uterotrophic assay, an ER binding assay and a E-Screen, he had identified styrene as a possible candidate, noting that some points are yet to be checked in the 2-generation study (in particular the developmental neuro-toxicity). In addition he proposed that the data provided by Japan with the Uterotrophic Bioassay and two in vitro screening tests (hER receptor binding assay and hER-alpha reporter gene assay) should also be used as support for the specificity of the Uterotrophic Bioassay.

6. Gary Timm informed the VMG of the recommendations of the EPA' Endocrine Disrupter Methods Validation Advisory Committee (EDMVAC) that the EPA support the development of a TG only with regard to the use of the assay for estrogenic chemicals.

7. The VMG agreed that the most important was to identify one or two additional negative substances. For oestrogen antagonist, Japan may be able to provide a report with existing data to show the specificity. Experience with Test Guidelines would provide information on substances with enhanced activity due to metabolism.

8. After a long discussion about what the criteria for a "negative" substance should be, the VMG agreed that the acceleration of vaginal opening is a valuable criterion to consider in addition to increased uterine weight in immature females. It also agreed that styrene might be a good candidate. In addition, a review of the results of (i) uterotrophic assays and (ii) 1 or 2-generation assays carried out in Japan might provide one or more other negative substances. Peter Gelbke will review the data provided by Japan and prepare a paper, if possible for the EDTA meeting.

Agenda Item 4: Development of the new Test Guideline for the Uterotrophic Assay

9. Peter Gelbke presented the Draft Test Guideline. The VMG agreed that Jun Kanno will check whether the text that is already included in the proposed TG with respect to mice use is sufficient and he

will provide a report supporting the use of mice. The TG might be revised at a later stage on the basis of this report.

10. The VMG provided advice on a number of issues for which specific guidance might be necessary. In particular, the VMG agreed that there was no need to specify criteria for negative versus positive, or weak versus strong estrogens, and recommended that the TG should contain guidance on oestrogen content in the feed and on control uterine weights in immature females.

11. Peter Gelbke will revise the draft on the basis of the comments made by the VMG and include the testing for anti estrogenic effect in an appendix as an option. This will be done in consultation with Japan, which is the lead country, with the Secretariat, for this project.

Agenda Item 5: Background Review Document to support the Hershberger Assay

12. The Background Review Document was not available for the meeting; therefore it was not discussed. However, the VMG took note of the status of validation work and discussed how to finalize it.

13. First, Willie Owens presented a status report on the Phase 3 validation of the assay with the castrate model (determination of inter- and intra-laboratory variability and laboratories blinded to identity of test chemicals). The VMG agreed that the Hershberger bioassay is valid for androgen receptor agonists and antagonists and appears feasible for 5α-reductase inhibitors. The VMG took note of the fact that for antagonists, the co-administration of two compounds results in baseline variability, and that only one potent 5α-reductase inhibitor was tested; however, no more validation work was considered necessary for the castrate model.

14. Willie Owens then presented a status report on the validation of the assay with the weanling model. It appears that the weanling model may be just slightly less sensitive than the castrate with anti-androgens.

15. The VMG agreed on the following next steps regarding the validation of the assay with the castrate model: (i) drafting of the Background Review Document, (ii) drafting of the Phase 3 validation report (iii) peer review of the assay. For the assay with the weanling model, 6 laboratories are ready to start Phase 3 validation to evaluate the laboratory performance when the identity of the tested chemical is blinded. The VMG recommended that Phase 3 validation focus on antagonists and include two negative substances. Laboratories previously not involved in the work on the weanling model should run a pre-study before entering coded testing. The minimum requested by the VMG was response to TP and antagonism by flutamide. A validation report needs to be developed for the validation of the assay with the weanling model.

16. In order not to delay the development of a TG for the castrate, it was suggested to have two different Test Guidelines (like TG 203A and TG 203B). Whether a light peer review could be sufficient for the weanling will have to be discussed. The Secretariat referred to Document INF 17 and observed that the development of the Test Guideline is not yet included in the rolling work plan and there is no lead country for this activity.

Agenda Item 6 and 8: Validation of the enhanced TG 407 and Draft Test Guideline for the enhanced TG 407

17. Given the low sensitivity of the assay for identifying weakly estrogenic and anti-androgenic substances, the VMG discussed at length the added value of the assay. Different views were expressed. Some participants considered that the enhanced TG would provide additional information at an acceptable additional cost (estimated to be around 10% including thyroid function-related hormone work); other were

concerned that the results of the TG could be misinterpreted and misused, discouraging the use of other tests. The VMG agreed that the enhanced TG 407 should not be considered as an endocrine disrupters screen and should not be used as a substitute of the Uterotrophic or Hershberger Assays. Negative results of the enhanced 407, with respect to (anti)estrogenic or (anti)androgenic modes of action, should not indicate that there is no need for further investigations concerning potential (anti)estrogenic or (anti)androgenic activity. However, considering that (i) the test method is intended to be a general toxicity assay to screen a wide variety of effects rather than a specific mechanism of action (ii) the test method will be used frequently and (iii) it is better to be informed than not informed of any test positive results, the VMG finally agreed on the recommendation to develop an updated Test Guideline that would replace the current one, subject to the insertion in the Test Guideline of a clear text describing the TG limitations. This text is included in Annex 2.

18. The VMG also agreed on the proposed changes to the Draft final report as included on Document INF 13. The report will be further revised to reflect the above discussion and to include corrections sent by the US.

19. There is no project in the rolling work plan for the development of the updated TG 407 and no lead country; therefore, according to the recent agreement on how to include a project in the Test Guidelines rolling work plan, a lead country or the European Commission should submit a proposal.

20. If the decision to update the Test Guideline is taken, the VMG recommends to convene an international group of pathologists to give detailed guidance on (i) interpretation of subtle hormone changes in target tissue without frank toxicity [for hormone dependant tissues, (male) mammary gland and oestrous synchronisation of female reproductive organs] (ii) dissection procedures for critical tissues, and (iii) evaluation of vaginal smears). Such guidance would improve the assay power and sensitivity. The VMG discussed whether T3, T4 and TSH hormones should be measured routinely and agreed that measurement should be triggered by thyroid histopathology.

21. The VMG recommended that the EDTA Task Force discussed the feasibility of establishing an inventory for all tests carried out according to the updated TG.

Agenda Item 7: Information on peer review process

22. Gary Timm presented the meeting document MD4 that includes the proposal to manage peer review by contract. From experience with the peer review of the Uterotrophic Assay validation, it appears that it is very important that the panel members first agree on what the validation principles should be for the *in vivo* or *in vitro* assay. Expertise should be the most important criteria for selecting the 6 to 10 panel members and a laboratory representative should be available to respond to the panel member questions. All data, and decision rationales related to the validation should be provided to the peer review panel.

Agenda Item 9: Detailed Review Paper (DRP) on Thyroid Hormone Disruption Assay

23. Shirlee Tan presented the DRP. The Secretariat indicated that the DRP would be posted on the EDTA Website for the end of April meeting.

Agenda Item 10: Level 5 Studies

24. Several presentations were made on activities related to the enhancement/improvement of reproductive toxicity tests, aiming to replace the 2 generational toxicity test (OECD TG 416). Based on an extensive database of 2-generation studies, it appears that examination of the F2 generation does not provide increased sensitivity (compared to examination of F1 animals) at detecting the effects of endocrine

disrupters. Furthermore, the sacrifice of F1 and F2 at very young ages prevents the detection of late onset pathology that comes with pathology senescence.

25. James Lamb (US) presented the ILSI/HESI project "The Life stages F1-extended one-generation development and reproductive test to develop an alternative test to the current or standard two-generation reproductive toxicity test". The test is designed as a tiered approach and would evaluate toxicity to reproductive, neural and immune system development. It would greatly reduce the number of animals required for detailed testing of pesticides compared to current requirements.

26. Dr. Aoyama (Japan) presented a project of the Japanese Ministry of Environment "The Enhanced one-generation reproductive toxicity study in rats for predicting low-dose effects". In addition to classical endpoints, molecular responses, in particular expression of estrogens, androgen receptors and ER- and AR responsive genes in the uterus and prostate gland were also evaluated.

27. Jun Kanno (Japan) presented a research project on a definitive testing method "The Rodent one life-span test", which is part of Screening and Testing Scheme for endocrine disrupting chemicals, adopted by the Japanese Ministry of Health Labour and Welfare. Based on the knowledge that traditional one-gen and two-gen protocols are not designed for the detection of receptor-mediated toxicity/low dose effects, the objective of this approach is to capture low dose effects that can be declared "adverse" by evaluating specific endpoints, such as the early onset of persistent oestrus in female mice; therefore, the work is more directed to developing new protocols than rearranging or modifying pre-existing protocols so that it is less advanced in terms of proposing methods/strategies. This activity may lead to a proposal that is different from the proposal made by the US and it was suggested that the outcome of the work on real low doses adverse effect would influence the US proposal at a later stage.

28. The VMG took note of the current work, and agreed to wait for the US retrospective analysis before discussing what the role of the VMG could be with respect to the development of a new or enhanced test, considering that a presentation on the life-steps test was already made at the OECD Working Group on Pesticide.

Agenda Item 11: Intact Male Assay

29. D. Bergfelt (US) and J. O'Connor (BIAC) presented progress with the validation of the 15-day intact adult male rat assay. The VMG took note and welcomed the development of this test. It noted the differences with the enhanced 407, in particular in terms of the number of animals (no less than 15 per group) and the capability of the laboratories to try and control for extraneous factors (e.g. method and time of blood collection and animal stress) that may affect the various hormone concentrations determined in this assay.

Agenda Item 12: Declassification of validation reports and peer review panel reports

30. The VMG supported the declassification of the reports. For the report of the peer review panel, it recommended to keep it anonym. The validation report of the enhanced TG 407 should be checked to ensure that all laboratory names have been removed.

Agenda Item 13: Other issues

31. The VMG was informed that the chemical repository, at the TNO, would be closed after Phase 3 validation of the Hershberger Assay using weanlings. Some countries or the other VMGs might be interested by the remaining chemicals. A proposal was made that the TNO should list the amounts of chemicals still available including the expiration dates. The VMG recommended that this issue be discussed at the EDTA Task Force meeting.

ANNEX 1

List of Participants

Meeting of the Fifth Validation Management Group for Mammalian Testing
Washington

4 April 2006 - 5 April 2006

Available to Government representatives only

ANNEX 2

Initial Considerations and limitations

The TG 407 has been modified to include endpoints to identify chemicals that interfere with thyroid physiology and affect the male and/or female reproductive organs in young adult animals, while still investigating all other toxicological parameters required under the prior TG 407. On the basis of data generated in the validation process, it must be emphasized that the sensitivity of this assay is not sufficient to identify all substances with (anti)androgenic or (anti)estrogenic modes of action. Consequently, the absence of effects in these endpoints can not be taken as evidence for the lack of such effects.

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

19-Apr-2006

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/TG/EDTA/A(2006)2
For Official Use

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

DRAFT AGENDA OF THE MEETING OF THE TASK FORCE ON ENDOCRINE DISRUPTERS TESTING AND ASSESSMENT

26-27 April 2006, Sundbyberg, Sweden

Ms. Laurence MUSSET
Tel: +33 (0)1 45 24 16 76; Fax: +33 (0)1 45 24 16 75; Email: laurence.musset@oecd.org

JT03207734

English - Or. English

9th Meeting of the Task Force on Endocrine Disrupters Testing and Assessment, Sweden, 26-27 April 2006

Draft Agenda

Wednesday 26 April 2006			
09h00	1	**Opening of the meeting by Mike Wader and Ethel Forsberg, Introduction of participants**	
09h15	2	**Adoption of the Draft Agenda** The reports of the three VMG meetings are provided as background documents.	**ENV/JM/TG/EDTA/A(2006)2**
09h20	3	• **Confirmation of the approval of the summary report of the 8th EDTA Task Force meeting** The Task Force will be invited to confirm the approval of the Summary Record of the 8th meeting. • **Information by the Secretariat** The Secretariat will report on issues related to the Test Guidelines Programme, which were discussed or agreed by the WNT and the Joint Meeting since the last EDTA Task Force Meeting.	**ENV/JM/TG/EDTA/M(2005)1** **INF. 1** **ENV/JM(2006)7**
	4	**Follow-up to the VMG-mammalian**	**Meeting Document 3**
09h30	**4a**	**Uterotrophic bioassay** The Chair of the VMG-mammalian (or the Secretariat) will report on the outcome of the discussions at the VMG Meeting; Peter Gelbke will present a retrospective study on negative substances and the draft Test Guideline. The Task Force will be invited to provide comments.	**ENV/JM/TG/EDTA(2006)9** **ENV/JM/TG/EDTA(2006)12**
10h15	**4b**	**Hershberger bioassay** The Chair of the VMG-mammalian (or the Secretariat) will report on the outcome of the VMG meeting. The Task Force will be invited to agree on a timeline for the finalization of the validation reports of the Hershberger bioassay (castrate and weanling models). The VMG proposal on how to proceed with the peer review of the castrate model validation will be also discussed under Item 5c. An SPSF should be submitted by a lead country or the EC for this project.	

10h30	*Coffee Break*		
11h00	**4c**	**Enhanced TG 407** The Secretariat will present the outcome of the VMG-mammalian discussions (including the possibility to create an inventory of tests results) and present the changes made to the validation report and the draft Test Guideline. The Task Force will be invited to endorse the validation report and comment on the draft Test Guideline and on the possible inventory. The VMG proposal on how to proceed with the Peer Review will also be discussed under Item 5c.	**Meeting Document 1** **Meeting Document 2** **INF. 9** **ENV/JM/TG/EDTA(2006)10**
11h30	**4d**	**Level 5 studies** The Chair of the VMG (or the Secretariat) will report on the VMG discussion. After the VMG meeting, a preliminary proposal (informal SPSF) was submitted by the US (INF 13). The EDTA will be invited to comment and make a recommendation on how to proceed with this issue.	**INF. 10** **INF. 11** **INF. 12** **INF. 13** **INF. 14**
12h00	*Lunch Break*		
	5	**Common issues to all VMGs activities**	
14h00	**5a**	**Detailed Review Paper on Thyroid Hormone Disruption Assays** The US will present the DRP. The Task Force will be invited to comment on the DRP for which WNT approval will be requested.	**Meeting Document 4** **Meeting Document 5** **Meeting Document 6**
14h30	**5b**	**Role and Utility of the Amphibian Metamorphosis Assay** Germany, co-lead country with the US and Japan, will present Document ENV/JM/TG/EDTA(2006)2.	**INF. 3** **INF. 4** **ENV/JM/TG/EDTA(2006)2**
15h00	**5c**	• **Issues related to validation** Gary Timm will present document INF 7. The Secretariat will introduce the short document ENV/JM/TG/EDTA(2006)13. The Task Force will be invited to discuss and consider (i) whether it is possible to develop guidance on how to apply flexibility when implementing DG 34, and (ii) how to develop such guidance. • **Peer Review Process for ED-related assays** The Task Force will be invited to discuss and comment on processes for peer review and on a strategy for the	**INF. 7** **ENV/JM/TG/EDTA(2006)13** **ENV/JM/TG/EDTA(2006)3**

		coming years. The discussion will be finalized on the second day if necessary. (See also Agenda items 4b, 4c, 6a and 7b)	
16h30	*Coffee Break*		
17h00	**5d**	**Chemical Selection** Gary Timm will present Document ENV/JM/TG/EDTA(2006)11 and the Task Force will be invited to address the questions included in Paragraph 3 of the document.	**ENV/JM/TG/EDTA(2006)11**
17h30	**5e**	**Scope of the Detailed Review Paper on In Vitro Fish Assays** This issue will be presented by Japan (or the Secretariat). The Task Force will be invited to endorse the scope of the document.	**INF. 15**
18h00	*Meeting adjourns for the day*		
Thursday 27 April			
	6	**Follow-up to the VMG-eco**	**ENV/JM/TG/EDTA/M(2005)4**
09h00	**6a**	**21-day Fish Screening Assay: peer-review and TG development** The Secretariat will introduce the issue. Japan will propose the action plan and timelines. The Task Force will be invited to endorse the VMG recommendation to proceed to peer-review and TG development (in parallel).	**ENV/JM/TG/EDTA(2006)4** **ENV/JM/TG/EDTA(2006)3** **INF. 5** **INF. 6** **INF. 8** **INF. 17**
10h00	**6b**	**Fish Sexual Development Test: current status** Denmark will provide a short status report. This is for information and discussion.	**ENV/JM/TG/EDTA(2006)5**
10h30	*Coffee Break*		
11h00	**6c**	**Fish Full-Life-Cycle and 2-Generation Test** The US or Japan will present the comparison study on medaka. This is for information.	
11h10	**6d**	**Validation of the Amphibian Metamorphosis Assay** The Task Force will be invited to provide advice on further work that might be needed for the validation of the assay.	**ENV/JM/TG/EDTA(2006)6**

11H30	**6e**	**Validation for invertebrates tests** The Secretariat will present a status report on this activity.	**ENV/JM/TG/EDTA/M(2005)4**
12h00	*Lunch Break*		
	7	**Follow-up to the VMG-non animal**	**Meeting Document 8**
14h00	**7a**	**Draft DRP on the Use of Metabolizing Systems for In Vitro Testing of Endocrine Disrupters** Walter Janssens (Belgium) will present an update on the scope, the recommendations and the comments received. The Task Force will be invited to take note of the DRP current status.	**Meeting Document 7**
14H30	**7b**	**ER Stably Transfected Assay** This test method was developed and validated by Japan. The Secretariat will present an update of the preliminary validation assessment of the assay. The Task Force will be invited to agree on a strategy regarding independent scientific review (the strategy should be proposed by Japan).	**INF. 2** **INF. 16**
15h00	*Coffee Break*		
15h30	**8**	**Joint Meeting Declassification of validation and Peer Review reports** The Secretariat will introduce this issue. The Task Force will be invited to take note of the status of the reports and to provide comments on the document as appropriate.	**Meeting Document 9** **Reports posted on the EDTA Website, under "Reports"**
16h00	**9**	**Peer Review Process (continued)**	
16h30	**10**	**Any other issues** Location and dates of the next VMG meetings (Japan for the VMG - non animal in December, Spain for the VMG-Eco in November, Slovenia for the VMG-mammalian in January?)	
17h00	*Meeting adjourns*		

List of Documents

Meeting Documents		
Item	**Title**	**Reference**
2	Draft Agenda (Revised 19 April)	ENV/JM/TG/EDTA/A(2006)2
3	Draft Summary Record of the 8th Meeting of the EDTA Task Force	ENV/JM/TG/EDTA/M(2005)1
4	Follow-up to the meeting of the VMG-Mammalian (Draft Summary Record)	MD3
4a	Draft Test Guideline on the Uterotrophic Bioassay in Rodents	ENV/JM/TG/EDTA(2006)9
4a	Additional data on the specificity of the Uterotrophic Bioassay	ENV/JM/TG/EDTA(2006)12
4c	Draft Validation Report of Enhanced Test Guideline 407	MD1, MD2
4c	Draft Enhanced Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents; Updated with Parameters for Endocrine Effects	ENV/JM/TG/EDTA(2006)10
5a	Draft Detailed Review Paper on Thyroid Hormone Disruption Assays	MD4
5a	Appendix B - Draft Report on Comparison of Thyroid Activity Measures Across Datasets	MD5
5a	Comments and responses on Draft Detailed Review Paper on Thyroid Hormone Disruption Assays	MD6
5b	Role and utility of various assays related to the detection of thyroid active substances	ENV/JM/TG/EDTA(2006)2
5c	Discussion paper related to the validation of test methods and how flexibility could be applied	ENV/JM/TG/EDTA(2006)13
5c, 6a	Proposed approaches to peer-reviews of validated test methods	ENV/JM/TG/EDTA(2006)3
5d	OECD Reference Chemical Selection	ENV/JM/TG/EDTA(2006)11
6	Follow-up to the meeting of the VMG-Eco	ENV/JM/TG/EDTA/M(2005)4
6a	Draft Paper: Example of application of Guidance Document 34 validation criteria	ENV/JM/TG/EDTA(2006)4
6b	Fish Sexual Development Test: Current Status	ENV/JM/TG/EDTA(2006)5
6d	Validation of the Amphibian Metamorphosis Assay	ENV/JM/TG/EDTA(2006)6
7	Follow-up to the meeting of the VMG-Non-Animal	MD8
7a	Draft DRP on the Use of Metabolising Systems for In Vitro Testing of Endocrine Disrupters	MD7
8	Status of the Validation and Peer Review Reports	MD9
Information Documents		
3	Information on the Test Guidelines Programme	INF.1
3	Report from the WNT on refocusing the Test Guidelines Programme, including a revised Workplan	ENV/JM(2006)7
4c	Changes to the Draft Final Report of the Validation of the Updated Test Guideline 407 Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats	INF.9
4d	A Mammalian Life-Stages Generational Development and	INF.10

	Reproductive Test - An Alternative to the Current Two-Generation Test for Detecting Endocrine Disruptor Effects	
4d	Results of One-generation Tests in Evaluation of the Endocrine Disrupting Activities in Rodents	INF.11
4d	English Draft Report of Screening and Testing Scheme for Endocrine Disrupting Chemicals (MHLW) updated for OECD VMG mammalian, April 4-5, 2006 @D.C.	INF.12
4d	SPSF for a new mammalian level 5 test involving various life stages, submitted by Gary Timm and Don Bergfelt, US EPA	INF.13
4d	A Tiered Approach to Life Stages Testing for Agricultural Chemical Safety Assessment	INF.14
5b	SPSF for a new Test Guideline on Endocrine Disrupters Frog Screen and Test, submitted by the US, August 2000	INF.3
5b	BIAC Letter on Frog Metamorphosis Assay, 20 October 2005	INF.4
5c	Validation of Screening and Testing Assays Proposed for the EDSP	INF.7
5e	SPSF for the Development of a DRP on Availability of In Vitro Receptor Assays in Fish for Screening of Endocrine Modulating Activities of Environmental Chemicals, Submitted by Japan, UK and Sweden	INF.15
6a	Results of Assay and Tests in Evaluation of the Endocrine Disrupting Activities in Fish (Medaka), Japan	INF.5
6a	Draft report of Phase 2 of the Validation of the 21-day Fish Screening Assay- Negative Substances Testing	INF.6
6a	US EPA Fish Screening Assay Discussion Paper	INF.8
7b	Summary minutes of teleconference meeting held on 6 Feb 2006 The preliminary validation assessment panel of the 'Japanese multi-laboratories validation study of a stably transfected ERalpha mediated reporter gene assay in Japan'	INF.2
7b	Draft summary minutes of teleconference meeting held on 17 March 2006 The preliminary validation assessment panel of the 'Japanese multi-laboratories validation study of a stably transfected ERα mediated reporter gene assay in Japan'	INF.16
Background Documents		
8	Please see the reports posted on the EDTA protected Website	Reports
Presentations		
6a	Completing the Validation of the OECD Fish Screen - BIAC Perspective, Willie Owens, BIAC	For information only
7a	The Use of Metabolising Systems for In Vitro Testing of Endocrine Disrupters, Walter Janssens, Scientific Institute for Public Health, Belgium	

Draft Agenda

6th Meeting of the Validation Management Group for Mammalian Effects Testing (VMG-Mammalian) of the Task Force on Endocrine Disrupters Testing and Assessment (EDTA) 17-18 January 2007, Ljubljana (Slovenia)

Wednesday 17 January		
09h00	**Agenda Item 1: Opening of the Meeting, Introduction of Participants** The meeting will be opened by Dr. Mike Wade, Chair of the VMG- mammalian. The host will welcome the participants.	
09h15	**Agenda Item 2: Approval of the Draft Agenda**	Draft agenda
09h10	**Agenda Item 3: Information from the Secretariat**	**INF 10** (status of the validation reports)
09h45	**Agenda Item 4: Approval of the report on "Additional data supporting the Test Guideline on the Uterotrophic Bioassay in Rodents"** The Secretariat has developed a specific report addressing additional issues for the validation of the Uterotrophic Bioassay. This report is made of two parts: "Additional data on the specificity of the Uterotrophic Bioassay" and "Validation of the Uterotrophic Bioassay in mice by bridging data to rats". This document was sent to the VMG-mam and the EDTA for comments by late November. Only a few comments were received leading to slight changes to the part on specificity. The VMG will be invited to approve the report.	**MD 8** (specificity - revised document) and **MD 9** (mice) **INF 7** (compiled comments) **INF 8** (Tinwell et al., 2000) **INF 9** (Markey et al., 2001)
10h30	*Coffee break*	
11h00	**Agenda Item 5: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** The Secretariat circulated a draft version of the TG in May 2006 to the WNT for comments/approval. The Secretariat revised the draft TG on the basis of the comments received and identified technical issues to be discussed by the VMG-mam. The revised TG was sent to the WNT in July 2006 for a second commenting round. Following the second series of comments, the draft TG was revised and the antioestrogenic protocol was moved to a separate Guidance Document. The list of technical issues to be discussed by the VMG was completed. The VMG will be invited to discuss these issues and to approve the Guidance Document. With respect to the TG, which is now under discussion by the WNT, the VMG should restrict its comments to the technical issues raised during the commenting periods. **(1) Issues on measurement and influence of phytooestrogen level in the diet and bedding**	**MD 1** (draft TG revised 23 November 2006) **MD 2** (discussion paper) **MD 3** (draft GD on the antioestrogenic protocol) **INF 1** (compiled comments 1st round) **INF 2** (compiled comments 2nd round, revised 23 November) **INF 3** (comments on the discussion issues) **INF 12** (Thigpen et al., 2004) **INF 13** (Kato et al., 2004) **INF 14** (Kanno et al., 2002)

12h00	**Agenda Item 6: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** **(2) Issues on controls and criteria for significance of positive results**	
13h00	*Lunch Break*	
14h30	**Agenda Item 7: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** **(3) Other issues**	
15h30	**Agenda Item 8: Review of the Test Guideline of the Uterotrophic Bioassay after adoption** During the second commenting round, the US proposed that the Test Guideline be revisited after sufficient experience is gained with its use (i.e. after testing the first 50-100 chemicals). The US would welcome working with the OECD Secretariat and would be willing to take the lead in this review. Gary Timm will present the topic and the VMG will be invited to discuss on the modality of implementation of this process and how other countries could be involved.	
16h00	*Coffee Break*	
16h30	**Agenda Item 9: Approval of the Phase-3 report of the validation study of the Hershberger Bioassay – adult castrate model** The Phase-3 of the validation study of the Hershberger Bioassay was sent to the VMG-mam and the EDTA for comments by late September 2006. The Secretariat revised this document on the basis of the comments received. The VMG will be invited to approve the report, revised as appropriate.	**MD 4** (validation report) **INF 4** (compiled comments)
17h10	**Agenda Item 10: Background Review Document of the Hershberger Bioassay** The development of the Background Review Document is on the Work Programme with the U.S. as lead country. Gary Timm will present the document and will answer to questions from the VMG. This document was posted on the public website on 4 January 2007 for comments from the VMG and EDTA by 9 February 2007.	**MD 5** (BRD)
17h30	*Meeting Adjourns for the day*	

Thursday 18 January		
09h00	**Items from previous day – revised documents for approval.**	
10h30	**Agenda Item 11: Phase-3 of the validation study of the Hershberger Bioassay – weanling model** Willie Owens will present the validation work on the weanling model and first results (if available). The VMG will be invited to take note of the progress of the validation study on weanlings, comment and agree on a time schedule for the development of the validation report.	
11h00	*Coffee Break*	
11h30	**Agenda Item 12: New mammalian Level 5 test involving various life stages** Gary Timm will report progress on the work on this issue. The VMG will be invited to discuss the design of the Level 5 mammalian test and the need to form an expert group to look at this issue.	**INF 5** (SPSF) **INF 6** (Cooper et al., 2006)
12h15	**Agenda Item 13: Guidance on histopathology for the updated TG 407** At the last VMG meeting, the need of a guidance on histopathology to improve the assay power and sensitivity was discussed. It was also proposed to convene an international group of pathologists to provide information for this guidance. The VMG will be requested to confirm on the need of a guidance document on histopathology, to set the main axis of this guidance and to discuss of the constitution of the group (need for a meeting?).	
13h00	*Lunch Break*	
14h30	**Agenda Item 14: Validation white paper: Lessons Learned and Experience Offered** The EDTA task Force agreed during its last meeting to develop a paper which would present practical experience gained with validation and show how flexibility was applied. Willie Owens has developed a draft white paper on validation. Willie Owens will present the document. The VMG will be invited to give their initial thoughts on the history related in this document. The WNT will be asked whether they support the development of the document with recommendations as prepared by Willie Owens.	**MD 7** (Draft Validation White paper)

14h50	**Agenda Item 15: Endocrine disrupting chemicals screening and testing scheme project (MHLW)** Jun Kanho will present this topic and the VMG will be invited to take note on the progress made since the last VMG mammalian meeting and comment.	**INF 11**
15h10	**Agenda Item 16: Activities of the common chemical repository in the development and validation of the updated TG 407 and the Uterotrophic and Hershberger Assays.** Elard Jacob will present this topic and the VMG will be invited to take note and comment on the activities and the future of the repository.	
15h30	**Agenda Item 17: Other issues** Any new activity for the VMG? Issues to be forwarded to the EDTA Task Force Meeting; Any other issues; Date of the next meeting; Election or reelection of the chair.	
16h30	*Meeting adjourns*	

Item 15 **INF11**

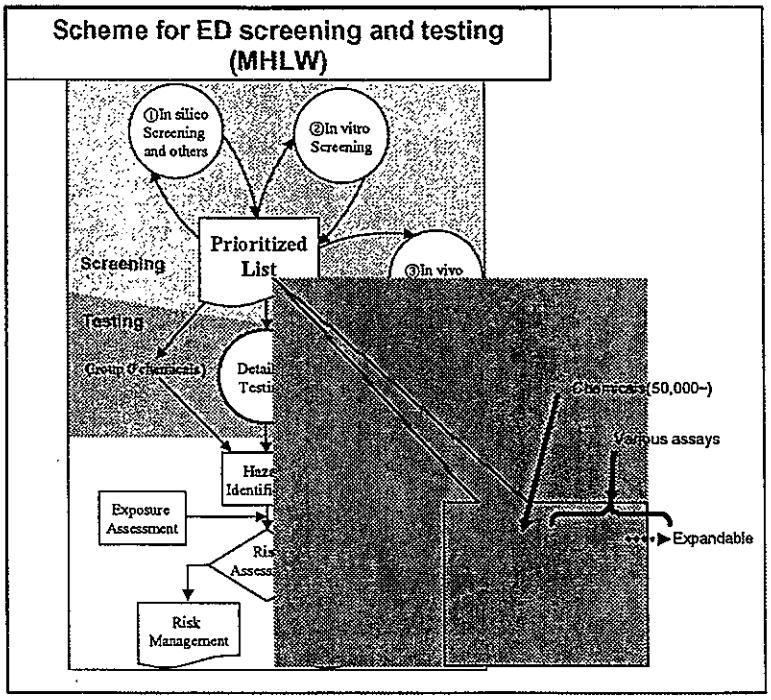
Draft Report of Screening and Testing Scheme for Endocrine Disrupting Chemicals (MHLW) **updated** for OECD VMG mammalian, *January 17-18, 2007 @Slovenia*

Scheme Working Group Members

Hiroshi Ono, FDSC
Yasuyuki Shimohigashi, Kyushu Univ.
Tohru Inoue, NCBSR, NIHS
Hajime Nawata, Kyushu Univ.
Yoshihiko Funae, Osaka City Univ.
Jun Kanno, NCBSR, NIHS

Drafted by, Jun Kanno
Division of Cellular and Molecular Toxicology,
Biological Safety Research Center,
National Institute of Health Science, Tokyo

1. Introduction



The Japanese Ministry of Health Labour and Welfare has adopted a Screening and Testing Scheme for Endocrine Disrupting Chemicals. The aim of the screening is to prioritize tens of thousands of chemicals with special reference to the hormonal activities. The prioritized chemicals are then subject to the Definitive Testing for the risk assessment and following risk management.

Since 1998, Research Groups have been assembled by the support of MHLW Health Science Research grants.

For the Screening, 1) in silico, 2) in vitro and 3) in vivo methods are prepared as a battery tests to prioritize the chemicals. The Chemicals in the list is sorted by the battery test data whenever the new measurement data are added to the list (a chemical with high hormonal activity goes to the top portion of the list, whereas a chemical with low activity goes down in position). It is noted that the total number of chemicals in this list does not decrease, and only the position of the chemicals are subject to changes, so that the list will mature according to the increase in data. The category for sorting chemicals can be increased on demand and can be give different weight for the sorting. For example, the product volume can be added so that both hormonal activity and production volume of the chemicals are incorporated in the process of prioritization.

Top chemicals of the prioritized list are subject to the definitive testing, risk assessment, and risk management. The chemicals proven to be negative by the definitive testing will be kept in the hold box until any new scientific finding on possible disruption emerges.

2. Current status of the development of the Screening and Testing Methods.

Screening methods

1) In silico screening:

The receptor binding ability is predicted by the 3D-SAR using the docking model. Fully automated ER alpha and ER beta docking models are developed, and a series of virtual screenings have been conducted. About 5,000~6,000 possible binders are selected from 20,000 chemical list. Current attempt includes calculation of relative binding affinity (RBA) against 17 beta estradiol. The disadvantage of the docking model is the need of structural information of the receptor molecule and also chemicals. To reduce the possibility of false negative, the crystallographic data of the ER with antagonist is used (the binding site is wider than that of agonist binding ER). The advantage of the docking model is that it does not need teaching molecule so that chemicals of any structure can be calculated (no chemical domain is considered, except for metals). Our study showed that the docking model can be utilized with low false negative rate so that it is useful for the screening purpose.

2) In vitro screening:

i. Cell culture system:

a. Reporter gene assay using mammalian cell lines

Screening methods which monitors the hormone receptor-dependent transcription were developed and tested. Hela cell was used for ER alpha, ER alpha-antagonist, ER beta, ER beta-antagonist, and CHO cell was used for AR, AR-antagonist, TR (TR beta + RXR alpha), TR-antagonist. The number of tested compounds on each assay was shown in Table1.

Table.1 Number of compound tested in NIHS (MHLW) by TA assay

NR	Assay system		No. of compounds tested
ERalpha	stable	agonist assay	350
		antagonist assay	350
	transient	agonist assay	170
ERbeta	transient	agonist assay	170
AR	stable	agonist assay	150
		antagonist assay	150
TRbeta*	transient	agonist assay	150
		antagonist assay	150

* TRbeta / RXR co-transfected CHO cell was used for assay

b. Other assays

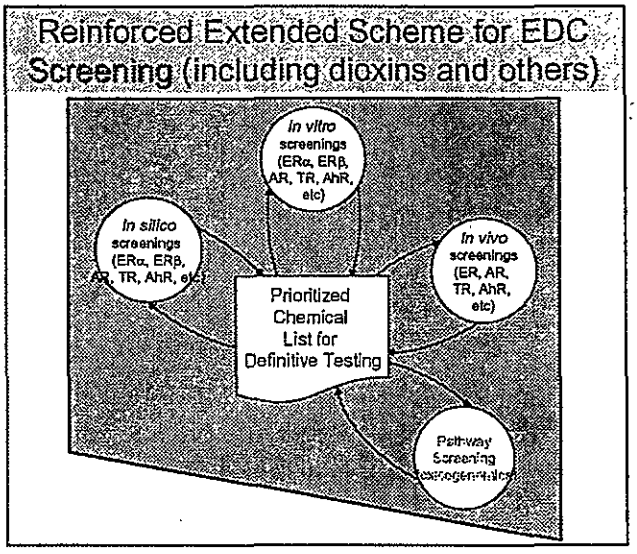
Aromatase assay using human granulosa tumor cell line (KGN cells) have been established and tested for 100 chemicals to detect aromatase activators and inhibitors. For androgenic activities, intranuclear AR dot localization assay, activin receptor assay, chemotactic assay for anti-androgens are under development.

ii. Cell-free system

Surface Plasmon Resonance system for measurement of molecular interaction between Receptor, Ligand, DNA responsive element, and co-factor are developed. Ligand dependent alteration in interaction between ER alpha and ERE sequence, ER beta and ERE sequence, ER alpha and cofactor TIF-2 LxxLL sequence, and ER beta and cofactor TIF-2 LxxLL sequence were tested for 300, 100, 300, 100 measurements respectively.

iii. Pathway screening

Additional comprehensive screening method would be the high-density cDNA microarray. We are planning to conduct a small scale in vivo microarray study to scan for the signaling pathways involved in the hormone receptor signaling system by referring to a large toxicogenomics database.

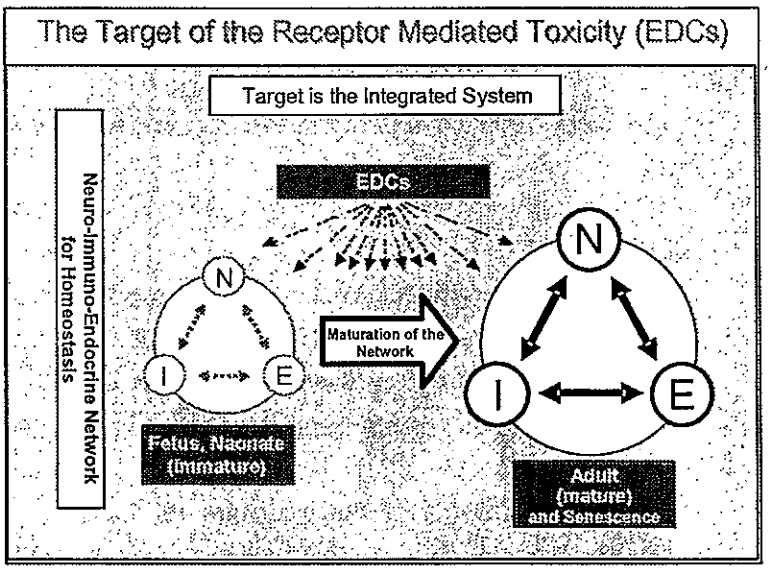


3) *In vivo* screening

The monitor in vivo activity of

the chemicals, uterotrophic assay for estrogenicity/ anti-estrogenicity and Hershberger assay for androgenicity/ anti-androgenicity are tested for 50 and 5 chemicals respectively. OECD TG407 has been tested for possible screening for thyroid hormone disruption.

Definitive Testing method ("Rodent one life-span test"):



It has been shown that multi-generation studies are relatively less sensitive to the known estrogens such as DES and 17beta-estradiol. On the other hand, so-called "low-dose effects" of hormonally active chemicals are likely to exist according to the recent studies on behaviors, sex organ functions and other non-reprotox endpoints including ageing-related phenotypes. Recognizing these facts, a 3-year research project (2005-2007 Leader: Dr. H. Ono) was organized aiming at gathering/ developing experimental protocols that are actually picking up low-dose effects in various systems and various endpoints covering the Neuro-Immuno-Endocrine network. This research project prioritize itself by obtaining real low dose data and gather them as proof of principle for the project approach of developing definitive testing method(s) suitable for receptor-mediated toxicity. A key, again, to this approach is to incorporate a concept of monitoring of the integrated system consisting of neuro-immuno-endocrine network at all stages of a life span, from conception, in utero development, growth, maturation to senescence (ageing).

The Research project consists of 18 scientists covering the research areas as follows;

- Definitive testing (rodent one life span test)
  - Neurological/ behavioral endpoints
    - Behavior effects of BPA perinatal exposure, its assessment and mechanism
    - Mouse operant conditioning for evaluation of higher brain functions Effects on sex differentiation of the brain
  - Immunological endpoints
    - Effects on immunological abnormality (autoimmune status, etc.)

- Endocrine endpoints
  - Effects on male reproductive organ development
  - Effects on female reproductive organ development
  - Effects on ageing processes of reproductive organs
- Supportive methodologies

  Carcinogenesis: Mammary gland epithelial system/ Nuclear receptor transcription, a high throughput detection system/ ES cell differentiation/ proliferation system/ Reproductive system signal transduction/ Nervous system development mechanism

■ Additional protocols for comprehensive screening/ testing scheme
- Ovary as a sensitive detection system
- Prostate system as assessment of androgen-estrogen signal crosstalk

■ OECD and other international collaborations/ communications

Biological Effects monitored so far by the research project are as follows (by the end of second year);

Low-dose effects

- Neuro/behavior: methamphetamine hypersensitivity by *in utero* BPA exposure (5 microg/kg p.o. to dam) .
- Neuro/behavior: BPA *perinatal (transplacental and translactational* 6dpc~PND20*)* low dose exposure (0.33~33 ppm in diet), has no effect on SCOB (Schedule-Controlled Operant Behavior) study (C57BL/6 male mouse).
- Neuro/behavior: Phytoestrogen low diet and behavior.
- Endocrine/ageing : early consistent estrus by *perinatal (transplacental and translactational* 6dpc~PND20*)* BPA exposure (0.5microg/kg p.o. and higher to dam) in Crj:CD-IGS rats.
- Endocrine/ageing : early consistent estrus by *postnatal (PND1~5)* DES p.o. exposure (the lowest tested 0.5 microg/kg p.o. and higher) (rat Crj:CD-IGS).
- Endocrine/ageing : early consistent estrus by *a single postnatal (PND1)* DES s.c. injection 1.5 microg/kg and higher.
- Reproductive system : increased multioocyte follicles by dietary phytoestrogen and perinatal DES exposure (C57BL/6 mouse)

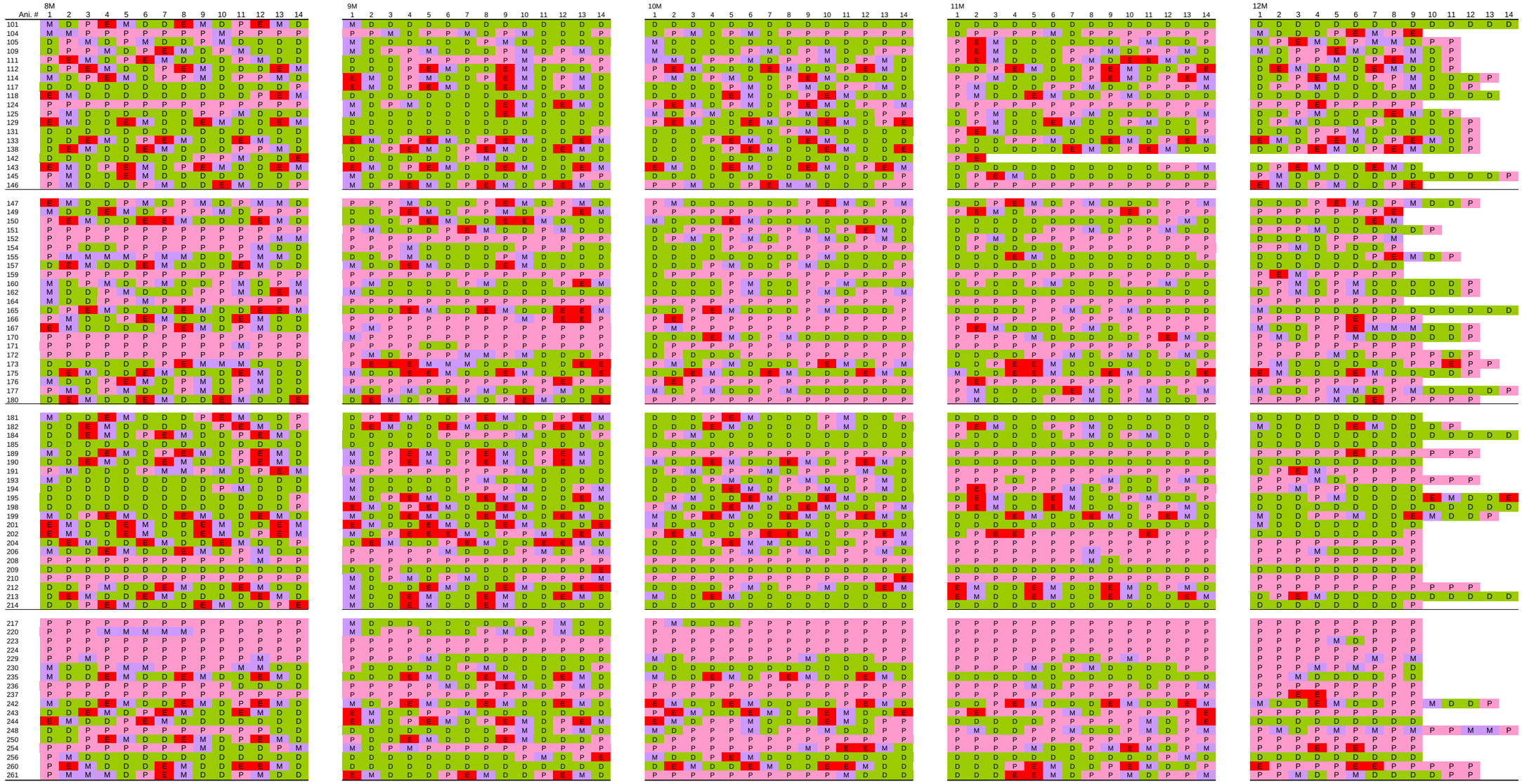
Immune system responses

- Anti-SRBC-IgG by neonatal DES exposure (high dose, 0.05microg/kg p.o.)
- Hyper Th1 and Th2 induction of LLNA by perinatal exposure to high dose EE (10microg/kg p.o.)

Table3-1　グラフ

3M
4M
5M
6M
7M
DosEs
Ani. #
1 2 3 4 5 6 7 8 9 10 11 12 13 14
VC 101 102 104 105 107 109 110 111 112 114 115 117 118 120 121 123 124 125 127 128 129 131 133 134 137 138 139 142 143 145 146
BPA 0.5 147 149 150 151 152 154 155 157 159 160 162 164 165 166 167 170 171 172 173 175 176 177 180
BPA 5 181 182 184 185 189 190 191 193 194 195 198 199 201 202 204 206 208 209 210 212 213 214
BPA 50 217 218 219 220 223 224 226 227 229 230 232 233 235 236 237 238 241 242 243 244 245 248 250 251 252 254 256 258 260 261

Table3-2　グラフ


8M
9M
10M
11M
12M
Ani. #
1 2 3 4 5 6 7 8 9 10 11 12 13 14
101 104 105 109 111 112 114 117 118 124 125 129 131 133 138 142 143 145 146
147 149 150 151 152 154 155 157 159 160 162 164 165 166 167 170 171 172 173 175 176 177 180
181 182 184 185 189 190 191 193 194 195 198 199 201 202 204 206 208 209 210 212 213 214
217 220 223 224 229 230 235 236 237 242 243 244 248 250 254 256 260 261