Special Review

Percellome Projectによる毒性トランスクリプトミクスの新しい試み

Percellome Project as a New Approach to Toxicology Transcriptomics

菅野 純　北嶋 聡　相崎健一　五十嵐勝秀　中津則之　高木篤也　小川幸男　児玉幸夫
Jun Kanno, Satoshi Kitajima, Ken-ichi Aisaki, Katsuhide Igarashi, Noriyuki Nakatsu, Atsuya Takagi, Yukio Ogawa, Yukio Kodama

身の回りの物質の毒性（有害性）を予測し，その被害を未然に防ぐのが毒性学の役割である．この精度向上を目指したトキシコゲノミクス研究を実施する際に，マイクロアレイなどから細胞１個当たりのmRNAコピー数を得るPercellome法を開発した．90化合物のマウス肝初期応答データを採取し終え，新たな対象（反復投与，胎児毒性，吸入毒性，多臓器連携）を加えたPercellome Projectを展開している．

key words
トキシコゲノミクス，分子毒性学，遺伝子発現カスケード，標準化，Percellome法，３次元多層(Millefeuille) データ

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　E-mail：kanno@nihs.go.jp
1985年東京医科歯科大学大学院医学研究科博士課程修了．人体病理学，実験病理学専攻．国立医薬品食品衛生研究所毒性部室長を経て，2002年より同部長．内分泌かく乱関連などの分子毒性学研究，トキシコゲノミクスプロジェクトなどを厚生労働所掌業務との有機的連携のもとに推進．

北嶋 聡，相崎健一，五十嵐勝秀，中津則之，高木篤也，小川幸男，児玉幸夫　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

はじめに

医薬品，食品，化粧品，生活関連用品など，身の回りの物質が我々の身体に取り込まれた際に生じる可能性のある毒性（有害性）を予測し，それらの使用に際しての被害を未然に防ぐのが毒性学の役割である[注1]（図1）．具体的には，人々の安全を確保するために使用法（用途）や使用量（残留量）を制限したり，場合によっては禁止したりするための科学的根拠を提供するが，その際，人の身代わりとして実験動物を用いる場合が多い．このような毒性学の精度向上の一環として，従来からの毒性研究（毒性症候学，毒性病理学，など）に加えてのトキシコゲノミクス（Toxicogenomics）研究が進められている．

トキシコゲノミクスでは，物質が生体に及ぼす影響をトランスクリプトームとして観測・解析する．その際，①分子毒性学を構築し種差や個体差の問題，複合暴露の問題などを解決するためには，遺伝子発現カスケードの全容解明を目指す必要がある，②形態学的に変化が現れた段階のトランスクリプトームは，遺伝子発現カスケードの最終段階に過ぎない，③形態変化の現れないごく初期段階を含む遺伝子発現カスケードを描出するためにはまとまった量のデータの蓄積が必須である，との観点から，筆者らは，マイクロアレイや定量PCRから細胞１個当たりのmRNAコピー数を得るPercellome手法と，そのデータ解析のための３次元多層（Millefeuille）システムを開発・実用化した．遺伝子発現量が共通の尺度，すなわち“コピー数／細胞”で表現されることから，検体間，実験間，マイクロアレイのバージョン間，異なったプラットホーム間，などのデータ比較が直接的に行えるようになり，数年かけて蓄積したデータの有機的活用が可能となった．現在，90種類の化学物質によるマウス肝の初期応答データを採取し終えたところである．新たな対象（反復投与，胎児毒性，吸入毒性，多臓器連携）を加えたPercellome Projectの概要を紹介する．

注1　環境への配慮も含まれる．

Ⅰ．Percellome法：細胞１個当たりのmRNA絶対量を得る方法

原理は単純である．サンプルの細胞数を計測し，外部標準mRNA（スパイクRNA）を細胞１個当たり決まった分子数だけそのサンプルに添加し，そしてRNA抽出，測定に移る．サンプルのRNAの測定値を，スパイクRNAの値を基準に，細胞1個当たりのコピー数に換算する．実際には細胞数を直接計測するのが困難なことが多いため，その代替指標として細胞核内のゲノムDNA量を用いる[1), 2)]．定量性・直線性の検証にはLBM標準サンプル（肝[L]と脳[B]を100：0，75：25，50：50，25：75および0：100に混合した5サンプルから成るセット）を用いる．なお，スパイクRNAは，5種類の枯草菌遺伝子のmRNAを濃度公比３で混合したカクテル（dose-graded spike cocktail；GSC）として用意した．高精度を要求されるDNA定量法は手作業プロトコールおよび自動ロボット（PerkinElmer JANUS）のプロトコールを準備

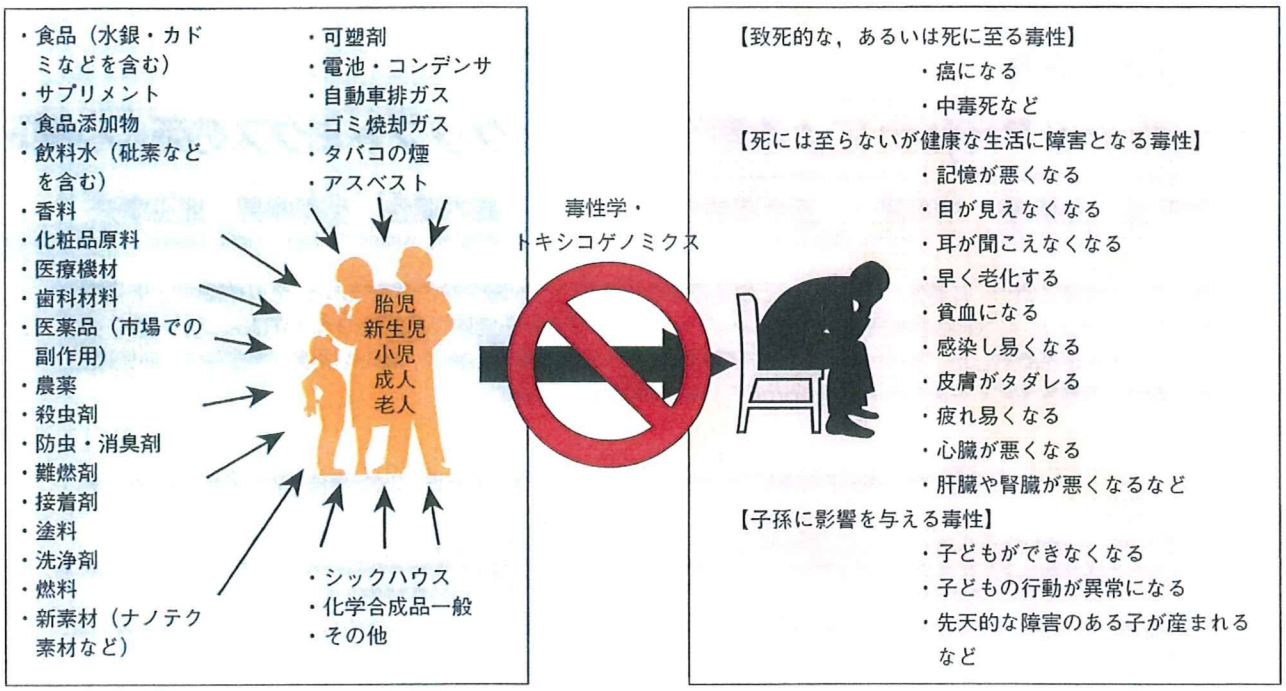

図1. 毒性学の対象

毒性学は，身の回りの物質が引き起こす障害を予測し，その発生を未然に防ぐことを目的としている．トキシコゲノミクス（毒性ゲノミクス）は，最先端の網羅的遺伝子発現解析技術を用いて，従来の毒性学の予測の精度を著しく向上，迅速化させることで，国民の健康安全の確保にさらに貢献することを目指している．

中である．カクテルとも共同研究ベースで供給可能である（連絡先：kanno@nihs.go.jp）．また，ERCC（The External RNA Control Consortium）と連絡をとるとともに，国際的標準化への関与を深めるため平成18年度厚労科研費「医薬品などの有効性・安全性評価に資する遺伝子発現解析の国際的標準化に関わる研究（H18-特別-指定-023）」を立ち上げた．現在，この他にシックハウス症候群を考慮した低用量域での吸入毒性トキシコゲノミクス，1匹のマウスから多臓器を採取しそれらの連携状況をトランスクリプトームから解析する多臓器トキシコゲノミクスを開始し，特徴的な遺伝子について組織内の発現分布を*in situ*ハイブリダイゼーションで確認する作業を並行している．また，下記の3次元データをweb公開するサーバを整備し，一部の化合物から3次元多層（Millefeuille）データを順次閲覧可能とした（http://toxicomics. nihs.go.jp/db/）．

Ⅱ．3次元多層（Millefeuille）データシステム：生物系研究者に優しいデータ可視化と解析

医薬品を含む毒性既知の90化合物について単回経口投与後のトランスクリプトームデータを取得して，初期応答遺伝子カスケードを解析するための基盤データベースを構築した．現在，第二段階として反復暴露データ集積を開始した．データは，用量軸，時間軸，および遺伝子発現軸から成る3次元表示により，遺伝子発現の用量および時間に依存した変化を1枚の曲面として表すことで可視的に変化を判別しやすいように配慮した（図2）．これにより，コンピュータが選び出した遺伝子クラスターの中身を確認する際，特に，mRNAの合成分解のスピードなどの知見から生物学的にありえないパターン（用量軸の方向にも時間軸の方向にもジグザグな変化など）を排除する際に威力を発揮している．

1つの実験から排出されるGeneChip約50枚のデータを一括処理する能力を持ったPercellome自動換算・データ品質管理（QC）に関わるソフトウエアに加えて，3次元多層（Millefeuille）データに最適化した，発現パターン類似性による候補遺伝子検索，およびそれを発展させた教師無しクラスタリング[3]を中心とした解析システム（MF System，MFシリーズ，開発：相﨑 健一）を独自に実用化し，開発継続中である（図3）．これらにより，データQCはその日のうちに，基本的な発現情報検索から全遺伝子の教師無しクラスタリングまでを3日間で完遂できるものとなっている．

この基本解析を用いて，発現パターンによって分類された候補遺伝子リストが多数生成される．一部の幸運な例ではただちに新規と思われる毒性関連反応を見いだすことができた．またそうでない場合のための1つの補強手段とし

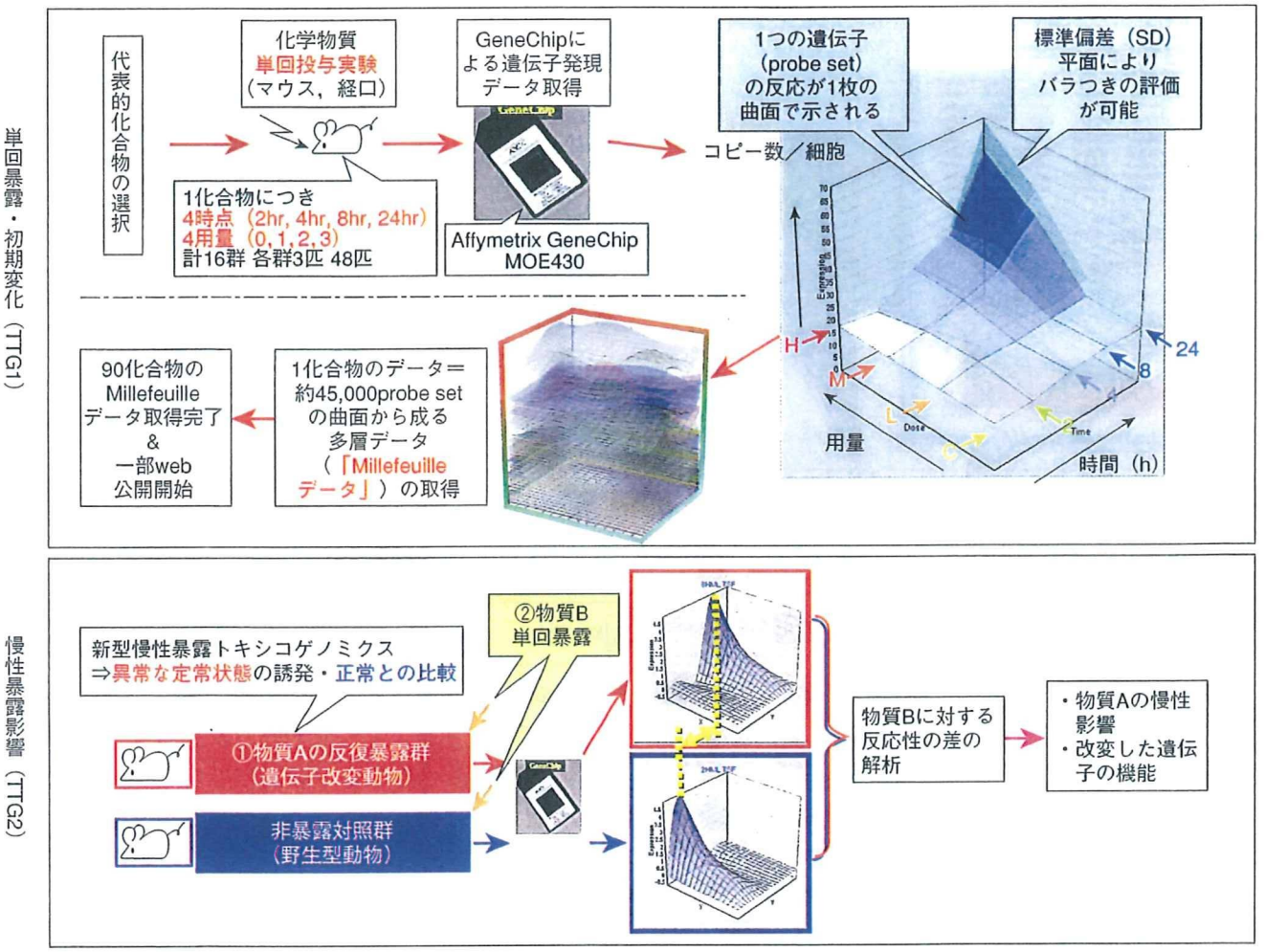

図2. Percellome法と3次元表示による多層（Millefeuille）データシステムを用いたプロジェクトの根幹部分の概要
単回投与による遺伝子発現初期変化データを90化合物について取得（上段）．現在，反復投与の影響を検討中（下段）．H；高用量（high），M；中用量（medium），L；低用量（low），C；コントロール（control）．

て，Gene Ontologyなどの既存知識を利用して候補遺伝子リストの理解を支援するソフトウエア（MF GoPlot）を用意した．このツールは一種の化合物クラスタリングとしても利用することができる．

さらに候補遺伝子リストを基に複数化合物間比較を行い，複数条件下においても同期して発現する遺伝子群を自動抽出するシステムも開発済みである．本システムで得られた同期遺伝子群はシグナルカスケードの構成単位である可能性があり，データベース化しつつ，その解析を進めている（5TB規模のデータベース部分および，大量計算アルゴリズム実装は（株）NTTコムウェアおよび（株）日本NCR/Teradataとの共同開発による）．

Ⅲ．Percellome手法のリアルタイムPCRを含む他のプラットホームへの適用

Percellome手法は，GSCの受け入れ条件を整えることにより，様々なプラットホームに適用可能である．その1つとして最も定量性が高いとされるリアルタイムPCR（ABI PRISM 7900 HT・96ウェルプレート）への適用例を示す．現行のRT-PCR絶対定量法では，遺伝子ごとに検量線が必要であり，多数のサンプルについて多数の遺伝子を検討するには不向きである．Percellome RT-PCRでは，マイクロアレイと同様の原理を用いる．すなわち，サンプル破砕液に，その細胞数に比例する量のスパイクカクテル（GSC）を添加し，それらのCt値をPCRプレートごとの検量線とすることにより，測定したい遺伝子のCt値を細胞1個当たりのmRNAコピー数に換算する．これにより，GAPDHやActinなどのハウスキーピング遺伝子が変動してしまう際の問題，例えば，少数の遺伝子を検討する際にGlobal normalization法を適用し難い問題などが解決される．共通サンプルを測定しデータを比較することにより，Affymetrix GeneChipのPercellome結果と9割程度の整合性が確認され，

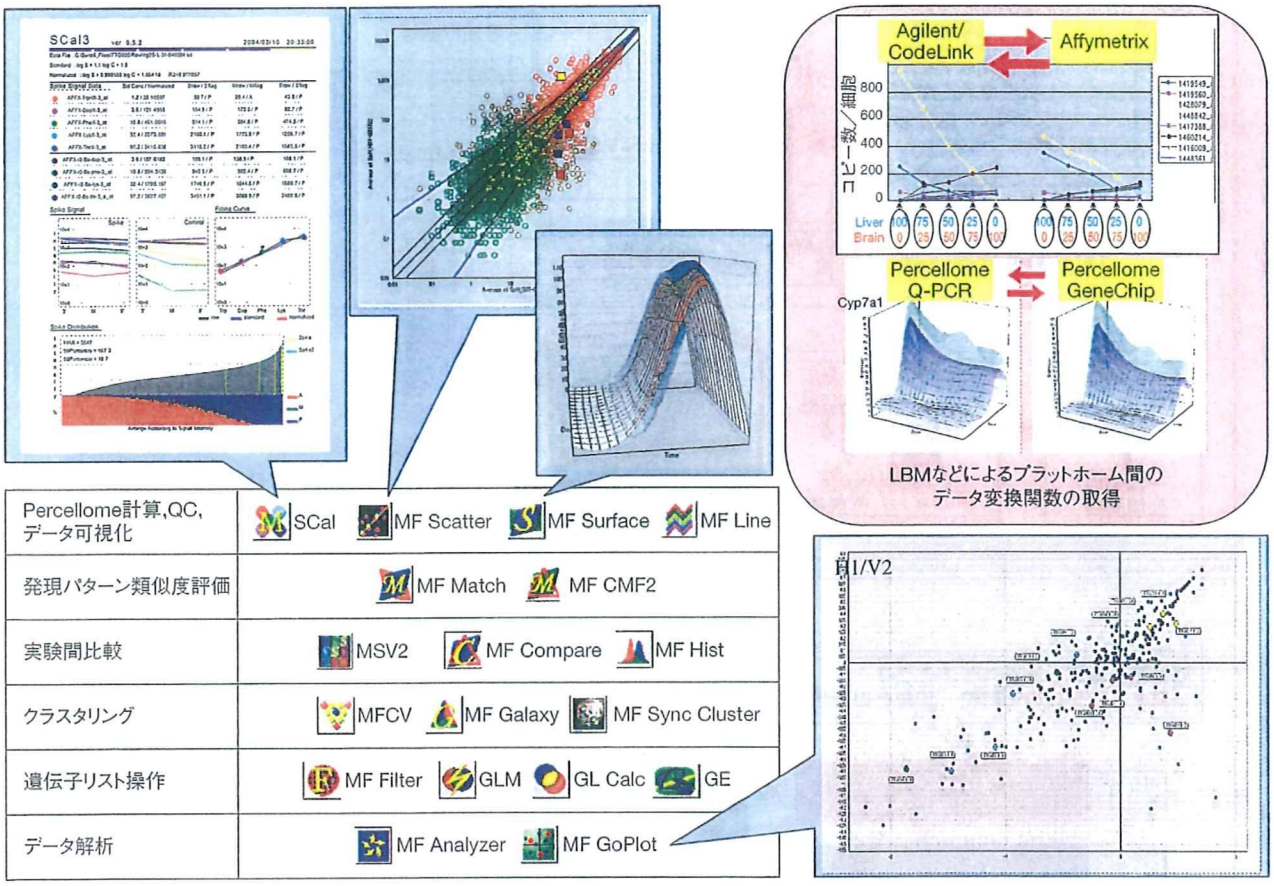

図3．3次元多層（Millefeuille）データの解析などに用いる独自開発プログラム群

品質管理とともにPrecellome計算を自動的に実施するSCal，Plotソフトウエア，3次元曲面の描画ソフト（MF Surface），など．右上はプラットホーム間のデータ変換情報の得方を示す．LBMを用いる方法（上段）と，実際の実験サンプルを用いる方法（下段）がある．いずれも，一度，両方のプラットホームでそれらのサンプルを測定する必要がある．

GeneChipとPercellome RT-PCRとの間でのコピー数の換算式がいくつかの遺伝子について得られている．この他に，Agilent社製の単色マイクロアレイとCodeLinkアレイにGSCを測定可能なカスタムアレイを用意し終え，LBMサンプルのデータなどをもとに，これらとの間の換算式も得つつある（図3右上）．

Percellome法は，Affymetrixの新しいエキソンアレイの定量性・直線性の検討にも適応可能である．Affymetrix社のHuman Exon 1.0 ST Arrayと従来型の発現アレイHuman Genome U133 plus 2について，性質の異なるヒト癌細胞株2株から調製したLBM様標準サンプル（100：0，75：25，50：50，25：75および0：100混合5サンプル）による比較を行い，両アレイ間の相関性の高いprobe setを多数検出することができた．また，既知のエキソンに対して設計されたprobe setでは発現が見られ，イントロンに対して設計されたprobe setでは発現が見られない，あるいは，既知のsplicing variantに対応したprobe setの発現が検出された，などの基本性能が確認された．しかし，Percellome法を適用して未知のsplicing variantの検出力を向上させるためには，現状では各エキソン間の定量性に問題があることが示唆された．定量値を算出する補正アルゴリズムの開発など，何らかの対策が必要であることが考えられ，現在，Affymetrix社に確認を行っている．

Ⅳ．核内受容体原性毒性のPercellomeトキシコゲノミクス解析

受容体原性毒性とは，化学物質が受容体（リガンド依存的転写因子を含む）に選択的に結合してシグナルをかく乱し，その結果生じる有害性を指す．代表例としてはダイオキシンが挙げられる．AhR（Arylhydrocarbon receptor）ノックアウトマウスでは，ダイオキシンを大量に投与しても毒性がほとんど観察されない．すなわち，野生型マウスがダイオキシンで死ぬメカニズムには，AhRが必須であり，AhRからの異常なシグナルがマウスを死に至らせていることに

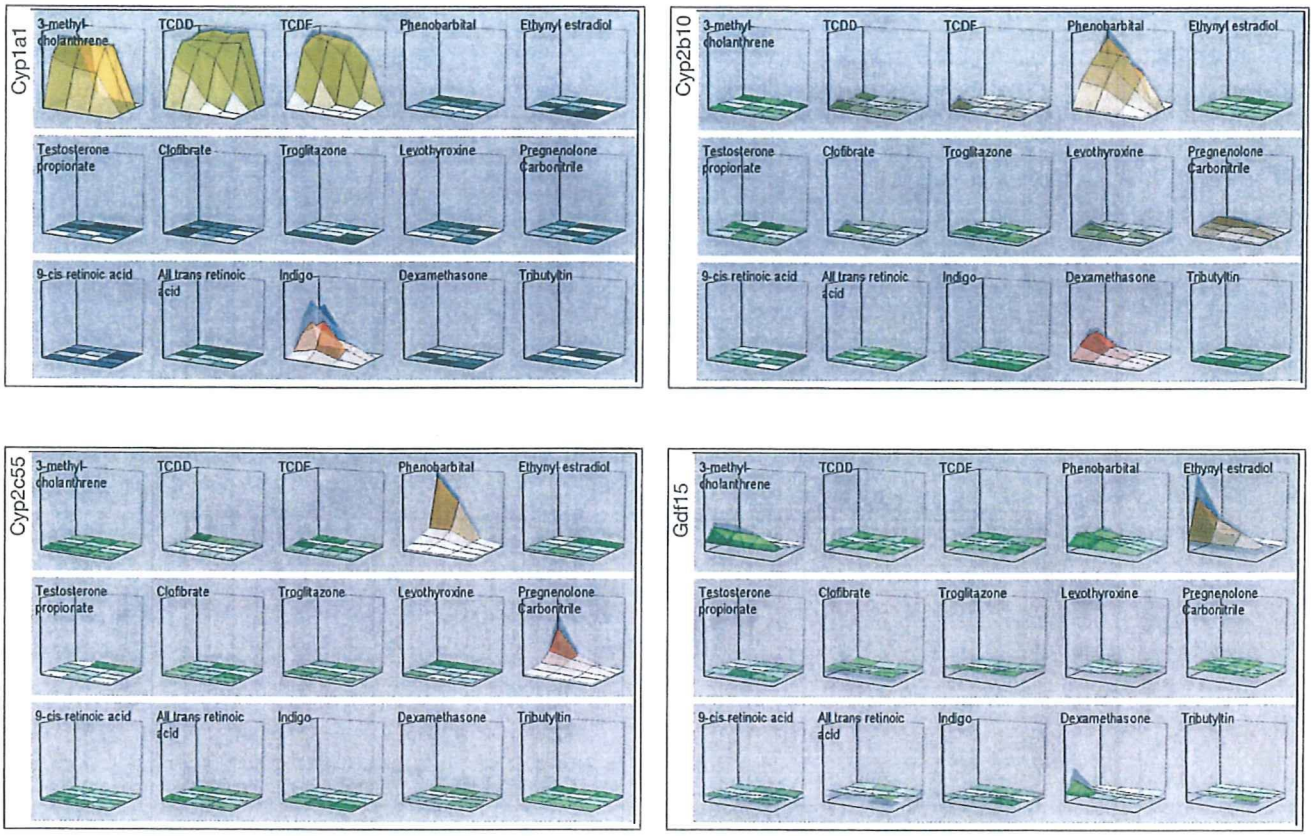

図4. 化合物間の発現比較

15種類の核内受容体リガンド化合物（各3次元グラフ内に表示）によるCyp1a1（左上），Cyp2c55（左下），Cyp2b10（右上）および，Gdf15（右下）の遺伝子発現を3次元表示したもの．各軸は，図2のとおり．縦軸のスケールは遺伝子ごとに共通．リガンドに選択的な遺伝子の発現が確認される．

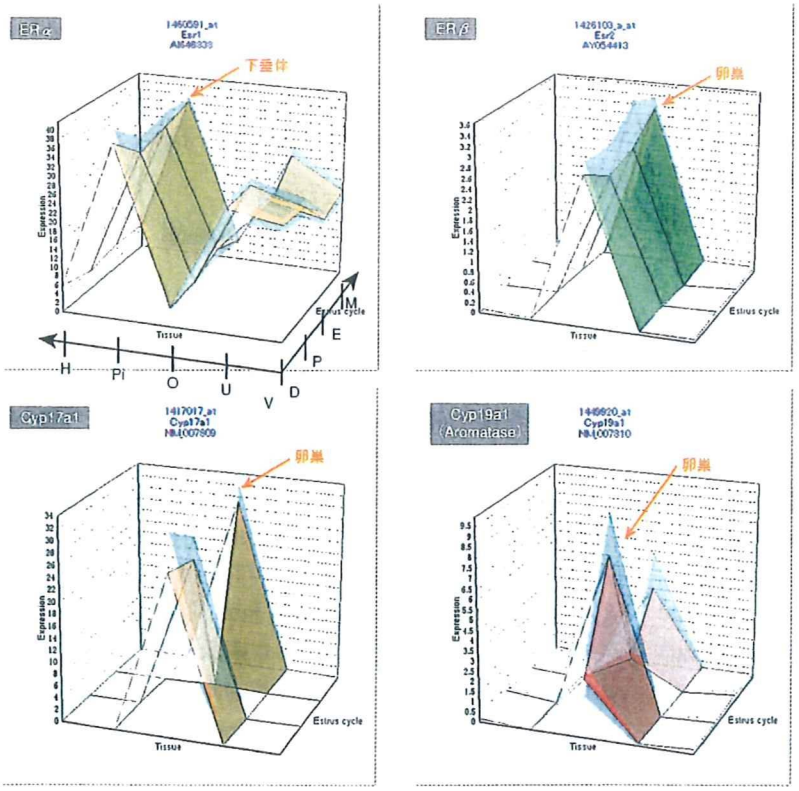

図5. 臓器間の発現比較

マウスの性周期（Diestrus，Proestrus，Estrus，Metestrusの4日間で1周期）ごとの視床下部（H），下垂体（Pi），卵巣（O），子宮（U）および膣（V）における，ERα，ERβ，Cyp17a1（steroid-17α-hydroxylase），およびCyp19a1（Aromatase）の遺伝子発現変動を3次元表示したもの．後二者の酵素は卵巣において周期性を持って発現している．

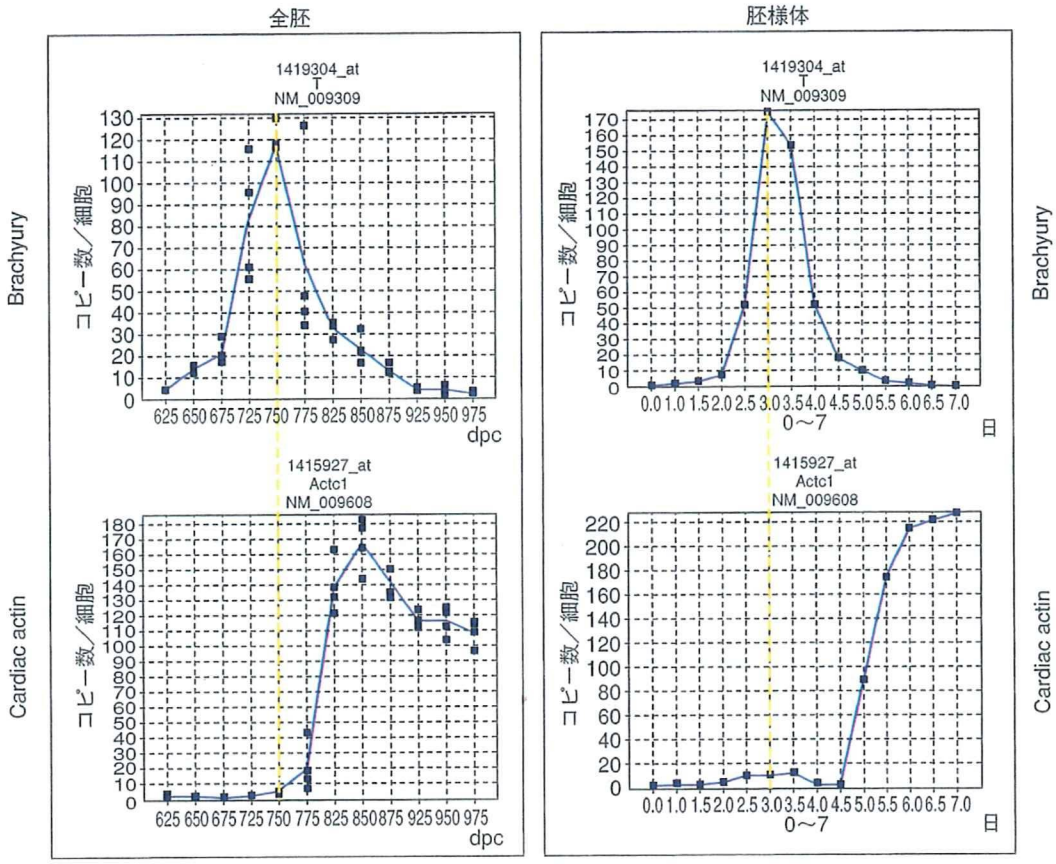

図6. マウス胎児（全胚）と胚様体の発現比較

マウス全胚の胎生6.25日～9.75日までの遺伝子発現と，胚様体の1日～7日目までの遺伝子変動の網羅的データベースから，初期中胚葉分化マーカーであるBrachyury遺伝子と，Cardiac actin遺伝子の経時変化を示す．

なる．エストロジェン活性化学物質による有害影響（内分泌かく乱化学物質問題）も同様にER（estrogen receptor）を介する受容体原性毒性と考えられ，胎生期にERを発現する組織が，低用量シグナルかく乱影響の重要標的であると考えられている．

ここでは，受容体原性毒性研究の基盤として，Percellome手法を適用して，①核内受容体作動性物質によるマウス雄肝臓の遺伝子発現変動，②性周期に伴うマウス雌生殖器遺伝子発現変動，③生後の発達過程におけるマウス雌生殖器遺伝子発現変動，の3種類のデータベースを構築した．例えば，①では10種類の核内受容体に作用する典型物質について，単回経口投与後，2，4，8，24時間目の変動を解析し，Ethynyl-estradiolがGDF15，TCDDがCyp1a1，9-cis Retinoic AcidがCyp26a1，DexamethasoneがCyp2b10，ClofibrateがCyp4a14，PCNがCyp2c55など，各々の受容体に特徴的な遺伝子発現を誘導するところがとらえられている（図4）．②の性周期データベースは視床下部，下垂体，卵巣，子宮，腟を対象としており，性周期との関連が網羅的にとらえられている（図5）．これらのデータベースは，今後，各種の候補物質が引き起こす変化を詳細に解析する際の基準として利用される．

V．発生トキシコゲノミクスへの応用

発生毒性学は，個体発生過程におけるダイナミックな遺伝子発現調節の分子機構を把握することにより，さらに正確なものに補強されると考える．現在，C57BL/6マウス胚の器官形成期初期にあたる胎生6.5～9.5日（プラグ確認日：0.5日）の，①全胚の遺伝子発現変動解析，②遺伝子欠失マウス全胚との比較，および③標的が明らかな既知発生毒性物質投与による本データベースの具体的な適用，を実施している．①についてはすでに0.25日間隔（Time point 計12点）の遺伝子発現データベースを得て，②遺伝子欠失胚のデータといくつかの注目すべき遺伝子についてはwhole mount ISHを用いた発現の検証を加えた．これと並行して，ES細胞からhanging drop法で得た胚様体の0.5日間隔の遺伝子発現データとの比較を実施している．個体発生に関与

する遺伝子群の多くは経時的に激しく変化しており，既知発生毒性物質投与実験については標的遺伝子シグナルカスケードを解析中である（図6）．

おわりに

ノーザンブロットでは実験サンプルにだけバンドが見られ，対照サンプルには遺伝子発現がないという結果を得ても，細胞1個当たりで定量してみると，対照が10コピーに対して実験サンプルが20コピーである場合がある．“無”が“有”になったのではなく，“10”が“20”になったのである．

さて，筆者らの属する毒性学でも，医学の分野でも，疾患概念や毒性概念が整理され，患者や実験動物を診断する際には，まず，そのどれに当てはまるかを検討する．すなわち，どの“典型”に近い症例であるかを検討することから始まることが多い．

しかし，最近の医学・生物学には多因子疾患・多因子形質発現制御の概念が導入され，今から何年かの後には，“21世紀初頭までは，患者の遺伝子多型を調べずして治療を行っていた時代”として，“血液型を調べずに輸血していた時代”と並び称されるようになる可能性がある．このような多因子概念が定着すると，その多くは，“有（100%）”“無（0%）”の組み合わせではなく，“70%”“50%”“90%”といった半端な数の組み合わせであることが考えられる．すなわち，今までの離散値的な“典型”例を基準とするアプローチから，連続値的な病態“スペクトラム”を直接扱うアプローチに変革していく可能性が考えられる．その際の網羅的データの解析とその蓄積の必要性を考えるとき，遺伝子発現データの定量化・標準化という問題は，今まで以上に重みを増すと考えられる．生命現象の網羅的解析にはトランスクリプトームだけでは不十分であることは自明であるが，この定量性を確保することは，これから実現されるであろう網羅的プロテオミクスなどの基盤としても重要ではないかと考える．

マイクロアレイなどから得られるトランスクリプトーム情報が，今後の医薬品審査や化学物質の安全性評価の際に必須なものとなる時代がすぐそこまで来ていることを念頭に，筆者らはPercellome法をさらに展開し，Percellome Projectデータベースを可能な限り高精度に保ちつつ毒性学的な内容を充実させるべく最大限の活動を継続して行く所存であるが，この技術，あるいは研究内容が毒性学以外の研究分野にもお役に立つことができれば幸甚である．

謝辞　本システムの開発とプロジェクトの遂行に当たっては，当毒性部の全メンバー，特に松田菜恵，辻 昌貴，森田紘一，今井あや子，安東朋子，安部麻紀，森山紀子，近藤優子，青柳千百合，相原妃佐子，渡辺 忍の各氏の卓越した働きに深謝する．本研究は厚生労働科学研究費補助金H13-生活-012，H13-生活-013，H14-トキシコ-001，H15-化学-002，H18-化学-一般-001などによる．

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Original

Observation of Preputial Separation is a Useful Tool for Evaluating Endocrine Active Chemicals

Shinsuke Yoshimura[1], Hajime Yamaguchi[1], Kazunori Konno[1], Noriko Ohsawa[1], Satoshi Noguchi[1], and Akiko Chisaka[1]

[1]Hatano Research Institute, Food and Drug Safety Center, 729–5 Ochiai, Hadano, Kanagawa 257–8523, Japan

Abstract: Flutamide, *p,p'*-dichlorodiphenyldichloroethylene, vinclozolin, diethylstilbestrol, ethynylestradiol and tamoxifen were administered by gavage to pregnant Sprague-Dawley rats on gestational days 14–17 or 18–21, and to male offspring on postnatal days 1–5, 17–21 or 35–39. The influence on the sexual maturation was assessed by preputial separation. Cleft phallus with hypospadias was induced by prenatal exposure to 10 mg/kg flutamide on gestational days 14–17 and 18–21, or administration of 100 mg/kg vinclozolin on gestational days 14–17 to the dams. The day of preputial separation in these offspring could not be determined, because complete separation did not occur. Prenatal exposure of males to other chemicals did not affect the preputial separation. Postnatal exposure of 10 and 30 mg/kg flutamide and 30 mg/kg vinclozolin led to delays of preputial separation. A marked delay was observed in males exposed to 100 μg/kg of ethynylestradiol or 3 mg/kg of tamoxifen on postnatal days 1–5. Diethylstilbestrol, 300 μg/kg, administration on postnatal days 1–5 and 35–39 caused a delay in preputial separation. These results indicate that observing preputial separation is useful for evaluating anti-androgen treatment in the prepubertal period, and estrogen-related chemical treatment from the neonatal period. (J Toxicol Pathol 2005; **18**: 141–157)

Key words: preputial separation, rat, flutamide, vinclozolin, ethynylestradiol, diethylstilbestrol

Introduction

Preputial separation, which is the separation of the prepuce from the glans penis, is used as an indication of puberty in the male rat. Histological observations on the progress of preputial separation after cornification at the lining of the prepuce and surface of the glans penis were well described using Long-Evans rats in 1942[1]. We showed similar histological changes in Sprague-Dawley rats examined from postnatal day (PND) 6 to PND 56[2]. Preputial separation is thought to be dependent on androgens, since castration blocks preputial separation and the addition of testosterone (TS) or dihydrotestosterone (DHT) nullifies the effect of castration[1,3]. In recent years, various *in vivo* screening assays have been developed for detecting endocrine disrupting chemicals. The uterotrophic assay is a method for detecting estrogenic or anti-estrogenic effects of chemicals on the weight of the uterus using immature or ovariectomized female rats. The Hershberger assay is a screening test to detect androgenic or anti-androgenic effects of chemicals on the weight of castrated male reproductive organs such as the ventral prostate and seminal vesicles. The enhanced OECD Test Guideline 407 is a draft, new version of the Repeated Dose 28-day Oral Toxicity Study in Rodents, and is designed to detect the endocrine effect of chemicals. Also, the rodent 20-day thyroid/pubertal male assay has been proposed for evaluating chemicals influencing male puberty. In this assay, weaning male rats are continuously dosed by gavage beginning one week before puberty (which occurs at about PND 40) until PND 53, and their puberty is measured by determining their age at preputial separation. Both estrogenic and anti-androgenic chemicals may induce delays in male puberty. Although the observation of preputial separation is a useful tool for detecting sexual maturation, the adequate administration period or relationship between dose and effect has not been sufficiently investigated. Accordingly, we performed a preliminary study to find out the suitable administration period or the most sensitive period using the following well-known chemicals: flutamide, *p,p'*-dichlorodiphenyldichloroethylene, vinclozolin, diethylstilbestrol, ethynylestradiol and tamoxifen. Flutamide (FLU)[4,5] is an anti-androgenic drug and is used in the Hershberger assay as the positive control agent. *p,p'*-dichlorodiphenyldichloroethylene (DDE)[4–7] and vinclozolin (VZ)[7,8] are anti-androgenic chemicals and are used in the OECD validation study of the Hershberger assay to verify

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Mailing address: Shinsuke Yoshimura, Laboratory of Toxicology, Hatano Research Institute, Food and Drug Safety Center, 729–5, Ochiai, Hadano, Kanagawa 257–8523, Japan
TEL: 81-463-82-4751 FAX: 81-463-82-9627
E-mail: yoshimura.s@fdsc.or.jp

the effectiveness of this screening assay. Diethylstilbestrol (DES) is an estrogenic compound and has been used in various experimental studies. Ethynylestradiol (EE) is a synthetic estrogen and is used as a positive control in the uterotrophic assay, and tamoxifen (TAM) is used as a positive control for the anti-estrogenic effect in this screening assay.

To determine the administration period, the following reports were used as references. Induction of hypospadias has been reportedly caused by anti-androgens[9]. Puberty is undetermined in males with hypospadias because complete separation in the glans penis is not evident in these animals. A sensitive prenatal period for hypospadias is known to exist, thus, we selected gestational days (GD) 18–21 as the low sensitive period for prenatal exposure. We selected GD 14–17 as the high sensitive period for comparative study, but the high dose group of some chemicals may not be used for the observation of preputial separation. To examine the effects on the neonatal period, newborn rats were orally administered test chemicals on PND 1–5. Leydig cells of neonatal rats are known as fetal Leydig cells, and adult type Leydig cells appear from about PND 14[10]. It was reported that male rat serum testosterone levels reach a maximum on GD 19, decrease on PND 12, and then increase[11]. Thus, we also chose to administer the test chemicals during PND 17–21 and PND 35–39 (prepubertal period). The 4 or 5 days of administration period in the present study is short compared to the Hershberger assay (10 days) or pubertal male assay (20 days), thus the effect on the animals is thought to be less than that seen in these assays. The purpose of this preliminary study was to find out the sensitive period for the screening assay, and more prolonged administration may show more pronounced effects on the animals. The number of animals in each group was determined referring to the other screening assays such as the uterotrophic assay (6 female rats in a group), the Hershberger assay (6 male rats in a group), and the Enhanced OECD Test Guideline 407 or pubertal male assay (10 male rats and 10 female rats). The number of pups and litters may be insufficient to determine the effects of unknown chemicals, but the purpose of the present study was to obtain the suitable period of administration for a screening study by the observation of preputial separation, thus we performed the preliminary study using the experimental design described in the following section.

Materials and Methods

Animals and housing conditions

Sprague-Dawley rats (Crj:CD (SD) IGS), 210 males and 421 females, 11 weeks of age, were obtained from Charles River Japan, Inc. (Atsugi, Japan). All animals were acclimatized to laboratory conditions and quarantined for about one week before mating. Rats used for this study were selected based upon their general appearance and behavior during the acclimatization period. Animals were housed individually in wire-bottom metal cages (220 × 270 × 190 mm) and kept in a barrier sustained animal room that was maintained at 21.0 – 25.0°C and 40.0 – 75.0% relative humidity with a 12-hour artificial light cycle (lighting from 7:00 to 19:00). The room air was changed fifteen times per hour, and a commercial diet, CE-2 (CLEA Japan, Inc., Tokyo, Japan), and water (Hadano City) were available *ad libitum* throughout the study. The Animal Use Committee of the Hatano Research Institute approved the study protocol.

To obtain pregnant animals, 12-week-old females were cohabited overnight on a 1:1 basis with males 12 weeks of age or older. Females were considered to be at GD 0 when daily examination revealed a vaginal plug. All pregnant animals were housed in cages with animal bedding, PAPER CLEAN® (Japan SLC, Inc., Shizuoka, Japan), from GD 18 and allowed to give birth. The dams and pups were housed in wire-bottom metal cages after postpartum day 10.

For prenatal exposure, pregnant females were randomly assigned to groups consisting of 3 to 5 based on body weight before administration. For PND 1–5 exposure (PND 0 is the day of delivery), all female pups were discarded on PND 1 and the number of males per litter was adjusted to 5. The litters were then allocated to groups consisting of 3 or 4 litters (except for one group consisting 2 litters) based on pup mean body weight. On PND 6, the number of pups for prenatal or neonatal exposure and that for premature exposure was adjusted to 4 males per litter. For PND 17–21 and 35–39 exposure, litters were allocated to groups a few days before administration based on mean body weight. The numbers of litters or pups were decided referring to the other screening assays.

Chemicals and treatment

FLU, DES, EE and TAM were purchased from Sigma-Aldrich (St. Louis, MO), DDE was obtained from Aldrich Chemical (Milwaukee, WI), and VZ from Wako Pure Chemical (Osaka, Japan). Each chemical was dissolved in corn oil (Nacalai Tesque, Inc., Kyoto, Japan). Dosage levels for FLU were 1, 10 and 30 mg/kg/day, for DDE 10, 30, 100 and 300 mg/kg/day, for VZ 10, 30 and 100 mg/kg/day, for DES 0.1, 1, 10, 100 and 300 μg/kg/day, for EE 10 and 100 μg/kg/day, and for TAM 0.03 and 0.1 mg/kg/day (prenatal exposure) or 0.3, 1 and 3 mg/kg/day (postnatal exposure). The dosages employed in this study were based on the results from preliminary studies or those reported in the literature.

The chemicals were orally administered by gavage (5 or 10 mL/kg BW) to pregnant female rats on GD 14–17 or 18–21, or to males on PND 1–5, 17–21 or 35–39 (Fig. 1). In addition, EE was administered on PND 6–10 or 11–15. The control animals were administered vehicle corn oil orally, at the same time periods and volume as the test group. An ATOM indwelling feeding tube (Atom Medical, Tokyo, Japan) was used for neonatal administration as described by Watanabe[12] and a stomach tube was used for adult and premature animals.

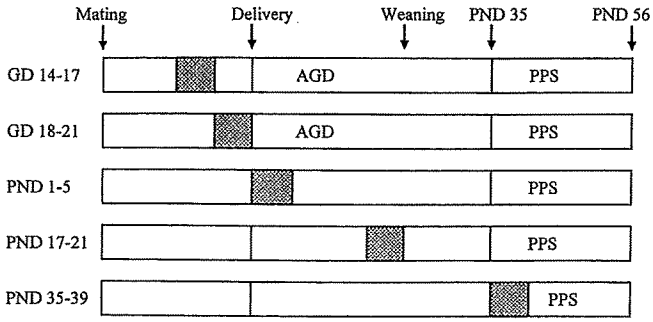

Fig. 1. Schedule of prenatal and postnatal treatment.
▒: period of chemical exposure, GD: gestational day, PND: postnatal day, AGD: measurement of anogenital distance on PND 6, PPS: observation of preputial separation from PND 35.

Examination and measurement

Body weights of pregnant females were measured on GD 0, on the day of grouping before administration, and during the administration period. Body weights of males were measured on PND 0, 6, 22, 35, 56, the day of complete preputial separation, on the day of grouping, and during the administration period. Body weights on PND 0 to 6 were measured as mean value of each litter, and after PND 22 they were measured individually. The anogenital distance (AGD) of prenatally exposed male pups was measured on PND 6 before adjusting to 4 males per litter, with a digital micrometer (reproducible precision of 0.01 mm, Digimatic caliper CD-15CP, Mitutoyo Corporation, Kanagawa, Japan). The progress of preputial separation in the males was observed macroscopically[13] from PND 35. Surviving males were sacrificed under anesthesia on PND 56 and autopsy was performed. Male fatalities were autopsied as early as possible after they were discovered. Following the macroscopic examination, the testes, epididymides, ventral prostate, seminal vesicles, prepuce and penis were excised and fixed in 0.1 mol/L phosphate buffered 10% formalin solution. Weights of the testes, epididymides, ventral prostate, and seminal vesicles of sacrificed animals were measured on the next day. Representative organs of 169 cases were embedded in paraffin, and the sections were then stained with hematoxylin-eosin (H&E) for histopathological examination. To observe early stage hypospadias some of the discarded males were sacrificed on PND 6 and examined histologically as described above.

Statistical analysis

Body weights on the day of preputial separation and autopsy, the day of preputial separation, organ weights, AGD, and correction values of AGD [AGD (mm)/ $\sqrt[3]{\text{body weight (g)}}$ of pups] were statistically analyzed using the litter as the unit. Body weights on the day of preputial separation, the day of preputial separation, and organ weights in postnatally administered males were also analyzed using the individual values. These data were analyzed using Bartlett's test. When homogeneity of variance was confirmed, one-way analysis of variance was applied to detect significant differences among the groups. If a significant difference was detected among the groups, Dunnett's test was applied for multiple comparisons. When variance was not homogeneous, the Kruskal-Wallis analysis of ranks was applied. If a significant effect was detected among the groups, Dunnett's test was applied for multiple comparisons. The day of preputial separation and the body weights on the day of preputial separation between the two groups were analyzed by Student's or Welch's t-test. Comparisons between groups were made using $P \leq 0.05$ as the level of significance. When preputial separation was not complete on the day of autopsy, the day of separation was set as day 56 for the analysis. The correlation between the day of preputial separation and body weight was analyzed using Pearson's correlation coefficient.

Results

Prenatal exposure

1) Effects on the pregnant females

In the groups exposed to 300 mg/kg of DDE on GD 14–17 or GD 18–21, body weights decreased during the administration period and either the pregnant females or their pups died. Depression of the weight gain was also observed in the 100 mg/kg DDE group exposed on GD 18–21, and all the pups in 3 litters out of 4 died. Deaths of pregnant females or pups were observed in the VZ, DES and TAM groups. Although depression of weight gain was observed in the EE group, all females survived and delivered pups. In the VZ, DES and TAM groups, one or two females delivered before the last day of administration period, and these females were excluded from the experiment.

2) Malformations and inflammatory lesions of the genital organ

The incidence of malformations and inflammatory lesions after prenatal exposure to chemicals are summarized in Table 1. On macroscopic examination, the glans penis of control males was covered with prepuce, and the prepuce could be completely retracted to expose the glans penis before PND 56 (Figs. 2A, 2B). The prepuce of males from dams exposed to 10 mg/kg of FLU on GD 14–17 had a cleft at the ventral aspect (cleft prepuce) and the glans penis was observed from the cleft. The ventral part of the penis was incompletely formed (cleft phallus) and the os penis was often exposed. The external urethral orifice of males with a cleft phallus opened at the ventral surface of the penis (hypospadias). Hypospadias was usually observed with cleft phallus. The incidence of a cleft prepuce was 25% (3 cases in 2 litters) and the incidence of a cleft phallus was 58% (7 cases in all of the 3 litters), although a cleft prepuce was usually observed with a cleft phallus. Five males had no cleft on their prepuce or phallus. Although there was no cleft at the prepuce of males from dams exposed to FLU on GD 18–21, the ventral part of the penis was incompletely formed (cleft phallus); the incidence of the cleft phallus in this group was 25% (3 cases in one litter).

Table 1. Malformation and Inflammatory Lesion in Genital Organ of Male Rats Prenatally Exposed to Chemicals

Chemical	Dosing period	Group	Males	Litters	Cleft prepuce	Cleft phallus	Ectopic testis	Hypoplasia of prostate	Prostatitis/ Vesiculitis
FLU	GD 14-17	Control	12	3	0	0	0	0	0
		1 mg/kg	12	3	0	0	0	0	0
		10 mg/kg	12	3	3 (2)	7 (3)	2 (2)	0	0
	GD 18-21	Control	12	3	0	0	0	0	0
		1 mg/kg	12	3	0	0	0	0	0
		10 mg/kg	12	3	0	3 (1)	0	0	7 (3)
DDE	GD 14-17	Control	12	3	0	0	0	0	0
		10 mg/kg	12	3	0	0	0	0	0
		30 mg/kg	16	4	0	0	0	0	0
		100 mg/kg	16	4	0	0	0	0	0
	GD 18-21	Control	14	4	0	0	0	0	0
		10 mg/kg	16	4	0	0	0	0	0
		30 mg/kg	14	4	0	0	0	0	0
		100 mg/kg	4	1	0	0	0	0	0
VZ	GD 14-17	Control	20	5	0	0	0	0	0
		10 mg/kg	20	5	0	0	0	0	0
		30 mg/kg	20	5	0	0	0	0	0
		100 mg/kg	20	5	17 (5)	17 (5)	1 (1)	1 (1)	0
	GD 18-21	Control	20	5	0	0	0	0	0
		10 mg/kg	16	4	0	0	1 (1)	0	0
		30 mg/kg	16	4	0	0	0	0	0
		100 mg/kg	15	4	0	0	0	0	0
DES	GD 14-17	Control	19	5	0	0	0	0	0
		0.1 μg/kg	12	3	0	0	0	0	0
		1 μg/kg	20	5	0	0	0	0	0
		10 μg/kg	16	4	0	0	0	0	0
		100 μg/kg	16	4	0	0	0	0	0
		300 μg/kg	12	4	0	0	0	0	0
	GD 18-21	Control	16	4	0	0	0	0	0
		0.1 μg/kg	20	5	0	0	0	0	0
		1 μg/kg	16	4	0	0	0	0	0
		10 μg/kg	20	5	0	0	0	0	0
		100 μg/kg	20	5	0	0	0	0	0
		300 μg/kg	7	2	0	0	0	1 (1)	0
EE	GD 14-17	Control	16	4	0	0	0	0	0
		10 μg/kg	16	4	0	0	0	0	0
		100 μg/kg	20	5	0	0	0	0	0
	GD 18-21	Control	19	5	0	0	0	0	0
		10 μg/kg	15	4	0	0	0	0	0
		100 μg/kg	16	4	0	0	0	0	0
TAM	GD 14-17	Control	16	4	0	0	0	0	0
		0.03 mg/kg	15	4	0	0	0	0	0
		0.1 mg/kg	7	2	0	0	0	0	0
	GD 18-21	Control	19	5	0	0	0	0	0
		0.03 mg/kg	12	3	0	0	0	0	0
		0.1 mg/kg	11	3	0	0	0	0	0

Value: number of cases (litters) with abnormality.
FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; GD: gestational day.

Cleft prepuce and cleft phallus were also observed in males from dams exposed to 100 mg/kg of VZ on GD 14–17 (Figs. 2C, 2D). The incidence of cleft prepuce and cleft phallus was 85% (17 cases in all of the 5 litters). However, there was no cleft at the prepuce or phallus of males from dams exposed to VZ on GD 18–21 (Figs. 2E, 2F). The time of sexual maturation is determined by complete separation of the prepuce from the ventral surface of the glans penis, but preputial separation could not determined in males with cleft phallus, since complete separation in the glans penis was not evident.

The testis of males from dams exposed to 10 mg/kg of

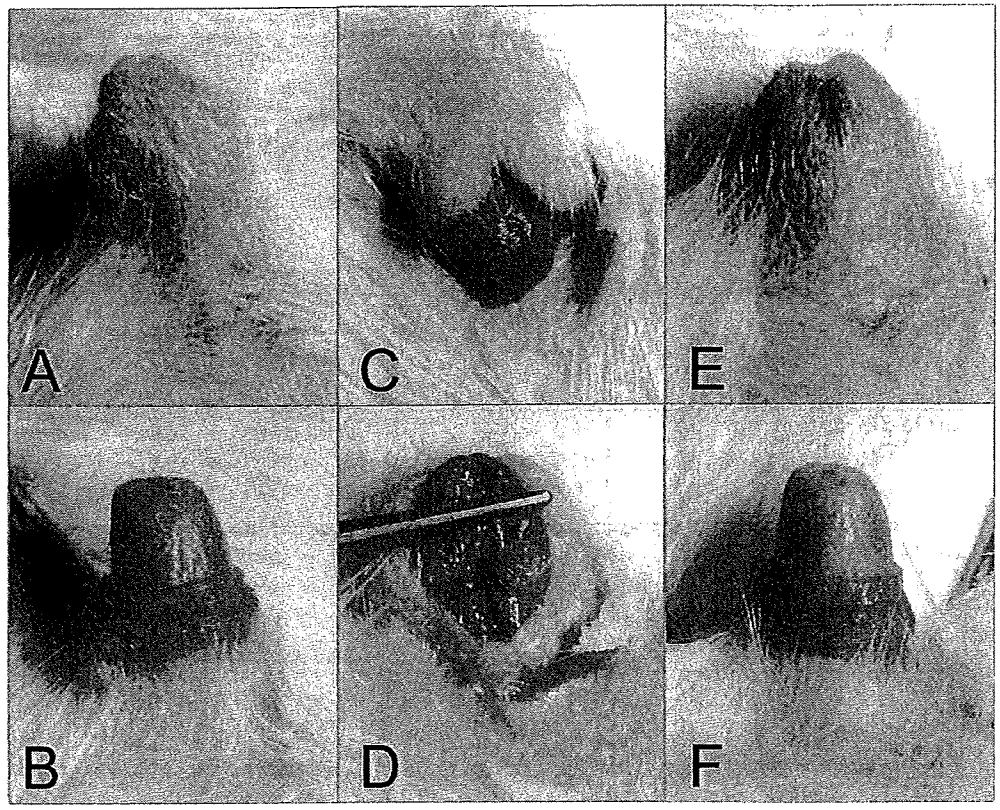

Fig. 2. External genitalia of male rats on PND 56.
A and B: Control male rat. Prepuce is completely retracted. C and D: Male rat from a dam exposed to 100 mg/kg vinclozolin on GD 14–17. Ventral side of the prepuce has a cleft and the glans penis is observed from the cleft (C). The ventral part of the penis is incompletely formed (cleft phallus, shown in D). E and F: Male rat from a dam exposed to 100 mg/kg vinclozolin on GD 18–21. There was no cleft on the ventral surface of the prepuce or penis.

FLU on GD 14–17 (17%, 2 cases in 2 litters) or 100 mg/kg of VZ on GD 14–17 (5%, one case in one litter) did not descend into the scrotum and was located in the ventral subcutis (ectopic testis) instead. One male from a dam exposed to 10 mg/kg VZ on GD 18–21 also had an ectopic testis. Ectopic testis was observed unilaterally. Marked inflammation in the prostate and seminal vesicle occurred in the group exposed to 10 mg/kg FLU on GD 18–21, and 5 of 12 males in this group died from the severe inflammation. Males with marked prostatitis and vesiculitis did not show hypospadias in their glans penis.

3) Preputial separation

Males with hypospadias were excluded from the preputial separation analysis, and the resulting days of preputial separation of males without hypospadias are summarized in Table 2. Preputial separation in males from dams exposed to 10 mg/kg of FLU was significantly delayed in both GD 14–17 and GD 18–21 treatment groups, and 2 males in the GD 18–21 treatment group had incomplete preputial separation on the day of autopsy, PND 56. In the group exposed to 100 mg/kg of DDE on GD 18–21, all pups of 3 litters died. There were no differences among the other 3 groups. In the 100 mg/kg VZ groups, male pups without hypospadias showed no difference from the control group, and the lower dose groups did not show any significant difference from the control. Two of 4 pregnant females administered 300 μg/kg of DES from GD 18 were excluded from the experiment because these females delivered their pups on GD 21 before the end of the administration period. Preputial separation of male offspring exposed to DES was not different from the control group. EE and TAM administered in any exposure period did not affect preputial separation.

Body weight on the day of complete preputial separation was significantly higher in males from dams exposed to FLU on GD 18–21, but there were no changes in other chemically-treated groups.

4) Measurement of AGD on PND 6

Table 3 shows the AGD, body weight, and correction values of AGD [AGD (mm)/ $\sqrt[3]{\text{body weight (g)}}$ of pups] on PND 6. Correction values of AGD were significantly decreased in the groups exposed to 10 mg/kg FLU on GD 14–17 or GD 18–21, and the group exposed to 100 mg/kg VZ on GD 14–17. DES induced a significant reduction in AGD of the 100 and 300 μg/kg groups exposed on GD 18–21. DDE, EE and TAM did not induce a significant

Table 2. Preputial Separation and Body Weights of Male Rats Prenatally Exposed to Chemicals

Chemical	Dosing period	Group	Males	Litters	PND of preputial separation	BW (g) on the day of PPS	Incomplete separation
FLU	GD 14-17	Control	12	3	42.8 ± 2.2	232.2 ± 26.5	0
		1 mg/kg	12	3	42.4 ± 0.3	216.5 ± 3.3	0
		10 mg/kg	5	2	49.1 ± 0.6 **	271.2 ± 41.3	0
	GD 18-21	Control	12	3	43.2 ± 1.4	227.0 ± 19.2	0
		1 mg/kg	12	3	44.5 ± 0.7	249.8 ± 12.7	0
		10 mg/kg	5	2	51.3 ± 0.5 **	322.2 ± 13.7 **	2 (1)
DDE	GD 14-17	Control	12	3	42.8 ± 2.2	232.2 ± 26.5	0
		10 mg/kg	12	3	42.6 ± 0.4	242.1 ± 23.3	0
		30 mg/kg	16	4	43.1 ± 1.0	248.3 ± 20.1	0
		100 mg/kg	16	4	44.6 ± 3.0	242.5 ± 14.0	0
	GD 18-21	Control	14	4	43.8 ± 1.5	231.7 ± 26.7	0
		10 mg/kg	16	4	43.3 ± 1.6	233.7 ± 19.6	0
		30 mg/kg	14	4	43.0 ± 1.0	239.3 ± 16.2	0
		100 mg/kg	4	1	43.5	233.5	0
VZ	GD 14-17	Control	20	5	44.4 ± 1.2	245.3 ± 10.5	0
		10 mg/kg	20	5	43.7 ± 1.6	243.7 ± 16.4	0
		30 mg/kg	20	5	44.5 ± 1.7	246.2 ± 13.7	0
		100 mg/kg	3	1	43.7	254.1	0
	GD 18-21	Control	20	5	44.1 ± 0.3	252.1 ± 36.7	0
		10 mg/kg	16	4	43.8 ± 1.5	248.2 ± 17.3	0
		30 mg/kg	16	4	43.3 ± 1.9	240.8 ± 11.4	0
		100 mg/kg	15	4	45.1 ± 1.7	259.2 ± 22.3	0
DES	GD 14-17	Control	19	5	44.4 ± 1.7	246.4 ± 12.9	0
		0.1 μg/kg	12	3	43.3 ± 0.5	242.3 ± 12.7	0
		1 μg/kg	20	5	43.3 ± 1.3	243.3 ± 17.6	0
		10 μg/kg	16	4	42.8 ± 1.3	244.2 ± 19.9	0
		100 μg/kg	16	4	43.7 ± 1.4	257.9 ± 8.4	0
		300 μg/kg	12	4	46.1 ± 1.0	260.8 ± 20.5	0
	GD 18-21	Control	16	4	44.6 ± 1.1	246.5 ± 16.0	0
		0.1 μg/kg	20	5	45.1 ± 1.1	255.9 ± 22.9	0
		1 μg/kg	16	4	43.3 ± 1.2	252.5 ± 18.5	0
		10 μg/kg	20	5	43.4 ± 1.4	241.6 ± 7.5	0
		100 μg/kg	20	5	44.0 ± 0.6	245.3 ± 9.1	0
		300 μg/kg	7	2	46.8 ± 1.1	239.9 ± 1.7	0
EE	GD 14-17	Control	16	4	44.4 ± 1.0	254.6 ± 11.9	0
		10 μg/kg	16	4	45.6 ± 2.0	240.2 ± 6.2	0
		100 μg/kg	20	5	45.8 ± 2.2	261.6 ± 20.2	0
	GD 18-21	Control	19	5	43.6 ± 1.7	246.6 ± 9.6	0
		10 μg/kg	15	4	43.3 ± 0.5	248.3 ± 10.1	0
		100 μg/kg	16	4	42.8 ± 1.6	247.6 ± 12.3	0
TAM	GD 14-17	Control	16	4	44.4 ± 1.0	254.6 ± 11.9	0
		0.03 mg/kg	15	4	44.5 ± 1.3	252.7 ± 25.2	0
		0.1 mg/kg	7	2	43.5 ± 0.3	244.2 ± 11.6	0
	GD 18-21	Control	19	5	43.6 ± 1.7	246.6 ± 9.6	0
		0.03 mg/kg	12	3	43.2 ± 1.0	242.7 ± 32.3	0
		0.1 mg/kg	11	3	43.7 ± 0.6	257.5 ± 30.3	0

Value: Mean ± S.D. calculated using the litter as the unit.

FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin, DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; GD: gestational day; PND: postnatal day; BW: body weight; PPS: preputial separation.

Incomplete separation: number of animals (litters) with incomplete separation on PND 56.

**: significantly different from control, $p<0.01$.

Table 3. Anogenital Distance (AGD) of PND 6 Male Rats Prenatally Exposed to Chemicals

Chemical	Dosing period	Group	Males	Litters	AGD (mm)	Correction value of AGD (mm/$\sqrt[3]{g}$)	Body weight (g)
FLU	GD 14-17	Control	12	3	7.07 ± 0.59	2.85 ± 0.11	15.4 ± 3.1
		1 mg/kg	20	3	6.21 ± 0.09	2.63 ± 0.03	13.3 ± 1.0
		10 mg/kg	26	3	5.53 ± 0.39 **	2.35 ± 0.12 **	13.0 ± 1.0
	GD 18-21	Control	19	3	6.70 ± 0.25	2.93 ± 0.07	11.9 ± 0.7
		1 mg/kg	20	3	7.04 ± 0.71	2.89 ± 0.21	14.4 ± 1.3
		10 mg/kg	22	3	5.82 ± 0.45	2.42 ± 0.22 *	14.1 ± 1.2
DDE	GD 14-17	Control	12	3	7.07 ± 0.59	2.85 ± 0.11	15.4 ± 3.1
		10 mg/kg	23	3	6.84 ± 0.38	2.84 ± 0.01	14.0 ± 2.2
		30 mg/kg	30	4	6.67 ± 0.59	2.78 ± 0.13	13.8 ± 2.3
		100 mg/kg	23	4	6.32 ± 0.44	2.71 ± 0.11	12.8 ± 1.6
	GD 18-21	Control	19	4	7.55 ± 0.86	3.10 ± 0.20	14.5 ± 2.3
		10 mg/kg	26	4	6.96 ± 0.56	2.93 ± 0.20	13.4 ± 1.1
		30 mg/kg	26	4	7.29 ± 0.45	3.00 ± 0.09	14.5 ± 2.9
		100 mg/kg	5	1	7.40	3.04	14.5
VZ	GD 14-17	Control	35	5	6.75 ± 0.39	2.86 ± 0.14	13.2 ± 0.9
		10 mg/kg	38	5	6.60 ± 0.14	2.72 ± 0.08	14.5 ± 1.9
		30 mg/kg	28	5	6.46 ± 0.22	2.73 ± 0.08	13.2 ± 0.4
		100 mg/kg	34	5	4.87 ± 0.48 **	2.02 ± 0.22 **	14.1 ± 2.2
	GD 18-21	Control	43	5	6.25 ± 0.75	2.65 ± 0.29	13.2 ± 2.0
		10 mg/kg	28	4	6.14 ± 0.38	2.59 ± 0.15	13.3 ± 0.8
		30 mg/kg	24	4	6.59 ± 0.31	2.70 ± 0.06	14.7 ± 1.8
		100 mg/kg	26	4	5.74 ± 0.54	2.41 ± 0.20	13.6 ± 1.4
DES	GD 14-17	Control	36	5	6.25 ± 0.41	2.66 ± 0.06	13.0 ± 1.9
		0.1 μg/kg	20	3	6.35 ± 0.13	2.60 ± 0.09	14.6 ± 0.8
		1 μg/kg	45	5	6.35 ± 0.20	2.66 ± 0.06	13.8 ± 1.7
		10 μg/kg	21	4	6.64 ± 0.54	2.75 ± 0.14	14.1 ± 1.8
		100 μg/kg	26	4	6.40 ± 0.62	2.63 ± 0.19	14.4 ± 1.3
		300 μg/kg	17	4	5.77 ± 0.83	2.54 ± 0.30	11.6 ± 0.9
	GD 18-21	Control	26	4	6.35 ± 0.46	2.68 ± 0.15	13.3 ± 0.9
		0.1 μg/kg	44	5	6.22 ± 0.26	2.64 ± 0.09	13.2 ± 1.4
		1 μg/kg	35	4	6.28 ± 0.28	2.63 ± 0.06	13.7 ± 2.2
		10 μg/kg	36	5	6.17 ± 0.31	2.62 ± 0.09	13.1 ± 0.9
		100 μg/kg	27	5	5.47 ± 0.44 **	2.38 ± 0.10 **	12.2 ± 1.6
		300 μg/kg	9	2	4.45 ± 0.35 **	2.08 ± 0.16 **	9.7 ± 0.0
EE	GD 14-17	Control	30	4	6.73 ± 0.60	2.82 ± 0.13	13.6 ± 2.1
		10 μg/kg	26	4	6.16 ± 0.52	2.76 ± 0.15	11.1 ± 1.2
		100 μg/kg	37	5	6.58 ± 0.19	2.80 ± 0.07	13.0 ± 1.0
	GD 18-21	Control	32	5	6.78 ± 0.41	2.81 ± 0.15	14.1 ± 0.6
		10 μg/kg	35	4	6.98 ± 0.34	3.00 ± 0.16	12.6 ± 0.5
		100 μg/kg	31	5	7.07 ± 0.54	2.89 ± 0.22	14.7 ± 1.5
TAM	GD 14-17	Control	30	4	6.73 ± 0.60	2.82 ± 0.13	13.6 ± 2.1
		0.03 mg/kg	25	4	6.50 ± 0.88	2.71 ± 0.21	13.8 ± 2.7
		0.1 mg/kg	9	2	6.52 ± 0.19	2.73 ± 0.02	13.7 ± 0.9
	GD 18-21	Control	32	5	6.78 ± 0.41	2.81 ± 0.15	14.1 ± 0.6
		0.03 mg/kg	16	3	6.84 ± 0.06	2.73 ± 0.12	15.8 ± 1.7
		0.1 mg/kg	25	4	6.57 ± 0.46	2.68 ± 0.14	15.0 ± 2.6

Value: Mean ± S.D. calculated using the litter as the unit.

FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; GD: gestational day; PND: postnatal day.

Correction value of AGD: AGD(mm)/ $\sqrt[3]{\text{body weight (g)}}$.

*: significantly different from control, $p<0.05$; **: significantly different from control, $p<0.01$.

Table 4. Relative Organ Weights and Body Weights of PND 56 Male Rats Prenatally Exposed to Chemicals

Chemical Dosing period	Group	Males	Litters	Body weight (g)	Testes (mg/g)	Epididymides (mg/g)	Ventral prostate (mg/g)	Seminal Vesicles (mg/g)
FLU								
GD 14-17	Control	12	3	350.2 ± 10.6	8.674 ± 0.105	1.439 ± 0.054	0.791 ± 0.007	1.464 ± 0.188
	1 mg/kg	12	3	331.1 ± 2.2	8.433 ± 0.313	1.481 ± 0.026	0.829 ± 0.060	1.605 ± 0.065
	10 mg/kg	12	3	341.4 ± 27.5	7.982 ± 0.819	1.410 ± 0.091	0.818 ± 0.105	1.657 ± 0.284
GD 18-21	Control	12	3	333.0 ± 15.5	8.364 ± 0.084	1.499 ± 0.066	0.855 ± 0.023	1.651 ± 0.227
	1 mg/kg	12	3	350.0 ± 21.9	8.164 ± 0.115	1.456 ± 0.083	0.798 ± 0.143	1.694 ± 0.164
	10 mg/kg	7	3	346.6 ± 29.3	8.447 ± 0.438	1.460 ± 0.080	0.641 ± 0.115	1.642 ± 0.278
DDE								
GD 14-17	Control	12	3	350.2 ± 10.6	8.674 ± 0.105	1.439 ± 0.054	0.791 ± 0.007	1.464 ± 0.188
	10 mg/kg	12	3	359.1 ± 38.6	8.330 ± 0.125	1.447 ± 0.113	0.862 ± 0.092	1.703 ± 0.206
	30 mg/kg	16	4	368.9 ± 38.3	7.763 ± 0.492**	1.431 ± 0.062	0.946 ± 0.082	1.672 ± 0.201
	100 mg/kg	16	4	340.4 ± 23.4	8.785 ± 0.229	1.465 ± 0.097	0.743 ± 0.102	1.601 ± 0.280
GD 18-21	Control	14	4	338.5 ± 15.6	8.291 ± 0.374	1.444 ± 0.095	0.882 ± 0.062	1.817 ± 0.032
	10 mg/kg	16	4	342.9 ± 15.6	8.308 ± 0.186	1.490 ± 0.058	0.758 ± 0.052	1.794 ± 0.270
	30 mg/kg	14	4	352.7 ± 21.6	7.927 ± 0.285	1.443 ± 0.075	0.903 ± 0.120	1.785 ± 0.142
	100 mg/kg	4	1	339.9	9.027	1.582	1.003	1.816
VZ								
GD 14-17	Control	20	5	355.2 ± 19.5	7.177 ± 0.212	1.463 ± 0.073	0.982 ± 0.089	1.729 ± 0.262
	10 mg/kg	19	5	352.9 ± 6.1	7.486 ± 0.358	1.514 ± 0.080	0.931 ± 0.047	1.752 ± 0.068
	30 mg/kg	19	5	344.6 ± 18.9	7.809 ± 0.699	1.489 ± 0.139	0.992 ± 0.124	1.840 ± 0.308
	100 mg/kg	20	1	369.2 ± 6.4	7.404 ± 0.608	1.489 ± 0.062	0.755 ± 0.093**	1.675 ± 0.213
GD 18-21	Control	20	5	363.8 ± 49.2	7.766 ± 0.955	1.523 ± 0.169	0.970 ± 0.131	1.869 ± 0.210
	10 mg/kg	16	4	358.5 ± 32.8	7.768 ± 0.493	1.596 ± 0.166	1.007 ± 0.178	1.996 ± 0.327
	30 mg/kg	16	4	357.4 ± 16.1	7.525 ± 0.405	1.597 ± 0.084	0.934 ± 0.095	1.830 ± 0.339
	100 mg/kg	15	4	357.2 ± 19.7	7.609 ± 0.309	1.517 ± 0.066	0.963 ± 0.118	1.771 ± 0.119
DES								
GD 14-17	Control	19	5	347.0 ± 23.9	7.980 ± 1.152	1.454 ± 0.106	0.983 ± 0.132	1.922 ± 0.086
	0.1 μg/kg	12	3	353.6 ± 11.7	7.802 ± 0.485	1.581 ± 0.043	1.091 ± 0.147	1.902 ± 0.239
	1 μg/kg	20	5	358.3 ± 22.7	7.639 ± 0.619	1.527 ± 0.105	1.004 ± 0.081	1.850 ± 0.092
	10 μg/kg	16	4	363.0 ± 23.6	7.966 ± 0.554	1.626 ± 0.088	1.037 ± 0.102	2.120 ± 0.187
	100 μg/kg	16	4	373.5 ± 13.7	7.337 ± 0.523	1.532 ± 0.084	0.913 ± 0.029	1.888 ± 0.243
	300 μg/kg	12	4	350.4 ± 20.1	7.888 ± 0.320	1.592 ± 0.062	0.718 ± 0.058**	1.885 ± 0.251
GD 18-21	Control	16	4	343.8 ± 11.2	8.006 ± 0.610	1.609 ± 0.102	1.115 ± 0.040	1.872 ± 0.144
	0.1 μg/kg	20	5	353.3 ± 20.7	8.369 ± 1.418	1.491 ± 0.072	1.100 ± 0.199	1.853 ± 0.263
	1 μg/kg	16	4	370.1 ± 25.7	7.110 ± 0.642	1.517 ± 0.161	1.033 ± 0.094	1.866 ± 0.238
	10 μg/kg	20	5	354.8 ± 15.0	7.959 ± 0.389	1.633 ± 0.116	1.050 ± 0.113	1.974 ± 0.290
	100 μg/kg	20	5	354.9 ± 10.2	7.457 ± 0.337	1.577 ± 0.079	0.753 ± 0.081**	1.743 ± 0.136
	300 μg/kg	7	2	316.5 ± 2.8	8.228 ± 0.183	1.664 ± 0.025	0.668 ± 0.134**	1.991 ± 0.364
EE								
GD 14-17	Control	16	4	364.1 ± 19.7	7.912 ± 0.032	1.583 ± 0.122	0.954 ± 0.095	1.918 ± 0.116
	10 μg/kg	16	4	333.8 ± 21.5	7.693 ± 0.280	1.477 ± 0.076	0.967 ± 0.145	1.877 ± 0.185
	100 μg/kg	20	5	354.9 ± 17.0	7.745 ± 0.189	1.538 ± 0.086	0.980 ± 0.165	1.993 ± 0.197
GD 18-21	Control	19	5	358.5 ± 14.1	7.677 ± 0.424	1.477 ± 0.112	0.949 ± 0.092	1.967 ± 0.185
	10 μg/kg	15	4	372.0 ± 16.2	7.752 ± 0.454	1.539 ± 0.073	1.036 ± 0.124	2.106 ± 0.062
	100 μg/kg	16	4	364.8 ± 6.2	7.791 ± 0.475	1.618 ± 0.062	0.923 ± 0.089	2.070 ± 0.173
TAM								
GD 14-17	Control	16	4	364.1 ± 19.7	7.912 ± 0.032	1.583 ± 0.122	0.954 ± 0.095	1.918 ± 0.116
	0.03 mg/kg	15	4	356.8 ± 26.7	7.808 ± 0.311	1.491 ± 0.116	0.996 ± 0.159	1.948 ± 0.285
	0.1 mg/kg	7	2	360.7 ± 13.4	7.320 ± 0.038	1.458 ± 0.117	0.903 ± 0.014	1.659 ± 0.076
GD 18-21	Control	19	5	358.5 ± 14.1	7.677 ± 0.424	1.477 ± 0.112	0.949 ± 0.092	1.967 ± 0.185
	0.03 mg/kg	12	3	359.8 ± 44.0	7.142 ± 0.660	1.469 ± 0.030	0.932 ± 0.174	1.869 ± 0.277
	0.1 mg/kg	11	3	376.4 ± 49.8	7.475 ± 0.202	1.513 ± 0.020	0.949 ± 0.036	1.731 ± 0.093

Value: Mean ± S.D. calculated using the litter as the unit.

FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; GD: gestational day; PND: postnatal day.

**: significantly different from control, $p<0.01$.

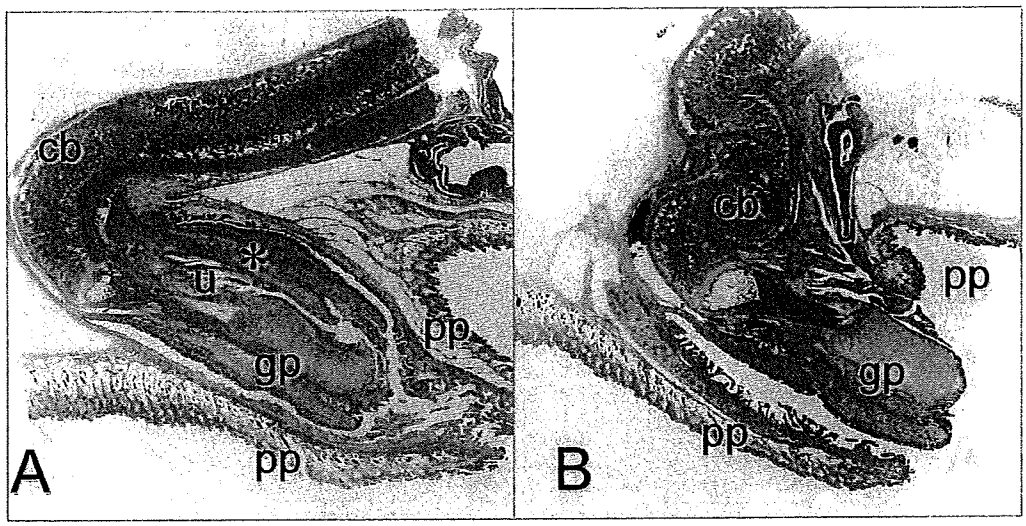

Fig. 3. Sagittal sections of the prepuce and penis of male rats sacrificed on PND 56.
A: Control male rat. The prepuce (pp) is separated from the glans penis (gp). The urethra (u) is located in the center of the glans penis. *: ventral half of the glans penis. cb: cavernous body. H&E staining, magnification: × 6. B: Male rat from a dam exposed to 100 mg/kg vinclozolin on GD 14–17. The prepuce (pp) is separated from the glans penis (gp) at the dorsal part, and the urethra (u) is located between the glans penis and subcutis. The prepuce is hypoplastic and the glans penis is not completely covered by the prepuce. The cavernous body (cb) of the penis is tortuous and bent. H&E staining, magnification: × 6.

difference in AGD. Body weights at the time of AGD measurement did not show any significant difference.

5) Organ weight

Relative organ weights of males prenatally exposed to chemicals are shown in Table 4. Changes in absolute organ weights were similar to changes in relative organ weights. Relative weights of the ventral prostate were decreased significantly in males from dams exposed to 100 mg/kg of VZ on GD 14–17, 300 μg/kg of DES on GD 14–17, and 100 and 300 μg/kg of DES on GD 18–21. Although there was a significant decrease in relative organ weight of the testes in the DDE group, a dose dependent relationship was not detected.

6) Pathological examination

Histopathological examination of representative males from dams exposed to 10 mg/kg of FLU or 100 mg/kg of VZ on GD 14–17 revealed a defect in the ventral half of the glans penis (cleft phallus). The urethra was not located in the center of the glans penis, but instead was observed at the ventral surface of the penis (Fig. 3B). The dorsal surface of the glans penis and prepuce of PND 56 males were covered with keratinized stratified squamous epithelium, and the prepuce was separated from the glans penis. The ventral part of the glans penis and ventral epithelium were not formed between the urethra and subcutis, the ventral surface of the glans penis was not covered with squamous epithelium, and preputial separation did not progress at the ventral aspect. The external urethral orifice opened at the ventral surface of the penis (hypospadias). These males with cleft phallus showed a tortuous cavernous body of the penis (Fig. 3B). On PND 6, hypoplasia of the ventral half of the glans penis and tortuous cavernous body were observed in male pups from dams exposed to 100 mg/kg of VZ on GD 14–17 (Fig. 4B).

Ectopic testes were induced by FLU and VZ, and showed severe atrophy of the seminiferous tubule in PND 56 males. Severe prostatitis and seminal vesiculitis were observed in males from dams exposed to 10 mg/kg of FLU on GD 18–21. Neutrophils, lymphocytes and macrophages infiltrated the prostate, seminal vesicles, and surrounding tissues of five animals which died (PND 44, 45, 52, 55 and 56) and two sacrificed (PND 56) males. In some cases, hemorrhage was observed in the muscular layer of the urinary bladder. The males with severe prostatitis and vesiculitis did not show hypoplasia in the ventral half of the glans penis.

Postnatal exposure

1) Preputial separation

The day of preputial separation and the body weights on the day of preputial separation were statistically analyzed using both the litter as the unit and the individual data. There were no significant changes on the day of preputial separation of males exposed to FLU, DDE or VZ on PND 1–5 (Table 5). On the other hand, the day of preputial separation was significantly delayed in groups exposed to 10 and 30 mg/kg of FLU and 30 mg/kg of VZ on PND 35–39. Statistical analyses using the individual data revealed additional significances in the delay of preputial separation in the groups exposed 300 mg/kg of DDE on PND 17–21 and 100 mg/kg on PND 35–39, and 100 mg/kg of VZ on PND 35–39. In the DDE group, 10 of 12 males exposed to 300 mg/kg of DDE on PND 35–39 died before maturation.

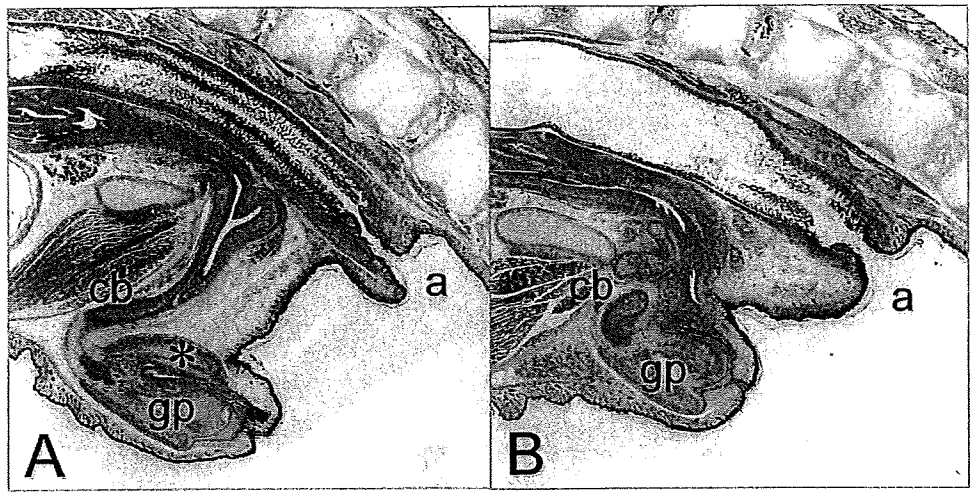

Fig. 4. Sagittal sections of the genital tubercle of males sacrificed on PND 6.
A: Control male rat. Glans penis (gp) is bordered with dorsal and ventral epithelium. The urethra is located in the center of the glans penis. a: anus. *: ventral half of the glans penis. H&E staining, magnification: × 8. B: A male rat from a dam exposed to 100 mg/kg vinclozolin on GD 14–17. The dorsal part of the glans penis is bordered with epithelium. The urethra is observed at the ventral surface of the glans penis (gp) and the cavernous body (cb) shows tortuous structure. a: anus. H&E staining, magnification: × 8.

Preputial separation in two surviving males (PND 48 and 50) was delayed compared to controls. A significant delay was not detected in males exposed to FLU or VZ on PND 17–21.

DES induced a significant delay in the 300 μg/kg group exposed on PND 1–5 or PND 35–39, while 2 of 16 males in the 100 μg/kg group and 3 of 16 males in the 300 μg/kg group exposed on PND 1–5 showed incomplete preputial separation on PND 56. In statistical analyses using the individual data, significant delays of preputial separation were also observed in the group exposed to 100 μg/kg of DES on PND 1–5 or 35–39. There were no significant delays in males exposed on PND 17–21.

In EE treatment, preputial separation was significantly delayed in males of the 100 μg/kg group exposed on PND 1–5, and 1 of 12 males in the 10 μg/kg group and 10 of 12 males in the 100 μg/kg group had incomplete separation on PND 56. Administration of 10 μg/kg of EE on PND 6–10 or PND 11–15 was added to the experimental protocol to define the sensitive period, because PND 1–5 exposure induced a marked effect on preputial separation. Although a delay in preputial separation was observed after PND 6–10 exposure, there was no significant difference between the PND 11–15 exposed group and controls. The 3 mg/kg TAM treated groups exposed on PND 1–5 also showed a significant delay in preputial separation, and 5 of 16 males in the 1 mg/kg group and 14 of 15 males in the 3 mg/kg group had incomplete separation on PND 56. Slight delay in preputial separation was also observed in the 0.3 mg/kg TAM group, and 2 of 16 males had incomplete separation on PND 56. In the statistical analyses using the individual data, significant delays of preputial separation were also observed in the groups exposed to 10 μg/kg of EE and 1 mg/kg of TAM on PND 1–5. No influence of EE and TAM treatments on PND 17–21 or PND 35–39 was detected.

Body weights on the day of preputial separation showed a higher value in the groups with a delay of separation.

2) Organ weight

Relative organ weights of males postnatally exposed to the chemicals are presented in Table 6 using the litter as the unit and in Table 7 using the individual data. Absolute organ weights showed similar changes to those of relative organ weights. Relative weights of the ventral prostate were decreased significantly in males exposed to 30 mg/kg of FLU on PND 35–39. No significant changes were observed in males exposed to FLU on PND 1–5 or 17–21, and DDE and VZ in any period.

DES caused a significant reduction in the relative weight of the ventral prostate in the 100 and 300 μg/kg groups exposed on PND 1–5. No significant changes were observed in males exposed to DES on PND 17–21 or PND 35–39. EE caused reductions in the testes, ventral prostate and seminal vesicles of males in the 100 μg/kg group and seminal vesicles of males in the 10 μg/kg group exposed to EE on PND 1–5. No significant changes in relative weight were observed in males exposed on PND 17–21 or PND 35–39. TAM treatment on PND 1–5 led to reductions in body and reproductive organ weights in the 3 mg/kg group and the ventral prostate weight in the 1 mg/kg group. No weight reductions were observed in the reproductive organs of males exposed to TAM on PND 17–21 or PND 35–39. Although the statistical analyses using the individual data showed significant results in the lower dose group and the other organs, there were no significances in the groups exposed to VZ.

Table 5. Preputial Separation and Body Weights of Male Rats Postnatally Exposed to Chemicals

Chemical	Dosing period	Group	Litters	PND of preputial separation	BW (g) on the day of PPS	Males	PND of preputial separation	BW (g) on the day of PPS	Incomplete separation
FLU	PND 1-5	Control	4	44.4 ± 2.3	268.4 ± 8.9	16	44.4 ± 2.4	268.4 ± 21.2	0
		1 mg/kg	4	42.7 ± 2.4	246.5 ± 21.6	16	42.7 ± 2.3	246.5 ± 22.4	0
		10 mg/kg	4	42.8 ± 2.1	257.6 ± 15.2	16	42.8 ± 2.1	257.6 ± 21.4	0
		30 mg/kg	4	43.9 ± 1.1	252.7 ± 19.6	15	44.0 ± 2.0	252.7 ± 27.9	0
	PND 17-21	Control	3	44.3 ± 0.4	258.3 ± 7.9	12	44.3 ± 1.4	258.3 ± 19.0	0
		1 mg/kg	3	45.0 ± 2.9	256.7 ± 20.1	12	45.0 ± 3.2	256.7 ± 29.7	0
		10 mg/kg	3	43.2 ± 1.9	250.6 ± 17.5	12	43.2 ± 2.0	250.6 ± 23.5	0
		30 mg/kg	3	43.6 ± 2.5	256.5 ± 28.2	12	43.6 ± 2.5	256.5 ± 26.9	0
	PND 35-39	Control	4	43.5 ± 1.4	253.1 ± 16.9	16	43.5 ± 1.7	253.1 ± 18.6	0
		1 mg/kg	4	44.6 ± 0.8	272.2 ± 9.6	16	44.6 ± 1.4	272.2 ± 20.4	0
		10 mg/kg	4	46.8 ± 1.2**	287.8 ± 9.2*	15	46.8 ± 2.0**	287.9 ± 19.6**	0
		30 mg/kg	4	47.1 ± 1.4**	294.5 ± 24.7*	16	47.1 ± 2.2**	294.5 ± 30.0**	0
DDE	PND 1-5	Control	3	42.3 ± 1.1	239.8 ± 8.6	12	42.3 ± 1.7	239.8 ± 13.5	0
		10 mg/kg	3	42.0 ± 0.7	243.3 ± 8.4	12	42.0 ± 1.3	243.3 ± 17.5	0
		30 mg/kg	3	40.9 ± 1.2	227.3 ± 7.5	12	40.9 ± 1.7	227.3 ± 15.4	0
		100 mg/kg	3	42.1 ± 0.2	242.2 ± 14.3	11	42.1 ± 1.2	240.8 ± 19.9	0
		300 mg/kg	3	42.2 ± 0.6	248.5 ± 13.5	12	42.2 ± 0.9	248.5 ± 17.8	0
	PND 17-21	Control	3	43.6 ± 0.1	243.4 ± 2.6	12	43.6 ± 0.8	243.4 ± 16.2	0
		10 mg/kg	3	43.8 ± 0.9	256.4 ± 10.5	12	43.8 ± 1.4	256.4 ± 15.5	0
		30 mg/kg	3	43.9 ± 1.1	246.1 ± 16.9	11	43.9 ± 1.6	245.1 ± 21.2	0
		100 mg/kg	3	44.5 ± 0.3	246.7 ± 3.8	12	44.5 ± 1.3	246.7 ± 19.5	0
		300 mg/kg	4	45.6 ± 1.4	261.0 ± 20.3	13	45.5 ± 1.4**	259.1 ± 21.5	0
	PND 35-39	Control	3	42.1 ± 1.2	210.0 ± 21.7	12	42.1 ± 1.3	210.0 ± 22.2	0
		10 mg/kg	3	43.1 ± 1.4	238.4 ± 19.7	12	43.1 ± 1.3	238.4 ± 20.0**	0
		30 mg/kg	3	43.4 ± 2.7	232.3 ± 11.1	12	43.4 ± 2.5	232.3 ± 18.1*	0
		100 mg/kg	3	46.4 ± 3.1	264.7 ± 5.1**	12	46.4 ± 3.3**	264.7 ± 16.4**	0
VZ	PND 1-5	Control	4	41.9 ± 0.9	244.3 ± 9.5	16	41.9 ± 1.5	244.3 ± 16.3	0
		10 mg/kg	3	42.8 ± 0.9	253.7 ± 19.0	12	42.8 ± 1.4	253.7 ± 24.9	0
		30 mg/kg	4	42.5 ± 1.6	252.7 ± 17.9	15	42.3 ± 1.7	251.2 ± 19.2	0
		100 mg/kg	4	41.6 ± 0.1	242.4 ± 13.1	16	41.6 ± 1.0	242.4 ± 15.9	0
	PND 17-21	Control	4	44.6 ± 1.4	249.3 ± 14.9	16	44.6 ± 2.2	249.3 ± 19.6	0
		10 mg/kg	4	44.1 ± 0.9	253.6 ± 6.1	16	44.1 ± 1.8	253.6 ± 13.4	0
		30 mg/kg	4	43.8 ± 0.9	257.7 ± 10.5	16	43.8 ± 1.8	257.7 ± 19.2	0
		100 mg/kg	4	42.6 ± 0.8	242.2 ± 14.6	16	42.6 ± 1.2**	242.2 ± 16.1	0
	PND 35-39	Control	4	43.6 ± 1.9	248.0 ± 4.7	16	43.6 ± 2.0	248.0 ± 14.2	0
		10 mg/kg	3	43.9 ± 0.8	256.4 ± 17.1	12	43.9 ± 1.4	256.4 ± 18.7	0
		30 mg/kg	3	46.8 ± 0.7*	267.4 ± 22.2	12	46.8 ± 1.4**	267.4 ± 29.0	0
		100 mg/kg	3	45.5 ± 1.1	261.5 ± 12.5	12	45.5 ± 1.7*	261.5 ± 17.9	0
DES	PND 1-5	Control	4	44.4 ± 1.9	259.5 ± 27.5	16	44.4 ± 2.5	259.5 ± 30.4	0
		10 μg/kg	4	44.9 ± 0.8	261.4 ± 25.1	16	44.9 ± 1.5	261.4 ± 27.5	0
		100 μg/kg	4	48.9 ± 3.4	311.8 ± 42.0	16	48.9 ± 4.0**	311.8 ± 46.0**	2 (2)
		300 μg/kg	4	49.9 ± 3.0*	288.0 ± 33.5	16	49.9 ± 3.9**	288.0 ± 39.8	3 (2)
	PND 17-21	Control	4	44.5 ± 1.3	242.7 ± 16.0	15	44.5 ± 2.1	241.2 ± 21.7	0
		10 μg/kg	3	46.3 ± 1.6	250.4 ± 7.2	12	46.3 ± 2.3	250.4 ± 19.9	0
		100 μg/kg	4	44.8 ± 0.9	243.3 ± 11.3	16	44.8 ± 1.1	243.3 ± 15.3	0
		300 μg/kg	4	45.4 ± 1.0	253.1 ± 12.7	16	45.4 ± 2.0	253.1 ± 22.2	0
	PND 35-39	Control	4	44.4 ± 1.9	251.6 ± 19.3	16	44.4 ± 2.2	251.6 ± 24.6	0
		10 μg/kg	3	43.9 ± 0.8	240.8 ± 32.7	12	43.9 ± 1.6	240.8 ± 32.5	0
		100 μg/kg	4	46.3 ± 1.4	243.3 ± 14.8	16	46.3 ± 2.4*	243.3 ± 19.8	0
		300 μg/kg	4	48.7 ± 2.2*	267.5 ± 34.1	16	48.7 ± 2.6**	267.5 ± 34.0	0
EE	PND 1-5	Control	4	43.3 ± 0.6	267.5 ± 4.8	16	43.3 ± 1.6	267.5 ± 17.4	0
		10 μg/kg	3	47.9 ± 4.3	282.7 ± 38.6	12	47.9 ± 4.6*	282.7 ± 39.3	1 (1)
		100 μg/kg	3	55.4 ± 1.0*	365.8 ± 9.1	12	55.4 ± 1.5**	365.8 ± 16.3**	10 (3)
	PND 6-10	Control	3	43.8 ± 1.1	245.7 ± 15.3	12	43.8 ± 1.8	245.7 ± 18.3	0
		10 μg/kg	4	48.4 ± 1.2**	287.3 ± 10.3**	16	48.4 ± 3.5**	287.3 ± 35.2**	1 (1)
	PND 11-15	Control	3	43.2 ± 0.9	257.0 ± 10.9	12	43.2 ± 1.2	257.0 ± 12.3	0
		10 μg/kg	4	44.1 ± 1.0	264.5 ± 14.1	16	44.1 ± 1.2	264.5 ± 19.3	0
	PND 17-21	Control	3	44.0 ± 0.8	243.7 ± 22.0	12	44.0 ± 1.1	243.7 ± 23.7	0
		10 μg/kg	2	43.8 ± 0.7	239.4 ± 20.9	8	43.8 ± 1.9	239.4 ± 18.8	0
		100 μg/kg	3	43.8 ± 0.8	252.7 ± 6.6	12	43.8 ± 1.7	252.7 ± 13.0	0
	PND 35-39	Control	4	44.3 ± 0.4	251.7 ± 16.9	16	44.3 ± 1.1	251.7 ± 22.5	0
		10 μg/kg	4	43.4 ± 1.5	240.0 ± 18.1	16	43.4 ± 1.9	240.0 ± 22.6	0
		100 μg/kg	4	45.9 ± 1.9	254.0 ± 24.2	16	45.9 ± 2.2	254.0 ± 28.3	0
TAM	PND 1-5	Control	4	43.3 ± 0.6	267.5 ± 4.8	16	43.3 ± 1.6	267.5 ± 17.4	0
		0.3 mg/kg	4	46.1 ± 4.5	276.8 ± 51.9	16	46.1 ± 4.6	276.8 ± 54.0	2 (1)
		1 mg/kg	4	49.8 ± 5.1	291.3 ± 47.9	16	49.8 ± 5.0**	291.3 ± 50.2	5 (2)
		3 mg/kg	4	55.8 ± 0.5**	323.0 ± 23.5	15	55.7 ± 1.0**	325.2 ± 29.6**	14 (4)
	PND 17-21	Control	4	44.6 ± 1.4	249.3 ± 14.9	16	44.6 ± 2.2	249.3 ± 19.6	0
		0.3 mg/kg	3	43.3 ± 1.3	244.4 ± 15.1	11	43.2 ± 2.0	243.3 ± 25.6	0
		1 mg/kg	4	43.9 ± 1.8	243.1 ± 3.8	15	43.7 ± 3.2	243.2 ± 27.3	0
		3 mg/kg	3	43.7 ± 2.1	246.5 ± 14.4	12	43.7 ± 2.3	246.5 ± 17.8	0
	PND 35-39	Control	4	43.6 ± 1.9	248.0 ± 4.7	16	43.6 ± 2.0	248.0 ± 14.2	0
		0.3 mg/kg	3	45.2 ± 0.8	246.0 ± 27.7	12	45.2 ± 2.1	246.0 ± 29.7	0
		1 mg/kg	3	44.5 ± 1.3	235.4 ± 13.9	12	44.5 ± 3.2	235.4 ± 26.3	0
		3 mg/kg	3	44.6 ± 1.3	231.6 ± 10.2	12	44.6 ± 1.6	231.6 ± 14.2	0

Value: Mean ± S.D. using the litter as the unit or using individual data.

PND: postnatal day; PPS: preputial separation; FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; BW: body weight.

Incomplete separation: number of animals (litters) with incomplete separation on PND 56.

*: significantly different from control, $p<0.05$; **: significantly different from control, $p<0.01$.

Table 6. Relative Organ Weights and Body Weights of PND 56 Male Rats Postnatally Exposed to Chemicals (Using the Litter as the Unit)

Chemical	Dosing period	Group	Litters	Body weight (g)	Testes (mg/g)	Epididymides (mg/g)	Ventral prostate (mg/g)	Seminal Vesicles (mg/g)
FLU	PND 1-5	Control	4	384.1 ± 19.6	7.281 ± 0.714	1.400 ± 0.079	1.025 ± 0.137	2.008 ± 0.289
		1 mg/kg	4	378.1 ± 15.6	7.536 ± 0.457	1.511 ± 0.121	0.986 ± 0.175	1.895 ± 0.239
		10 mg/kg	4	389.8 ± 28.3	7.441 ± 0.713	1.443 ± 0.074	0.998 ± 0.112	1.774 ± 0.216
		30 mg/kg	4	371.4 ± 17.0	7.594 ± 0.583	1.438 ± 0.087	1.050 ± 0.099	1.806 ± 0.244
	PND 17-21	Control	3	376.2 ± 5.9	7.446 ± 0.481	1.438 ± 0.082	0.989 ± 0.051	1.758 ± 0.220
		1 mg/kg	3	358.8 ± 11.3	7.389 ± 0.075	1.489 ± 0.214	1.007 ± 0.093	1.835 ± 0.266
		10 mg/kg	3	372.1 ± 14.1	7.508 ± 0.469	1.451 ± 0.062	0.951 ± 0.188	1.918 ± 0.064
		30 mg/kg	3	376.4 ± 16.4	7.707 ± 0.172	1.484 ± 0.040	1.043 ± 0.041	1.923 ± 0.349
	PND 35-39	Control	4	377.3 ± 30.6	7.654 ± 0.302	1.520 ± 0.039	1.068 ± 0.076	2.065 ± 0.132
		1 mg/kg	4	382.8 ± 15.8	8.108 ± 0.773	1.603 ± 0.153	1.097 ± 0.112	2.108 ± 0.099
		10 mg/kg	4	378.5 ± 19.9	7.742 ± 0.725	1.407 ± 0.118	1.051 ± 0.121	1.774 ± 0.235
		30 mg/kg	4	381.1 ± 15.5	7.951 ± 0.378	1.398 ± 0.093	0.866 ± 0.106*	1.788 ± 0.131
DDE	PND 1-5	Control	3	362.5 ± 6.2	7.760 ± 0.192	1.447 ± 0.068	0.809 ± 0.084	1.942 ± 0.217
		10 mg/kg	3	382.2 ± 10.3	7.122 ± 0.178	1.395 ± 0.067	0.852 ± 0.113	1.874 ± 0.138
		30 mg/kg	3	367.2 ± 27.2	7.670 ± 0.261	1.443 ± 0.091	0.928 ± 0.074	1.893 ± 0.259
		100 mg/kg	3	369.8 ± 19.6	7.458 ± 0.358	1.421 ± 0.052	0.826 ± 0.020	1.792 ± 0.049
		300 mg/kg	3	374.0 ± 13.3	8.024 ± 0.356	1.398 ± 0.086	0.749 ± 0.011	1.783 ± 0.185
	PND 17-21	Control	3	358.6 ± 11.8	8.522 ± 0.979	1.539 ± 0.052	0.815 ± 0.136	1.724 ± 0.319
		10 mg/kg	3	377.0 ± 7.0	7.804 ± 0.504	1.465 ± 0.128	0.893 ± 0.006	1.820 ± 0.284
		30 mg/kg	3	359.7 ± 12.9	7.864 ± 0.469	1.497 ± 0.098	0.864 ± 0.058	1.674 ± 0.171
		100 mg/kg	3	362.8 ± 2.4	8.263 ± 0.261	1.502 ± 0.012	0.840 ± 0.059	1.751 ± 0.095
		300 mg/kg	4	361.9 ± 33.6	8.344 ± 0.668	1.423 ± 0.088	0.848 ± 0.127	1.550 ± 0.114
	PND 35-39	Control	3	333.5 ± 26.1	7.844 ± 0.454	1.520 ± 0.116	0.925 ± 0.205	1.834 ± 0.318
		10 mg/kg	3	356.0 ± 22.3	7.362 ± 0.622	1.323 ± 0.115	0.875 ± 0.017	1.831 ± 0.110
		30 mg/kg	3	347.2 ± 40.7	7.500 ± 0.215	1.376 ± 0.028	0.908 ± 0.067	1.807 ± 0.172
		100 mg/kg	3	353.5 ± 23.0	7.823 ± 0.247	1.409 ± 0.108	0.847 ± 0.065	1.523 ± 0.249
VZ	PND 1-5	Control	4	367.5 ± 20.6	7.990 ± 0.894	1.601 ± 0.142	1.049 ± 0.151	2.151 ± 0.180
		10 mg/kg	3	382.0 ± 32.7	7.392 ± 0.531	1.477 ± 0.047	1.006 ± 0.055	2.059 ± 0.083
		30 mg/kg	4	380.1 ± 9.5	7.986 ± 0.644	1.500 ± 0.133	0.910 ± 0.118	2.049 ± 0.093
		100 mg/kg	4	377.9 ± 17.6	7.373 ± 0.152	1.582 ± 0.109	1.030 ± 0.094	2.116 ± 0.082
	PND 17-21	Control	4	357.5 ± 26.3	7.791 ± 0.654	1.432 ± 0.058	0.967 ± 0.094	1.903 ± 0.300
		10 mg/kg	4	366.8 ± 9.9	7.981 ± 0.225	1.562 ± 0.080	0.987 ± 0.018	2.041 ± 0.239
		30 mg/kg	4	378.3 ± 17.4	7.629 ± 0.610	1.550 ± 0.147	0.891 ± 0.105	1.931 ± 0.237
		100 mg/kg	4	373.2 ± 25.5	8.066 ± 0.507	1.532 ± 0.137	1.018 ± 0.103	2.105 ± 0.135
	PND 35-39	Control	4	369.8 ± 20.8	7.711 ± 0.696	1.502 ± 0.053	0.895 ± 0.041	1.752 ± 0.115
		10 mg/kg	3	373.3 ± 39.0	7.420 ± 0.420	1.572 ± 0.114	0.910 ± 0.048	1.911 ± 0.081
		30 mg/kg	3	361.7 ± 32.0	8.125 ± 0.253	1.546 ± 0.018	0.982 ± 0.042	1.885 ± 0.053
		100 mg/kg	3	363.1 ± 5.5	7.719 ± 0.280	1.508 ± 0.088	0.941 ± 0.058	1.907 ± 0.083
DES	PND 1-5	Control	4	367.5 ± 25.0	8.089 ± 0.473	1.560 ± 0.151	1.064 ± 0.097	1.948 ± 0.141
		10 μg/kg	4	365.7 ± 24.8	7.296 ± 0.588	1.391 ± 0.100	0.917 ± 0.139	1.870 ± 0.364
		100 μg/kg	4	383.6 ± 13.7	8.277 ± 1.331	1.431 ± 0.121	0.769 ± 0.088**	1.668 ± 0.537
		300 μg/kg	4	343.6 ± 19.7	7.840 ± 1.413	1.585 ± 0.206	0.829 ± 0.076*	1.323 ± 0.086
	PND 17-21	Control	4	343.3 ± 32.2	7.753 ± 0.315	1.554 ± 0.155	0.925 ± 0.046	1.952 ± 0.302
		10 μg/kg	3	337.2 ± 20.4	7.906 ± 0.530	1.432 ± 0.075	0.922 ± 0.083	1.781 ± 0.050
		100 μg/kg	4	348.9 ± 19.6	6.940 ± 0.696	1.455 ± 0.105	0.900 ± 0.095	1.939 ± 0.281
		300 μg/kg	4	352.8 ± 13.8	7.309 ± 0.751	1.417 ± 0.093	0.933 ± 0.099	1.935 ± 0.317
	PND 35-39	Control	4	363.7 ± 38.2	7.750 ± 0.452	1.542 ± 0.103	1.052 ± 0.045	2.303 ± 0.295
		10 μg/kg	3	344.9 ± 40.5	7.472 ± 0.696	1.593 ± 0.146	1.035 ± 0.113	1.963 ± 0.221
		100 μg/kg	4	330.4 ± 23.8	7.920 ± 0.561	1.556 ± 0.024	0.987 ± 0.160	1.910 ± 0.240
		300 μg/kg	4	336.5 ± 36.0	7.986 ± 0.322	1.611 ± 0.100	0.970 ± 0.075	2.017 ± 0.201
EE	PND 1-5	Control	4	391.7 ± 14.7	7.908 ± 0.276	1.533 ± 0.097	1.024 ± 0.084	1.947 ± 0.140
		10 μg/kg	3	367.0 ± 22.8	7.681 ± 0.568	1.477 ± 0.094	0.850 ± 0.087	1.548 ± 0.136*
		100 μg/kg	3	374.0 ± 6.1	6.920 ± 0.110*	1.394 ± 0.112	0.700 ± 0.096**	1.220 ± 0.123**
	PND 17-21	Control	3	353.9 ± 32.7	7.154 ± 0.245	1.456 ± 0.062	0.908 ± 0.127	1.978 ± 0.041
		10 μg/kg	2	357.0 ± 27.3	7.201 ± 0.472	1.366 ± 0.099	1.013 ± 0.143	1.875 ± 0.179
		100 μg/kg	3	373.1 ± 21.3	6.980 ± 0.316	1.521 ± 0.049	1.053 ± 0.160	2.118 ± 0.156
	PND 35-39	Control	4	357.4 ± 26.0	7.766 ± 0.479	1.567 ± 0.098	1.078 ± 0.174	1.954 ± 0.102
		10 μg/kg	4	353.6 ± 9.4	7.586 ± 0.739	1.569 ± 0.131	0.960 ± 0.084	1.910 ± 0.236
		100 μg/kg	4	347.1 ± 27.6	7.637 ± 0.700	1.517 ± 0.119	0.936 ± 0.104	1.783 ± 0.262
TAM	PND 1-5	Control	4	391.7 ± 14.7	7.908 ± 0.276	1.533 ± 0.097	1.024 ± 0.084	1.947 ± 0.140
		0.3 mg/kg	4	368.7 ± 20.3	7.153 ± 0.691	1.498 ± 0.084	0.813 ± 0.120	1.625 ± 0.271
		1 mg/kg	4	356.4 ± 22.4	6.756 ± 1.014	1.454 ± 0.198	0.790 ± 0.128*	1.601 ± 0.242
		3 mg/kg	4	325.6 ± 25.8**	5.011 ± 0.351**	1.159 ± 0.042**	0.596 ± 0.112**	1.197 ± 0.257**
	PND 17-21	Control	4	357.5 ± 26.3	7.791 ± 0.654	1.432 ± 0.058	0.967 ± 0.094	1.903 ± 0.300
		0.3 mg/kg	3	365.3 ± 22.5	7.788 ± 0.213	1.524 ± 0.155	0.935 ± 0.178	1.937 ± 0.160
		1 mg/kg	4	351.9 ± 31.7	7.990 ± 0.766	1.541 ± 0.119	0.972 ± 0.264	1.871 ± 0.139
		3 mg/kg	3	363.5 ± 15.9	7.457 ± 0.510	1.516 ± 0.095	0.927 ± 0.131	2.060 ± 0.090
	PND 35-39	Control	4	369.8 ± 20.8	7.711 ± 0.696	1.502 ± 0.053	0.895 ± 0.041	1.752 ± 0.115
		0.3 mg/kg	3	348.8 ± 37.5	7.357 ± 0.352	1.548 ± 0.074	0.900 ± 0.075	1.733 ± 0.126
		1 mg/kg	3	348.8 ± 37.4	8.189 ± 0.358	1.581 ± 0.136	0.959 ± 0.079	1.909 ± 0.235
		3 mg/kg	3	339.8 ± 10.0	8.159 ± 0.212	1.626 ± 0.133	0.934 ± 0.119	1.866 ± 0.138

Value: Mean ± S.D. calculated using the litter as the unit.

FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; PND: postnatal day.

*: significantly different from control, $p<0.05$; **: significantly different from control, $p<0.01$.

Table 7. Relative Organ Weights and Body Weights of PND 56 Male Rats Postnatally Exposed to Chemicals (Using the Individual Data)

Chemical	Dosing period	Group	Males	Body weight (g)	Testes (mg/g)	Epididymides (mg/g)	Ventral prostate (mg/g)	Seminal Vesicles (mg/g)
FLU	PND 1-5	Control	15	383.1 ± 24.2	7.294 ± 0.799	1.397 ± 0.105	1.014 ± 0.170	1.986 ± 0.304
		1 mg/kg	16	378.1 ± 21.8	7.536 ± 0.528	1.511 ± 0.130	0.986 ± 0.194	1.895 ± 0.255
		10 mg/kg	16	389.8 ± 34.8	7.441 ± 0.751	1.443 ± 0.111	0.998 ± 0.146	1.774 ± 0.249
		30 mg/kg	15	371.0 ± 27.8	7.574 ± 0.684	1.431 ± 0.107	1.049 ± 0.112	1.801 ± 0.276
	PND 17-21	Control	12	376.2 ± 15.8	7.446 ± 0.542	1.438 ± 0.124	0.989 ± 0.124	1.758 ± 0.292
		1 mg/kg	11	359.1 ± 21.3	7.388 ± 0.609	1.511 ± 0.205	1.016 ± 0.132	1.862 ± 0.307
		10 mg/kg	12	372.1 ± 27.8	7.508 ± 0.590	1.451 ± 0.084	0.951 ± 0.185	1.918 ± 0.170
		30 mg/kg	12	376.4 ± 20.4	7.707 ± 0.428	1.484 ± 0.100	1.043 ± 0.127	1.923 ± 0.388
	PND 35-39	Control	16	377.3 ± 33.2	7.654 ± 0.377	1.520 ± 0.067	1.068 ± 0.094	2.065 ± 0.238
		1 mg/kg	16	382.8 ± 26.3	8.108 ± 0.811	1.603 ± 0.165	1.097 ± 0.172	2.108 ± 0.301
		10 mg/kg	15	378.4 ± 26.7	7.778 ± 0.859	1.411 ± 0.139	1.053 ± 0.192	1.755 ± 0.324*
		30 mg/kg	16	381.1 ± 22.1	7.951 ± 0.487	1.398 ± 0.114*	0.866 ± 0.154**	1.788 ± 0.266*
DDE	PND 1-5	Control	12	362.5 ± 14.6	7.760 ± 0.369	1.447 ± 0.090	0.809 ± 0.130	1.942 ± 0.267
		10 mg/kg	12	382.2 ± 32.2	7.122 ± 0.498*	1.395 ± 0.108	0.852 ± 0.169	1.874 ± 0.226
		30 mg/kg	12	367.2 ± 26.8	7.670 ± 0.442	1.443 ± 0.102	0.928 ± 0.112	1.893 ± 0.292
		100 mg/kg	11	367.9 ± 28.3	7.460 ± 0.544	1.422 ± 0.074	0.826 ± 0.098	1.789 ± 0.214
		300 mg/kg	12	374.0 ± 26.9	8.024 ± 0.658	1.398 ± 0.096	0.749 ± 0.084	1.783 ± 0.288
	PND 17-21	Control	12	358.6 ± 24.0	8.522 ± 1.002	1.539 ± 0.142	0.815 ± 0.171	1.724 ± 0.334
		10 mg/kg	12	377.0 ± 25.9	7.804 ± 0.660	1.465 ± 0.131	0.893 ± 0.077	1.830 ± 0.308
		30 mg/kg	11	358.8 ± 24.7	7.830 ± 0.568	1.489 ± 0.109	0.865 ± 0.095	1.676 ± 0.174
		100 mg/kg	12	362.8 ± 18.3	8.263 ± 0.382	1.502 ± 0.064	0.840 ± 0.103	1.751 ± 0.211
		300 mg/kg	13	359.1 ± 38.0	8.731 ± 0.864	1.430 ± 0.124	0.841 ± 0.159	1.558 ± 0.178
	PND 35-39	Control	12	333.5 ± 26.1	7.844 ± 0.731	1.520 ± 0.124	0.925 ± 0.209	1.834 ± 0.331
		10 mg/kg	12	356.0 ± 24.1	7.362 ± 0.634	1.323 ± 0.112**	0.875 ± 0.084	1.831 ± 0.153
		30 mg/kg	12	347.2 ± 41.8	7.500 ± 0.372	1.376 ± 0.069*	0.908 ± 0.097	1.807 ± 0.238
		100 mg/kg	12	353.5 ± 26.9	7.823 ± 0.492	1.409 ± 0.156	0.847 ± 0.119	1.523 ± 0.247**
VZ	PND 1-5	Control	15	368.9 ± 30.7	7.917 ± 1.053	1.590 ± 0.158	1.039 ± 0.153	2.168 ± 0.277
		10 mg/kg	12	382.0 ± 35.0	7.392 ± 0.688	1.477 ± 0.100	1.006 ± 0.137	2.059 ± 0.160
		30 mg/kg	15	379.6 ± 16.8	8.020 ± 0.761	1.505 ± 0.136	0.917 ± 0.130	2.048 ± 0.302
		100 mg/kg	15	376.3 ± 24.8	7.361 ± 1.030	1.589 ± 0.134	1.025 ± 0.187	2.115 ± 0.195
	PND 17-21	Control	16	357.5 ± 28.1	7.791 ± 0.655	1.432 ± 0.086	0.967 ± 0.133	1.903 ± 0.317
		10 mg/kg	16	366.8 ± 24.6	7.981 ± 0.658	1.562 ± 0.126	0.987 ± 0.101	2.041 ± 0.302
		30 mg/kg	16	378.3 ± 26.0	7.629 ± 0.831	1.550 ± 0.182	0.891 ± 0.117	1.931 ± 0.287
		100 mg/kg	15	373.2 ± 30.3	8.056 ± 0.710	1.527 ± 0.146	1.022 ± 0.156	2.117 ± 0.277
	PND 35-39	Control	16	369.8 ± 25.8	7.711 ± 0.746	1.502 ± 0.098	0.895 ± 0.153	1.752 ± 0.190
		10 mg/kg	12	373.3 ± 35.7	7.420 ± 0.647	1.572 ± 0.122	0.910 ± 0.100	1.911 ± 0.231
		30 mg/kg	12	361.7 ± 31.7	8.125 ± 0.562	1.546 ± 0.076	0.982 ± 0.114	1.885 ± 0.154
		100 mg/kg	12	363.1 ± 14.2	7.719 ± 0.393	1.508 ± 0.134	0.941 ± 0.132	1.907 ± 0.203
DES	PND 1-5	Control	16	367.5 ± 31.8	8.089 ± 0.914	1.560 ± 0.174	1.064 ± 0.155	1.948 ± 0.262
		10 μg/kg	16	365.7 ± 26.6	7.296 ± 0.829	1.391 ± 0.152*	0.917 ± 0.167*	1.870 ± 0.390
		100 μg/kg	16	383.6 ± 23.0	8.277 ± 2.095	1.431 ± 0.127	0.769 ± 0.129**	1.668 ± 0.527
		300 μg/kg	15	343.0 ± 38.6	7.893 ± 2.723	1.569 ± 0.271	0.835 ± 0.120**	1.321 ± 0.232**
	PND 17-21	Control	15	340.4 ± 35.0	7.783 ± 0.497	1.566 ± 0.156	0.927 ± 0.097	1.962 ± 0.353
		10 μg/kg	12	337.2 ± 26.7	7.906 ± 0.674	1.432 ± 0.105*	0.922 ± 0.132	1.781 ± 0.209
		100 μg/kg	16	348.9 ± 24.8	6.940 ± 0.743**	1.455 ± 0.118	0.900 ± 0.109	1.939 ± 0.283
		300 μg/kg	16	352.8 ± 20.5	7.309 ± 0.846	1.417 ± 0.121**	0.933 ± 0.117	1.935 ± 0.346
	PND 35-39	Control	15	360.3 ± 36.2	7.793 ± 0.609	1.539 ± 0.168	1.052 ± 0.194	2.299 ± 0.488
		10 μg/kg	12	344.9 ± 39.7	7.472 ± 0.686	1.593 ± 0.155	1.035 ± 0.140	1.963 ± 0.240
		100 μg/kg	16	330.4 ± 26.3	7.920 ± 0.736	1.556 ± 0.089	0.987 ± 0.193	1.910 ± 0.351
		300 μg/kg	16	336.5 ± 36.5	7.986 ± 0.777	1.611 ± 0.153	0.970 ± 0.134	2.017 ± 0.260
EE	PND 1-5	Control	16	391.7 ± 17.3	7.908 ± 0.437	1.533 ± 0.131	1.024 ± 0.128	1.947 ± 0.269
		10 μg/kg	12	367.0 ± 22.9**	7.681 ± 0.690	1.477 ± 0.103	0.850 ± 0.110**	1.548 ± 0.270**
		100 μg/kg	10	372.7 ± 15.4*	6.919 ± 0.402**	1.387 ± 0.149*	0.692 ± 0.114**	1.223 ± 0.166**
	PND 17-21	Control	12	353.9 ± 32.3	7.154 ± 0.561	1.456 ± 0.126	0.908 ± 0.147	1.978 ± 0.207
		10 μg/kg	8	357.0 ± 27.4	7.201 ± 0.538	1.366 ± 0.101	1.013 ± 0.149	1.875 ± 0.277
		100 μg/kg	12	373.1 ± 23.1	6.980 ± 0.530	1.521 ± 0.101	1.053 ± 0.187	2.118 ± 0.245
	PND 35-39	Control	16	357.4 ± 29.4	7.766 ± 0.783	1.567 ± 0.118	1.078 ± 0.196	1.954 ± 0.286
		10 μg/kg	16	353.6 ± 17.8	7.586 ± 0.738	1.569 ± 0.149	0.960 ± 0.147	1.910 ± 0.347
		100 μg/kg	16	347.1 ± 31.1	7.637 ± 0.870	1.517 ± 0.144	0.936 ± 0.123 *	1.783 ± 0.267
TAM	PND 1-5	Control	16	391.7 ± 17.3	7.908 ± 0.437	1.533 ± 0.131	1.024 ± 0.128	1.947 ± 0.269
		0.3 mg/kg	16	368.7 ± 27.9*	7.153 ± 0.772	1.498 ± 0.115	0.813 ± 0.161**	1.625 ± 0.352*
		1 mg/kg	16	356.4 ± 28.8**	6.756 ± 1.135*	1.454 ± 0.219	0.790 ± 0.181**	1.601 ± 0.403*
		3 mg/kg	15	327.9 ± 29.0**	5.043 ± 0.550**	1.162 ± 0.114**	0.606 ± 0.124**	1.218 ± 0.371**
	PND 17-21	Control	16	357.5 ± 28.1	7.791 ± 0.655	1.432 ± 0.086	0.967 ± 0.133	1.903 ± 0.317
		0.3 mg/kg	11	364.5 ± 29.6	7.768 ± 0.452	1.530 ± 0.189	0.950 ± 0.196	1.936 ± 0.244
		1 mg/kg	15	354.0 ± 31.8	7.913 ± 0.812	1.530 ± 0.126	0.945 ± 0.243	1.873 ± 0.236
		3 mg/kg	12	363.5 ± 21.1	7.457 ± 0.901	1.516 ± 0.121	0.927 ± 0.212	2.060 ± 0.374
	PND 35-39	Control	16	369.8 ± 25.8	7.711 ± 0.746	1.502 ± 0.098	0.895 ± 0.153	1.752 ± 0.190
		0.3 mg/kg	12	348.8 ± 37.5	7.357 ± 0.457	1.548 ± 0.095	0.900 ± 0.102	1.733 ± 0.173
		1 mg/kg	12	348.8 ± 42.4	8.189 ± 0.787	1.581 ± 0.134	0.959 ± 0.141	1.909 ± 0.291
		3 mg/kg	12	339.8 ± 16.1*	8.159 ± 0.596	1.626 ± 0.134*	0.934 ± 0.139	1.866 ± 0.194

Value: Mean ± S.D. calculated using the individual data.

FLU: flutamide; DDE: *p,p'*-dichlorodiphenyldichloroethylene; VZ: vinclozolin; DES: diethylstilbestrol; EE: ethynylestradiol; TAM: tamoxifen; PND: postnatal day; *: significantly different from control, $p<0.05$; **: significantly different from control, $p<0.01$.