Honma M, Takahashi T, Asada S, Nakagawa Y, Ikeda A, Yamakage K.	*In vitro* clastogenicity and phototoxicity of fullerene (C60) nanomaterials in mammalian cells	Mutation Res	749	97-100	2012
小野 敦	効能の高い化粧品原料の安全性リスク評価に対する考え方	*Cosmetic stage*	9,(1)	21–26	2012
Ikeda A, Yamakage K.	*In vitro* clastogenicity and phototoxicity of fullerene (C60) nanomaterials in mammalian cells	Mutation Res.	749	97-100	2012

Ⅳ. 研究成果の刊行物・別刷り

効能の高い化粧品原料の安全性リスク評価に対する考え方

Considerations on the safety risk assessment for cosmetic ingredients with high efficacy.

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1　はじめに

化粧品には，基礎化粧品（化粧水など）やメークアップ化粧品（口紅，ファンデーションなど）など顔につけるものから，ボディ用化粧品に至るまで多岐に渡る製品が含まれる。近年，消費者のアンチエイジングへの関心から，美白やエイジングケアなどの機能を有する有効成分の開発が活発化している。一方，化粧品によるアレルギー反応，接触性皮膚炎，色素沈着，色素脱失などの事例は絶えない。

我が国において化粧品は，いわゆる一般化粧品と医薬部外品に分類される薬用化粧品に大別されるが，いずれとも薬事法では「人体に対する作用が緩和な物」とされている。しかし，緩和な作用についての基準はなく申請する側の判断に委ねられている。近年，より高い効能を求める消費者の要求や企業の開発競争により，もはや「人体に対する作用が緩和」とは言えない効能の高い有効成分も開発されており，十分なリスク評価に基づいた製剤設計を行わなければ，健康被害を引き起こす恐れがある。人体に対する作用が緩和であるとは，有効成分そのものの作用の強さよりむしろ，その製品が長期間に渡り，日常的に使用された場合の安全性が確保されていることが要求されていると解釈すべきである。

化粧品は，特定の病気の治療のため限られた期間，決められた用法・用量で使用される医薬品とは異なり，健康な人の肌に繰り返し長期間にわたり使用されるもので，不特定多数の人が使用し，その使用方法は人によって大きく異なる。医薬品は有効であれば多少の副作用も許容される可能性もあるが，化粧品には絶対的な安全性が要求されることからすれば，医薬品以上に安全性の確保が重要である。特に効能の高い有効成分では，その作用機序によっては，効能の延長線上で有害作用が起こる可能性もあり，注意深い安全性評価が必要である。

我が国では，化粧品の効能として認められている範囲[1)]を超える効能・効果を有する成分を含む化粧品は，医薬部外品として承認申請することが求められており，基本的には医薬品に準じた有効性・安全性に関する資料が要求される。すなわち医薬部外品であれば，一通りの安全性評価は実施されているはずであるが，近年，医薬部外品による大規模な健康被害も起きている。一方，医薬部外品以外の化粧品については，平成 13 年の薬事法改正に伴う大幅な制度改定（規制緩和）により，化粧品基準に示された一部の配合禁止成分や配合制限成分を除く成分については，原則として自由に配合が可能であり，製造販売における承認申請は求められていない。これは，化粧品であれば安全性評価が不要ということではなく，各企業には自己責任による自主的な安全性評価と管理に従って安全性を担保することが求められている。

効能の高い新規有効成分は，医薬部外品として安全性評価が実施されると想定されることから，以下では，主に医薬部外品の安全性リスク評価の考え方について記載するが，医薬部外品以外の化粧品であっても安全性リスク評価の基本原則は同じである。

2　リスク評価の原則

リスク評価とは物質が持っている有害性（ハザード）が，想定される暴露条件で起こるかどうかを見積もるこ

とである。すなわち，各種の毒性試験データにより有害性評価を行い，有害反応の用量反応関係より無毒性量を求め，求められた無毒性量より許容される暴露量を推定し，想定される暴露量との対比を行うことで安全性を確認する。通常使用による暴露量では問題が無い場合でも，使用者が使用後洗い流すべきものを流さず放置したり，用途外の部位に使用したりした場合の危険性も考慮するため，有害性評価においては，製品使用で想定される暴露量で有害性が無いことを確認するだけでは不十分であり，高濃度に暴露された場合に起こりうる有害反応のプロファイル（種類）や有害反応が起こる暴露量と起こらない暴露量（無毒性量）を明らかにすることが重要である。製剤への配合濃度については，ヒトにおける使用形態での暴露量を詳細に検討し，リスク評価により懸念がない濃度を，最終製品中の最大許容濃度とする。

リスク評価では，無毒性量と製品使用による暴露量の比を安全係数（＝無毒性量／製品使用による暴露量）として求める。どの程度の安全係数が確保すべきかについて明確な基準はないが，例えば，食品添加物や残留農薬の1日許容摂取量（ADI）の設定では，安全係数として100を用い，長期反復投与試験による無毒性量の1/100に設定される。この安全係数100の内訳としては，動物試験からの推定であることから種差に基づく係数として10倍，またヒトにおける感受性の個人差を包含するための係数として10倍と説明されている。化粧品の場合，製品の使用形態（皮膚に残る製品と洗い流される製品）や想定される有害作用の種類等にもよるが，おおよそ以下の4点から必要となる安全係数を検討する必要がある。

①動物間種差：動物試験からヒトにおいて許容される暴露量を推定する場合は種差に基づく係数として最大10倍を考慮する。ヒトにおける試験結果がある場合は，この項目を考慮する必要はない。

②個人間変動：年齢，性差，人種差，生まれ付きの皮膚防御能，遺伝的影響などによる感受性の変動を包含するため，最大10倍を考慮する。

③賦形剤，製品マトリックス効果：製品中に，エタノール，刺激物や浸透促進剤が含まれる場合は，最大10の係数を考慮する。

④製品の使用形態：製品が接触する部位のバリアー機能の状態や閉塞性などを考慮する。脇の下などの閉塞性の部位に使用する製品であれば，最大10を考慮する。

同一の有効成分を配合した複数の製品を使用する可能性がある場合には，その点も考慮する必要がある。一方で，安全であっても，期待される効果が発揮されなければ意味がない。ヒトにおける経皮吸収性や反応性などのデータを用いることで必要な安全係数を減らすことが可能な場合もある。長期に愛用される商品とするためには，十分な有効性が認められ，かつ，安全係数の大きい有効成分の開発や製剤設計が重要である。

3　安全性評価が必要な項目

医薬部外品の承認申請書に添付すべき安全性に関する資料の範囲は，平成11年7月26日医薬発第893号「医薬部外品等の製造又は輸入の承認申請に際して添付すべき資料について」にあげられている。医薬部外品の申請においては，大きく3つの区分があり，その区分により要求される安全性データの項目が異なる※。ヒトに医薬部外品として使用経験が無い新規の有効成分は，区分1であり，原則として以下の資料が要求されるが，科学的に妥当であると判断されれば，既に得られている知見等からの類推により省略出来る可能性がある。

1. 単回投与毒性（急性毒性）に関する資料
2. 反復投与毒性（亜急性毒性および慢性毒性）に関する資料
3. 生殖発生毒性に関する資料
4. 抗原性（皮膚感作性試験，光感作性試験等）に関する資料
5. 変異原性に関する資料
6. がん原性に関する資料
7. 局所刺激性（皮膚刺激性試験，粘膜刺激性試験等）に関する資料
8. 吸収・分布・代謝・排池に関する資料

※ 医薬部外品の申請区分及び添付すべき資料については，平成26年中に見直しが予定されている。

我が国においては医薬部外品有効成分承認のための有効性・安全性試験に関しての試験法ガイドラインは示されておらず，安全性試験は医薬品等の試験方法を参考に実施する。具体的には，医薬品毒性試験ガイドライン[2]，遺伝毒性試験ガイドライン[3]及び，がん原性試験ガイドライン[4]などで示された試験法に従って実施する。また，OECD（Organisation for Economic Cooperation and Development：経済開発協力機構）ガイドライン等で示された試験法に従って実施された試験結果であっても問題ない。

安全性試験を実施するにあたりGLPは必須ではないものの試験データの信頼性担保という観点から，信頼性が担保されたシステムのもとで実施されることが望ましい。一方，動物愛護の観点から動物実験の実施に際しては，「厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針について」（平成18年6月1日科発第0601001号）及びその他の動物実験等に関する法令等の規定を遵守することが求められている。動物試験をむやみに行うのでなく，後述する代替試験法の活用も検討したうえで評価に必要な情報を得るために必要最小限の試験デザインを検討するとともに動物に与える苦痛を軽減するよう努める必要がある。

4　安全性評価において注意すべき点

本項では，安全性試験実施にあたり考慮すべき点について概説する。個別の安全性試験法については，前述のガイドライン[2-4]及びQ&A[5]を参照されたし。

安全性試験を実施するにあたっては，要求される試験を漫然と実施するのではなく，試験対象の有効成分の物理化学的性質，類似の化学構造や作用メカニズムを有する化合物の安全性情報（化粧品成分のみだけではなく工業用化学物質などの情報も収集する）および予備試験成績等を考慮した上で，適正な評価を行うための最適な試験デザインを考慮することが重要である。

特に試験対象の有効成分の効能が生体分子との相互作用（薬理作用）の基づく作用である場合には，薬理作用の延長線上で起こりうる有害作用に注意した試験デザインを考慮すべきである。例えば，美白剤としての効能を有する成分のメカニズムとしては，

①チロシナーゼ活性を直接抑制する成分

②チロシナーゼ合成やチロシナーゼの成熟化を阻害する成分

③メラノサイト選択的な毒性を有する成分

等が挙げられるが，それぞれのメカニズムによって注意すべき点は異なってくる。そのため，安全性評価を行うにあたり，有効性試験において当該有効成分の作用メカニズムについて明らかにされていることも重要である。

有効成分の生体への影響について評価を行う上で適切な安全性試験デザインを検討する上で，試験対象とする成分の体内への取り込みの有無及び体内での挙動について把握することは重要である[6]。薬事法により化粧品や医薬部外品については「真皮層まで浸透する」という“広告表現”は禁じられているが，実際には多くの物質が真皮に移行することが知られており，新規成分について経皮吸収性の検討は必須である。経皮吸収による全身への移行が認められる場合や安全係数があまり大きくない場合には，分布・代謝・排泄及び蓄積性についても検討を行う必要がある。さらに，蓄積性が示唆される成分については，毒性が遅れて発現する可能性があるため，反復投与により，特定の組織や臓器への蓄積性についても確認する必要がある。また，製剤の添加物により皮膚透過性が向上する可能性がある場合には，その点も考慮する必要がある。

一方，経皮吸収が認められない場合でも，唇に適用する商品では直接経口摂取されることはもとより，眼元や顔全体へ適用する商品では，粘膜部位からの吸収について注意が必要である。さらに反復投与毒性試験等で経皮吸収による毒性が示唆された場合，得られた結果をヒトに外挿するため必要に応じてヒトにおける体内動態データの測定についても検討する。

通常，経皮吸収試験は，全身毒性の予測のため実施され，ターゲットである皮膚中での挙動についてはあまり配慮されていない。適用部位における有害影響については，皮膚刺激性や感作性等，他の試験で検討されるとはいえ，皮膚組織中における分布や排せつ，代謝物や分解物の影響について検討することで，動物試験デザインや結果の解釈に有益な情報や動物試験では検出が難しい生

体影響を示唆する知見が得られる可能性がある。

安全性試験において重要なことは，当該有効成分により引き起こされる可能性のある有害作用のプロファイル（毒性の種類や標的臓器）を明らかにするとともに用量反応関係から有害作用の起こらない用量を導き出すことであり，試験に用いる用量（濃度）は，明らかな毒性が認められる用量もしくは適用可能な最大量（もしくは試験ガイドラインで決められた限度用量）まで試験を行うことが望ましい。反復投与毒性試験や生殖発生毒性試験の投与経路については，実使用時の適用経路に準じて実施することとされている。しかし，経皮吸収性や蓄積性が認められる成分や強い急性毒性が認められる成分であって，経皮投与により明らかな毒性兆候が認められない場合には，毒性プロファイルを明らかにするため，より高用量の暴露が可能な投与経路での試験の必要性について，得られている試験結果から実使用時に想定される暴露量において十分な安全係数が担保出来るかどうかも含めて検討すべきである。一方，経皮吸収性の有無に関わらず，適正使用時の安全性評価のための局所毒性（抗原性（感作性），刺激性），事故的曝露を評価するための単回投与毒性，局所発がん性等を評価するための遺伝毒性は必須かつ重要である。特に感作性については，一度，感作誘導が起こると容易には脱感作されない場合もあるため低感作性物質の検出が可能な条件で試験実施に問題ない最高濃度まで試験を実施する必要がある。また，ヒトは，既に様々な化学物質に暴露されており，新規成分であったとしても，その類縁物質にすでに感作されている場合もあるため，構造類似物質についての感作性の報告についても調査する必要がある。感作性，刺激性に関しては，光感作性，光刺激性試験のデータが必要であるが，280 ～ 450nm の範囲で吸収極大の有無を確認し，紫外部吸収スペクトル（波長範囲：290 ～ 400nm）で吸収極大が認められない場合は省略できる。一方，吸収極大が認められた場合，要求されてはいないが，光遺伝毒性評価の必要性について検討すべきである。

さらに，具体的な試験項目として要求されていないが，色素沈着や白斑等の皮膚障害について構造や作用が類似する物質についての調査を行う必要がある。皮膚障害の種類によっては，通常の動物試験では検出が難しいため，*in vitro* 試験を含む特別にデザインされた試験が利用可能であれば，その実施を検討するとともに，動物試験で障害性が認められなかった場合でもヒト使用試験や市販後調査などにおいても引き続き注意が必要である。

5　いわゆる代替法試験の利用について

欧米を中心とした動物愛護の要請から EU においては，化粧品原料についても試験を実施した原料を含む製品の販売に関して動物を使用したすべての毒性試験の実施が 2013 年 3 月 11 日に禁止された。動物試験の禁止に先駆けて，安全性評価に必要な試験項目について代替法開発が進められており，既に幾つかの試験法が OECD ガイドライン化されている。我が国においても「OECD 等により採用された代替試験法あるいは適切なバリデーションでそれらと同等と評価された方法に従った試験成績であれば差し支えない」[5] とされており，積極的に活用していくべきであるが，ガイドライン化された手法であっても，それぞれの手法には限界があり，適用限界を理解した上で，試験対象とする有効成分が，その物性や類似物質の結果などから各 *in vitro* 手法の適用範囲であることを確認して使用する必要がある。これまでに OECD ガイドライン化された代替試験法のうち医薬部外品の申請に用いることが出来る試験法の例として Q&A[5] では，光毒性試験代替法として OECD 432：*in vitro* 3T3 NRU Phototoxicity Test 及び感作性試験の代替法である OECD 429：Skin sensitization, Local Lymph Node Assay が示されている。その他，皮膚吸収性試験（OECD 428：Skin Absorption：*in vitro* Method），皮膚腐食性試験（OECD 431：*in vitro* Skin Corosion：Human Skin Model Test）及び皮膚一時刺激性試験（OECD 439：*in vitro* Skin Irritation：Reconstructed Human Epidermis Test Method）等が，OECD ガイドライン化されており，使用可能な試験法は今後さらに増えるであろう。一方，目刺激性については，OECD 438：Isolated Chicken Eye Test 及び OECD 437：Bovine Corneal Opacity and Permeability Test がガイドライン化されているもののこれらの試験法は，比較的強い刺激性物質の検出に有効であるものの，医薬部外品に求められる弱刺激性，無刺激

性の評価における信頼性については十分に検証されておらず医薬部外品の評価に用いるには注意が必要である。

多くの試験法について *in vitro* 試験法の開発が進められているのとは対象的に，遺伝毒性試験については，*in vitro* 試験結果が発がん性データと比較した場合に偽陽性結果を示すことが多いこと等から，最終的な遺伝毒性の判断には動物を用いる *in vivo* 遺伝毒性試験の結果が重視されるようになりつつある。改訂 ICH ガイドライン[7]では，これまでより *in vivo* 試験にウエイトを置いたバッテリーが提示されたことから，我が国では，医薬品と同じガイドラインが適用される医薬部外品についても原則として同様のバッテリーが要求される。

代替試験法とともに，OECD では *in silico*（構造活性相関）予測評価技術の化学物質のリスク評価への活用を推進しており，加盟国が共通に利用できるプラットフォームとして OECD QSAR Toolbox[8] の開発を進めており，無償で利用出来る。現在の OECD QSAR Toolbox には，化合物を遺伝毒性や皮膚感作性について化学構造からプロファイルする機能が搭載されており，申請資料としては利用出来ないものの，試験実施に先立つ *in silico* 評価の実施は試験デザインの最適化や試験結果の解釈に有用である。

一方，動物試験を実施すればそれで完璧というわけではない。そもそも，動物試験自体が，ヒトの代替試験法であることを理解した上で，試験結果の評価を行うことが重要である。薬理作用に基づく生体の機能変化は，通常の毒性試験では明らかとはならない場合もあり，評価項目によっては，*in vitro* 試験法のほうが有用である場合もある。

6 ヒトでの評価

新有効成分を含有する薬用化粧品の承認申請時には，臨床試験として，ヒトパッチ試験及び効能・効果に関するヒト使用成績試験の実施が求められている。ただし，ヒト使用成績試験においては，有効性評価の他，有害事象の有無などの安全性についても評価することとされており，ヒトパッチ試験は必ずしも必要ない。とはいえ，ヒト使用成績試験は，あくまでも有効性評価を念頭に置いた試験デザインで実施されることが多く，安全性評価には十分でない可能性がある。

新規機能成分，抗老化および美白機能を有する既存成分の配合製品で，従来品に比べて意図的に経皮吸収性を高め，これらの機能を新たに持たせた製品，または当該機能を高めた製品の安全性評価については，日本香粧品学会安全性評価専門委員会による安全性評価ガイドライン[9]において，血中濃度測定や二重遮蔽法を含むヒト使用試験ガイドラインが示されている。

さらには，ヒトにおける長期使用時の安全性を確認するためには，医療用医薬品の外用剤に準じた長期安全性試験が必要であるとの指摘もあり，新有効成分含有医薬部外品の安全性評価に係る臨床試験のありかたについて，現在，厚生労働省で検討が進められているため本稿では割愛する。

7 おわりに

本稿では，効能の高い新規化粧品成分の安全性リスク評価の考え方について概説した。既に安全性評価済みの成分でも，より高用量の成分を含有した製剤や使用用途が異なる製剤，さらに物理化学特性の変更により経皮吸収性が著しく向上させた製剤の安全性リスク評価においても基本的な考え方は共通である。医薬品と違い，医薬部外品や化粧品では，万が一，事故が起きても公的な救済制度はなく，市販後の健康被害の補償は全て企業責任に委ねられている。特に，これまで使用経験のない新規成分については，想定しうる全ての項目について評価を行ったとしても安全性を完全に保証することは難しいと認識すべきである。市販前の安全性リスク評価の重要性はもちろんのこと，市販後においても常に安全性情報の把握に努めることも重要である。化粧品は，人々を幸せにする製品でなければならない。

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4）平成 11 年 11 月 1 日医薬審第 1607 号「医薬品のがん原性試験に関するガイドラインについて」
5）平成 18 年 7 月 19 日厚生労働省医薬食品局審査管理課事務連絡「医薬部外品の製造販売承認申請及び化粧品基準改正要請に添付する資料に関する質疑応答集（Q&A）について」
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Repeated Dose and Reproductive/Developmental Toxicity of Perfluorododecanoic Acid in Rats

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ABSTRACT: Perfluoroalkyl carboxylic acids (PFCAs) are a series of environmental contaminants that have received attention because of their possible adverse effects on wildlife and human health. Although many toxicological studies have been performed on perfluorooctanoic acid with carbon chain length C8, available toxicity data on PFCAs with longer chains are still insufficient to evaluate their hazard. A combined repeated dose and reproductive/developmental toxicity screening study for perfluorododecanoic acid (PFDoA; C12) was conducted in accordance with OECD guideline 422 to fill these toxicity data gaps. PFDoA was administered by gavage to male and female rats at 0.1, 0.5, or 2.5 mg/kg/day. The administration of PFDoA at 0.5 and 2.5 mg/kg/day for 42–47 days mainly affected the liver, in which hypertrophy, necrosis, and inflammatory cholestasis were noted. Body weight gain was markedly inhibited in the 2.5 mg/kg/day group, and a decrease in hematopoiesis in the bone marrow and atrophic changes in the spleen, thymus, and adrenal gland were also observed. Regarding reproductive/developmental toxicity, various histopathological changes, including decreased spermatid and spermatozoa counts, were observed in the male reproductive organs, while continuous diestrous was observed in the females of the 2.5 mg/kg/day group. Seven of twelve females receiving 2.5 mg/kg/day died during late pregnancy while four other females in this group did not deliver live pups. No reproductive or developmental parameters changed at 0.1 or 0.5 mg/kg/day. Based on these results, the NOAELs of PFDoA were concluded to be 0.1 mg/kg/day for repeated dose toxicity and 0.5 mg/kg/day for reproductive/developmental toxicity.
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Keywords: perfluorododecanoic acid; repeated dose toxicity; reproductive toxicity; developmental toxicity; screening test; rat; perfluoroalkyl carboxylic acid

INTRODUCTION

Perfluoroalkyl carboxylic acids (PFCAs) are a series of environmental contaminants that have recently received attention because of their possible effects on wild life and human health. They have been widely used as processing aids in the manufacture of fluoropolymers and as additives and components in consumer and industrial products (Prevedouros et al., 2006). Major sources of environmental pollution are considered to be fluoropolymer manufactures (Prevedouros et al., 2006). The stability and nonbiodegradability of PFCAs

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allow them to be persistent in the environment (Lau et al., 2007).

Many toxicological studies have been performed on the 8-carbon chain length PFCA, perfluorooctanoic acid (PFOA), which was the most widely used PFCA at least until the PFOA Stewardship Program was launched by the United States Environmental Protection Agency and eight major industrial companies in 2006 (US EPA, 2013). Repeat-dose toxicity studies of PFOA in rodents revealed a steep dose-response curve for mortality, reduced body weight, and hepatocellular hypertrophy and necrosis (Griffith et al., 1980; Perkins et al., 2004; UK COT, 2006). The incidences of hepatocellular adenomas, Leydig cell tumors, and pancreatic acinar cell tumors were shown to be increased in a 2-year bioassay of PFOA in rats (Biegel et al., 2001). The developmental and hormonal effects and immunotoxic potential of PFOA have also been established in rodents (Lau et al., 2007). The lowest NOAEL of PFOA was 0.06 mg/kg/day based on its effects on the liver in a 13-week feeding study in rats (Perkins et al., 2004).

Recent studies have been extended to other PFCAs, which can be used as alternatives to PFOA. Findings have indicated that the toxic potency of PFCAs increases with lengthening of the carbon chain, at least up to C9 (Permadi et al., 1993; Kudo et al., 2006). Since the bioaccumulation potential of PFCAs has also been reported to increase depending on their carbon number (Martin et al., 2003), long-chain PFCAs may cause serious environmental and/or human health concerns in the future; however, available toxicity data on such long-chain PFCAs are still insufficient to evaluate the hazard.

To evaluate longer chain PFCAs, the Ministry of Health, Labour and Welfare, Japan, conducted combined repeat dose and reproductive/developmental toxicity screening tests on several long-chain PFCAs (carbon chain lengths C11 to C18) under the Japanese safety programmes for existing chemicals between 2007 and 2011. We previously reported the results for perfluorooctadecanoic acid [PFOdA (C18)], which demonstrated that the toxic potency of PFOdA was relatively low, compared to the other PFCAs; the NOAELs were 40 mg/kg/day for repeated dose toxicity and 200 mg/kg/day for reproductive/developmental toxicity (Hirata-Koizumi et al., 2012). This study reported the recently obtained results for perfluorododecanoic acid [PFDoA (C12); CAS No. 16517-11-6].

PFDoA is a white powder with a melting point of 107–109°C. It has been reported that PFDoA was detected in the influent, effluent, or sludge in sewage and also in industrial wastewater treatment plants in Japan, Thailand, and Australia (Clara et al., 2008; Murakami et al., 2009; Kunacheva et al., 2011), in the water, sediment, or soil of rivers in Japan, China, and Australia (Nishikoori et al., 2011; Thompson et al., 2011; Wang et al., 2012), and in house dust and indoor air in Norway (Haug et al., 2011). PFDoA has been detected in various wildlife including the albatross, harbor seals, and porpoises in many different geographic locations throughout the world (Hoff et al., 2004; Tao et al., 2006; Van de Vijver et al., 2007; Ahrens et al., 2009; Thompson et al., 2011). PFDoA was found at the levels of a few pg/mL to hundreds of pg/mL in the blood of humans in various parts of the world (Guruge et al., 2005; Falandysz et al., 2006; Harada et al., 2011; Haug et al., 2009; Olsen et al., 2012) and at <10–41 pg/mL in breast milk in East Asia (Fujii et al., 2012).

The concentration of PFDoA in the river sediment or soil and in wildlife exceeded that of PFOA, which may reflect the lower water solubility and higher bioaccumulative properties of PFDoA (Martin et al., 2003; Inoue et al., 2012). Although there is no data available on the production volume and application, PFDoA sources may not only be from the manufacture and use of PFDoA, but also from where PFDoA is present as an impurity or where substances may degrade to form PFDoA (Prevedouros et al., 2006).

PFDoA was recently reported to affect the liver, leading to lipidosis and widespread disintegrated cell systems, and inhibited steroidogenesis in the testis and ovary in rats (Shi et al., 2007, 2009a,b, 2010a,b; Zhang et al., 2008; Ding et al., 2009); however, no data are available on how PFDoA affects other organs, reproductive performance, and development. The results of the combined repeated dose and reproductive/developmental toxicity screening test described here may provide a more comprehensive toxicity profile of PFDoA than has been reported previously.

MATERIALS AND METHODS

This study was conducted in accordance with OECD guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test" (OECD, 1996) at the Safety Research Institute for Chemical Compounds (Sapporo, Japan). All procedures involving the use and care of animals complied with the principles for Good Laboratory Practice (MOE et al., 2008) and applicable animal welfare regulations ["Act on Welfare and Management of Animals" (Japanese Animal Welfare Law, 2006), "Standards Relating to the Care, Management of Laboratory Animals and Relief of Pain" (MOE, 2006), and "Guidelines for Animal Experimentation" (JALAS, 1987)].

Animals and Housing Conditions

Crl:CD(SD) rats (8-week-old) were purchased from Atsugi Breeding Center, Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were maintained in an air-conditioned room with controlled temperature (22 ± 3°C) and humidity (50 ± 20%). Light was provided on a 12-h light/dark cycle (light: 8:00–20:00). Animals were housed in groups of two during the quarantine and acclimation periods, and after being assigned to each dose group, were reared individually, except for mating and nursing periods, in metal

bracket-type cages with wire-mesh floors. Regarding pregnant animals, individual dams and litters were reared from day 17 of gestation to day 4 of nursing using wood chips as bedding (White Flake; Charles River Laboratories Japan). All animals were fed *ad libitum* with a standard rat diet (CRF-1; Oriental Yeast, Tokyo, Japan), and had free access to tap water (Sapporo, Japan).

Rats were acclimated to the laboratory for 14 days, during which general conditions were observed once a day and body weights were measured on the day of receipt, the 8th day of acclimation, and the end of acclimation. No abnormality was seen in the general state or weight in either animal. Vaginal smears were prepared daily for female animals in order to examine the estrous cycle for 9 days before animals were assigned to each group. Abnormalities were identified in two females. Animals found to be in good health and showing normal estrous cycles were divided into each dose group by stratified random sampling to equalize the mean body weight. The body weights of animals selected for the study were from 368 to 424 g in males and from 228 to 279 g in females.

Chemicals and Dosing

PFDoA was purchased from Exfluor Research Corporation (TX, USA). The PFDoA (Lot No. 4103) used in this study was 97 % pure, and was kept in an airtight container in a cold and dark place (2–9°C). The test article was suspended in a 0.5% aqueous solution of carboxymethylcellulose sodium (CMC-Na; Maruishi Pharmaceutical, Osaka, Japan), and administered to the animals by gastric intubation with a disposable gastric tube and disposable syringe. Before the start of the study, the stability of PFDoA in a 0.5% CMC-Na aqueous solution at concentrations of 0.01 and 100 mg/mL was confirmed after 4-h storage at room temperature following a 15-day refrigerated storage; therefore, dosing solutions were prepared at least once every 15 days throughout the study and were kept in a cool (2.0–7.2°C) and dark place under airtight conditions until dosing. The concentrations of PFDoA in the formulations were analyzed at the first and last preparation using high-performance liquid chromatography-tandem mass spectrometry, and were confirmed to be 96.4–99.0% of the target.

Experimental Design

Rats were administered PFDoA daily by repeated oral administration. The dose levels were determined based on the results of a 14-day dose-finding study, in which the liver weight was increased at all doses (1 mg/kg/day and above), and more clear toxic effects, including inhibition of body weight gain, changes in blood biochemical, and hematological parameters and brown discoloration of the liver, were observed in rats given 3 and 5 mg PFDoA/kg/day. Considering the longer administration period in the present combined study, the maximum dose of PFDoA was set at 2.5 mg/kg/day, and 0.1 and 0.5 mg/kg/day were derived by one-fifth divisions. The daily application volume (10 mL/kg body weight) was calculated according to the latest body weight. Control rats were given the same volume of vehicle alone.

Twelve males in each dose group (0, 0.1, 0.5, and 2.5 mg/kg/day) were dosed for a total of 42 days, beginning 14 days before mating. Seven males in the control and 2.5 mg/kg/day groups and all males in the 0.1 and 0.5 mg/kg/day groups were necropsied the day after day 42 of the dose (main group). The remaining five males in the control and 2.5 mg/kg/day groups were assigned to a recovery group, and after 42-day administration, they were kept without administration for 14 days (recovery period) and then necropsied. Twelve females/dose were dosed from 14 days prior to mating (main group). The pregnant females were dosed for gestation and nursing periods until 5 days after delivery and then necropsied on day 6 of nursing. Pregnant females which did not deliver by day 25 of gestation and females which showed abnormal delivery (stillbirth) were necropsied on day 26 of gestation and on day 0 of nursing, respectively. As a recovery group, other five females per dose were dosed with 0 or 2.5 mg PFDoA/kg/day for a total of 42 days without mating, and then kept without administration for 14 days (recovery period). All females in the recovery group were necropsied on the day after the 14-day recovery period, and therefore, females given PFDoA without mating were not examined fully at the end of administration period.

The first date of administration was defined as day 1 of the doing. In the main group females, the day of successful copulation was designated as day 0 of gestation and the end of deliver as day 0 of nursing or postnatal day (PND) 0. In recovery group males and females, the day following 42-day administration was defined as day 1 of recovery period.

Observation and Examination

Repeated Dose Toxicity Data

In all animals, general status, including life or death, appearance and behavior, of individual rats was observed twice daily during administration period (before and after the administration) and during the recovery period (morning and afternoon), and once in the morning of the day of necropsy. In addition, detailed clinical observations were conducted using a standardized scoring system for all of the animals before start of administration and once a week throughout the administration and recovery periods. Food consumption and body weight were measured at regular intervals throughout the administration and recovery periods.

Functional observations were performed on day 40 of the administration and on day 8 of recovery periods for males and for females of the recovery group, and on day 4 of nursing for females of main group. Subjects for the observations were 5 males/dose selected to approximate to the mean body weight of each dose group, 5 females/dose in recovery group

and 5 females/dose having delivered earlier in the main group. Evaluations were conducted using a predetermined standardized scoring system, as follows: (i) Sensorimotor reactivity to visual, tactile, auditory, pain, proprioceptive stimuli, and air righting reflex was assessed on an examination table, (ii) Forelimb and hindlimb grip strength was measured three times with a CPU gauge (Aikoh Engineering, Osaka, Japan), and (iii) Spontaneous motor activity was recorded for 1 h at intervals of 10 min using an automated activity monitor system [SUPERMEX and CompAct AMS (Muromachi Kikai, Tokyo, Japan)].

Urine was collected at the end of the administration and recovery periods in a nonfasted condition from five males per dose selected for functional observations and from five females per dose in the recovery group in the metabolism cage (KN-646, B-1 type, Natsume Seisakusho, Tokyo, Japan). Fresh urine (3 h) was examined for pH, protein, glucose, ketone body, urobilinogen, bilirubin, occult blood, and color, and urine volume and specific gravity were measured using collected urine (21 h).

Blood samples were collected for hematology and blood biochemistry from the abdominal aorta at necropsy under ether anesthesia after starvation for 16–22 h. In the main group, five males per dose not used in the functional observations and five females per dose used in the functional observations were selected for blood sampling. All animals in the recovery group were subjected to the blood sampling. One portion of the blood was treated with ethylenediaminetetraacetic acid dipotassium (EDTA-2K,TERUMO CORPORATION., Tokyo, Japan) and examined for the red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte, platelet count, white blood cell count (WBC), and differential count of white blood cells. Prothrombin time (PT), and activated partial thromboplastin time (APTT) were measured using plasma separated from another blood sample treated with 3.8% sodium citrate. Plasma obtained from blood treated with heparin sodium [HEPARIN SODIUM INJECTION-N "Ajinomoto", 1000 unit/mL (AJINOMOTO CO., INC., Tokyo, Japan)] was analyzed for aspartate aminotransferase (AST) and glucose. Serum prepared by centrifuging the blood collected in tubes filled with a serum separation agent (SEPACLEAN-A, EIKEN CHEMICAL CO., LTD., Tokyo, Japan) was analyzed for alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γ-GTP), total cholesterol (T-Cho), triglycerides (TG), total bilirubin (T-Bil), blood urea nitrogen (BUN), creatinine (Crea), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphorus (IP), total protein (TP), protein fraction ratio, albumin/globulin (A/G) ratio, and albumin.

All surviving animals were euthanized by exsanguination after blood was collected under deep ether anesthesia and the external surfaces and organs and tissues of the whole body were examined macroscopically. The brain, pituitary gland, thyroid, heart, liver, kidney, spleen, adrenal gland, thymus, testes, epididymides, and ovaries were then weighed. The following organs and tissues were fixed and stored in 10% neutral-buffered formalin: the brain (cerebrum, cerebellum, and pons), spinal cord, pituitary gland, thymus, thyroid (including parathyroid), adrenal gland, spleen, heart, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum, trachea, lung, kidney, urinary bladder, prostate, seminal vesicle (including the coagulating gland), ovary, uterus (horn part and jugular), mammary gland (right abdomen), femur (right, including bone marrow), mesenteric lymph nodes, submandibular lymph nodes, sciatic nerve, and grossly abnormal tissues (including the boundary with normal tissues). The eyeball and Harderian gland were fixed and preserved with Davidson's fixative solution. The testis and epididymis were fixed with Bouin's solution and preserved in 70% ethanol. The lungs were fixed with immersion following the injection of fixing solution. In principle, organs with right and left parts were both fixed and preserved.

Histopathological examinations were conducted on the preserved organs and tissues of five males and five females in the control and high dose groups, and also dead and euthanized animals during the study. All grossly abnormal tissues were also histopathologically examined regardless of the dose groups. Since treatment-related gross or histopathological abnormalities in the high dose group were observed in the forestomach, glandular stomach, pancreas, liver, testis, epididymis, prostate gland, seminal vesicle, coagulating gland, uterine horn part, spleen, thymus, born marrow, and adrenal gland, these organs from all animals in all groups were examined histopathologically. The organs were sectioned after paraffin embedding, stained with hematoxylin–eosin, and examined under a light microscope. To confirm the findings of the liver, specimens stained with Hall stain and Oil red O were additionally prepared and examined microscopically.

Reproductive/Developmental Toxicity Data

The estrous cycle of all females was evaluated by sampling the vaginal lavage daily from the first day of administration until evidence of copulation in the main group and until the necropsy day in the recovery group. Vaginal smear specimens made by the Giemsa stain were observed under an optical microscope. Females having repeated 4–6 day estrous cycles were judged to have normal estrous cycles.

Each female in the main group was transferred to the home cage of a randomly chosen male from the same exposure group on day 14 of administration, and cohabited on a 1 : 1 basis until successful copulation occurred or a mating period of 2 weeks had elapsed. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as evidence of successful mating. Gestation was confirmed by the

presence of delivery and by counting the number of implantation sites in the uterus at necropsy. Following confirmation of mating, females were returned to their home cages and allowed to deliver spontaneously and nurse their pups. They were checked at least three times daily (9:00, 13:00, and 17:00) on days 21–25 of gestation, and the day on which dams held their pups under the abdomen in the nest by 9:00 was designated as the end of delivery. Gestational length was recorded, and the copulation index, fertility index, and gestation index were computed for each dose group.

All live and dead pups born were counted on PND 0, and the live birth index was calculated for each litter. Live pups were sexed and examined grossly on PNDs 0 and 4. Sex ratios were calculated for each litter. They were observed daily for general appearance and behavior, and the body weight of live pups was recorded on PNDs 0, 1, and 4. The viability index on PND 4 was calculated for each litter. All pups were euthanized on PND 4 by the inhalation of carbon dioxide and subjected to a gross external and internal (include the oral cavity) observation.

At necropsy of maternal animals, the numbers of corpora lutea and implantation in the uterus were recorded, and the implantation index and delivery index were calculated for each litter.

Statistical Analysis

The trend for detailed clinical and functional observations of each group, qualitative parameters of urinalysis, specific gravity of urine, and histopathological findings with multiple grades was evaluated by the Kruskal-Wallis test. If significant differences were identified ($p \leq 0.10$), data were compared between the control and each dosage group using the Mann-Whitney U test. The incidence of females with normal estrous cycles, copulation, fertility, and gestation indices, and histopathological findings with a single grade were analyzed using the chi-square test. If significant differences were found ($p \leq 0.10$), the data were compared between the control and each dosage group using the chi-square two sample test or the Fisher's exact probability test.

The mean and standard deviation were calculated for the other parameters and evaluated by the Bartlett's test for homogeneity of variances. The live birth index, neonatal sex ratio, viability index, and body weight of male and female pups were similarly analyzed using the litter as the experimental unit. When homogeneity was recognized ($p > 0.05$), a one-way analysis of variance was applied and data without homogeneity ($p \leq 0.05$) were subjected to the Kruskal-Wallis test. If a significant difference was identified ($p \leq 0.10$), the Dunnett's test or the Mann-Whitney test was used for pairwise comparisons between the control and individual treatment groups.

All statistical analyses comparing the control and individual treatment groups were conducted using the 5% level of probability as the criterion for significance.

RESULTS

Clinical and Functional Observations

In the 2.5 mg/kg/day group, soft feces was observed in one male on days 35–42 of the administration period and in another male only on day 4 of the recovery period. Although no deaths were observed in males, four females given 2.5 mg PFDoA/kg/day were found dead on days 18–22 of gestation. Hypothermia and vaginal hemorrhage were observed before death in one female. Three females were euthanized in the 2.5 mg/kg/day group due to a moribund condition on days 18–20 of gestation. In addition, two females were euthanized on day 26 of gestation because they did not deliver any pups, and two females were euthanized on nursing day 0 because of abnormal delivery (all pups stillborn). No clinical signs of toxicity were observed in females in the recovery group.

In detailed clinical observations, no significant difference was observed between the control and PFDoA-treated groups at any observation point. Although no significant differences were observed in functional observations for males on day 42 of the administration period between the control and PFDoA-treated groups, a significant decrease in forelimb grip strength was noted in males in the 2.5 mg/kg/day group at the end of the recovery period (Table I). A similar change was also observed in females of the recovery group. In females in the main group, the results of the 2.5 mg/kg/day group were exempt from statistical evaluation because only one female normally delivered pups and survived to the day of the functional observations (day 4 of nursing). Forelimb grip strength was slightly higher in this female than in control females. No significant change was observed in hindlimb grip strength in any of the treatment groups in either sex. In females given 2.5 mg PFDoA/kg/day in the recovery group, a significant decrease in motor activity was observed at 0–10 min, 10–20 min, and 20–30 min on week 6 of administration. Total motor activity for 60 min was also significantly decreased (Table I). Such an effect was not found on week 2 of the recovery period. No significant changes were observed in motor activity in any other groups.

Body Weight

Body weight was significantly lower in males at 2.5 mg/kg/day than in the controls from day 21 to the end of the 42-day administration period, and it remained significantly lower until the end of the 14-day recovery period (Fig. 1). Body weight in females in the recovery group showed similar time-dependent changes to those observed in the males, as shown in Figure 1. Body weight in females in the main group was significantly decreased at 2.5 mg/kg/day through the gestation period (Fig. 2). One female that normally delivered pups in the 2.5 mg/kg/day group had a lower body weight than the controls during the nursing period.

TABLE I. Grip strength and motor activity in male and female rats administered PFDoA

Dose (mg/kg/day)			0 (control)	0.1	0.5	2.5
MALES						
Number of animals examined			5	5	5	5
Administration week 6						
	Grip strength (g)	Forelimb	1459.28 ± 99.35	1532.14 ± 197.47	1632.80 ± 372.40	1313.72 ± 312.91
		Hindlimb	673.92 ± 240.73	636.16 ± 155.70	671.26 ± 84.61	526.92 ± 100.23
	Total motor activity	(60 min)	1459.3 ± 99.4	1169.4 ± 487.4	1340.0 ± 831.2	732.8 ± 442.6
Recovery week 2						
	Grip strength (g)	Forelimb	1576.54 ± 290.26			1217.00 ± 147.46*
		Hindlimb	669.16 ± 85.59			531.26 ± 119.71
	Total motor activity	(60 min)	1171.2 ± 344.9			9961.4 ± 362.2
FEMALES						
Number of animals examined			5	5	5	1
Day 4 of nursing (Main group)						
	Grip strength (g)	Forelimb	943.68 ± 140.49	922.20 ± 137.48	904.68 ± 124.73	1236.70[a]
		Hindlimb	502.66 ± 80.17	475.08 ± 88.55	478.46 ± 41.24	413.00[a]
	Total motor activity	(60 min)	1216.2 ± 234.2	1222.6 ± 494.5	1240.2 ± 853.1	1145.0[a]
Number of animals examined			5			5
Administration week 6 (Recovery group)						
	Grip strength (g)	Forelimb	1236.20 ± 260.53			998.20 ± 137.67
		Hindlimb	582.20 ± 51.34			472.54 ± 112.04
	Total motor activity	(60 min)	2785.6 ± 1000.2			1044.2 ± 563.3**
Recovery week 2 (Recovery group)						
	Grip strength (g)	Forelimb	1233.28 ± 221.67			834.58 ± 87.45**
		Hindlimb	630.68 ± 70.19			537.22 ± 105.37
	Total motor activity	(60 min)	1933.0 ± 1171.4			1117.8 ± 764.9

*Significantly different from the control, at $p \leqq 0.05$.
**Significantly different from the control, at $p \leqq 0.01$.
[a]Data from only one animal. In this group, other females did not deliver pups normally or survive to the day of the functional observations.

Food Consumption

A remarkable and significant decrease in food consumption was observed on days 28, 35, and 42 of the administration period at 2.5 mg/kg/day in males. However, no significant decreases were noted in food consumption in the 0.1 and 0.5 mg/kg/day groups. During the recovery period, no significant difference was observed in food consumption between the control and PFDoA-treated groups in males.

Females given 2.5 mg PFDoA/kg/day in the main group also consumed a significantly smaller amount of food from day 3 through day 20 of gestation. The amount of food consumed by one female that normally delivered pups in this group was lower than that of the control group during the nursing period. Food consumption in females in the 2.5 mg/kg/day recovery group showed similar time-dependent changes to those in the males.

Urinalysis

No significant difference was seen in any urinalysis parameters between the control and PFDoA-treated groups either at the end of the administration period or at the end of the recovery period.

Hematology

MCV and the reticulocyte ratio were significantly lower and MCHC was significantly higher in males given 2.5 mg

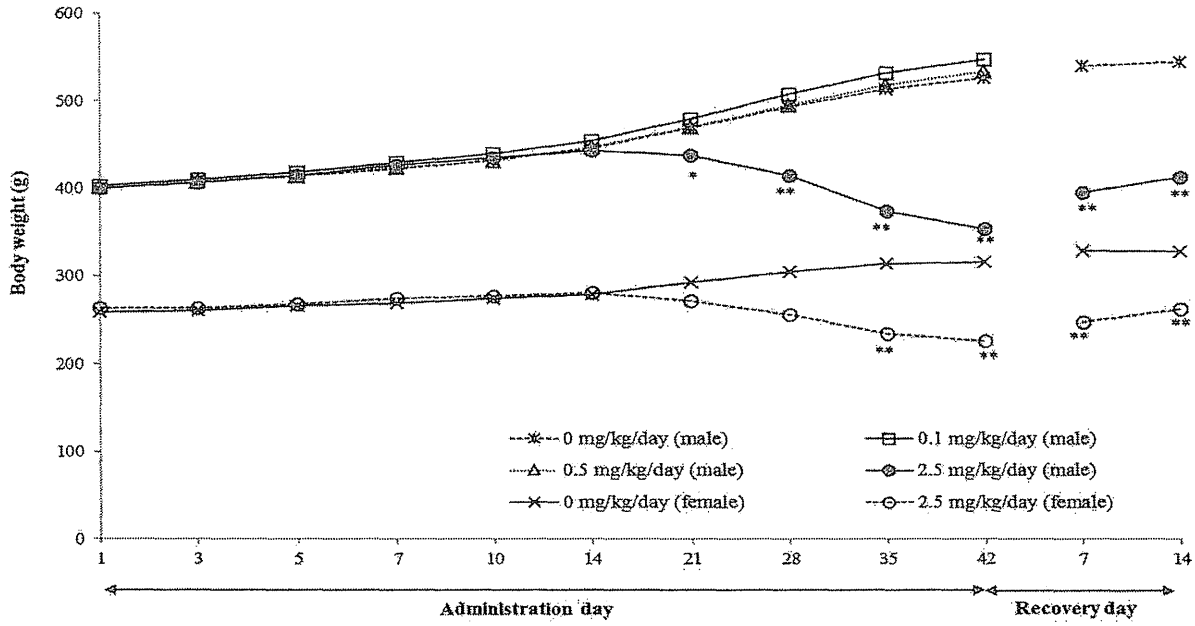

Fig. 1. Body weight changes in male rats in the main and recovery groups and female rats in the recovery group in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test for perfluorododecanoic acid. *Significantly different from the 0 mg/kg/day group at $p \leq 0.05$, **Significantly different from the 0 mg/kg/day group at $p \leq 0.01$.

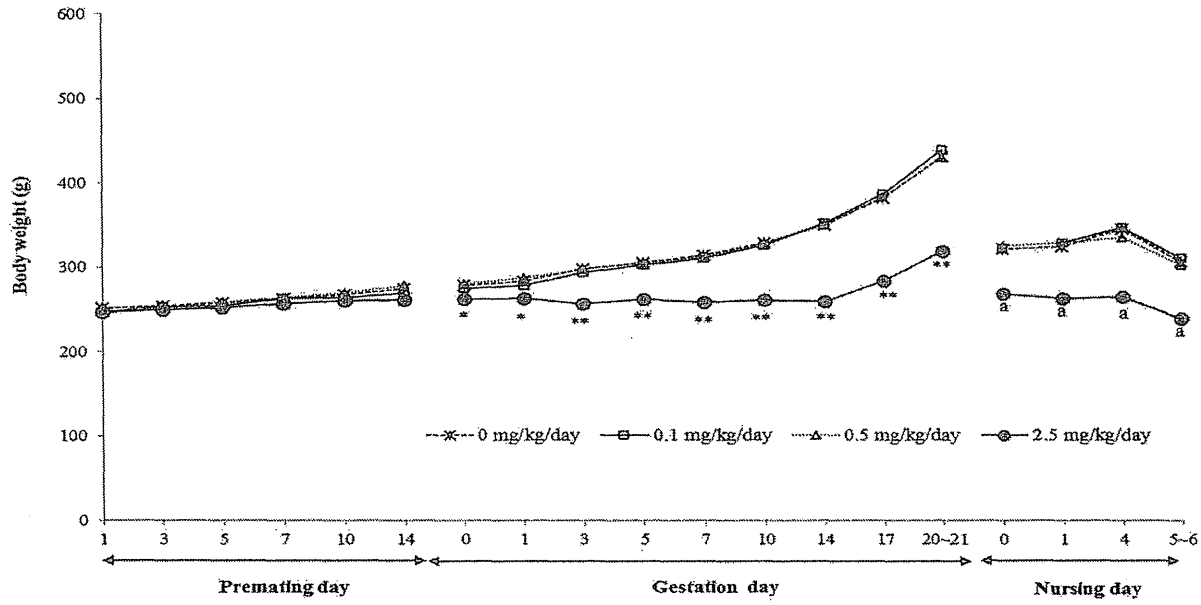

Fig. 2. Body weight changes in female rats in the main group in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test for perfluorododecanoic acid. *Significantly different from the 0 mg/kg/day group at $p \leq 0.05$, **Significantly different from the 0 mg/kg/day group at $p \leq 0.01$. a: The data were exempt from statistical evaluation because only one female normally delivered pups.

PFDoA/kg/day than in the control group at the end of the administration period (Table II). In this dose group, significant decreases were noted in WBC, RBC, HGB, HCT, and lymphocyte, monocyte, and eosinophil counts on a differential counts of WBC, and a significant increase was observed in the reticulocyte counts at the end of the recovery period.

Many changes, including decreases in HCT, MCV, and the reticulocyte count and an increase in MCHC, were found in one female given 2.5 mg/kg/day, as shown in Table II. In the 0.1 and 0.5 mg/kg/day groups, no significant changes were found in any parameters. In the recovery group, HGB, HCT, MCV, and MCH were significantly lower, while the neutrophil differential count of WBC was significantly higher in females given 2.5 mg PFDoA/kg/day than in the control group.

Blood Biochemistry

Serum TP and albumin levels were significantly decreased in males at 2.5 mg/kg/day at the completion of the administration period (Table III). Significant increases were observed in the albumin and γ-globulin ratios in the protein fraction, as well as a significant decrease in the α_1-globulin ratio at 2.5 mg/kg/day and a significant decrease in the α_2-globulin ratio at 0.5 mg/kg/day and above. ALP activity was

TABLE II. Hematological findings in male and female rats administered PFDoA

	Main Group				Recovery Group	
Dose (mg/kg/day)	0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES						
Number of animals examined	5	5	5	5	5	5
Red blood cells (10^4/μL)	918 ± 37	914 ± 18	927 ± 31	943 ± 58	978 ± 56	859 ± 41**
Hemoglobin (g/dL)	16.3 ± 0.6	16.2 ± 0.4	17.0 ± 0.6	16.1 ± 0.9	16.4 ± 0.6	14.2 ± 0.6**
Hematocrit (%)	46.3 ± 1.4	46.2 ± 1.6	48.2 ± 2.2	44.0 ± 1.9	46.0 ± 1.3	40.3 ± 13.0**
MCV (fL)	50.5 ± 2.8	50.6 ± 2.2	52.0 ± 2.0	46.7 ± 1.6	47.1 ± 1.6	47.0 ± 1.4
MCH (pg)	17.8 ± 1.1	17.7 ± 0.7	18.3 ± 0.5	17.1 ± 0.4	16.8 ± 0.4	16.5 ± 0.4
MCHC (g/dL)	35.2 ± 0.3	35.0 ± 0.4	35.2 ± 0.5	36.7 ± 0.8**	35.7 ± 0.5	35.2 ± 0.5
Reticulocytes (%)	3.18 ± 0.47	3.26 ± 0.55	3.35 ± 0.63	1.17 ± 0.67**	3.15 ± 0.31	4.63 ± 0.56**
Platelets (10^4/μL)	124.8 ± 8.6	112.5 ± 11.8	118.0 ± 9.0	116.7 ± 33.2	127.6 ± 15.6	169.9 ± 41.2
White blood cells (10^2/μL)	113.4 ± 28.2	148.2 ± 33.9	108.4 ± 19.6	135.0 ± 36.9	139.8 ± 14.2	112.5 ± 6.9**
Neutrophils (10^2/μL)	16.8 ± 4.1	17.9 ± 6.2	13.8 ± 3.1	21.2 ± 8.6	19.3 ± 8.4	15.3 ± 5.0
Lymphocytes (10^2/μL)	90.6 ± 24.5	123.7 ± 30.6	89.8 ± 17.9	108.0 ± 32.4	112.5 ± 15.0	93.0 ± 6.0*
Monocytes (10^2/μL)	4.24 ± 1.22	4.28 ± 0.65	3.56 ± 1.18	4.76 ± 3.85	5.36 ± 1.90	2.54 ± 0.78*
Eosinophils (10^2/μL)	1.68 ± 0.78	2.26 ± 0.73	1.22 ± 0.55	1.00 ± 0.58	2.60 ± 0.62	1.56 ± 0.56*
Basophils (10^2/μL)	0.04 ± 0.09	0.04 ± 0.05	0.02 ± 0.04	0.06 ± 0.09	0.06 ± 0.05	0.00 ± 0.00
PT (sec)	21.3 ± 4.3	22.7 ± 3.9	20.7 ± 3.5	19.1 ± 0.9	20.1 ± 3.4	18.5 ± 1.0
APTT (sec)	26.0 ± 2.3	26.9 ± 2.2	25.9 ± 1.6	22.0 ± 3.5	25.5 ± 4.5	23.0 ± 1.2
FEMALES						
Number of animals examined	5	5	5	1	5	5
Red blood cells (10^4/μL)	812 ± 24	828 ± 44	837 ± 26	803[a]	868 ± 27	836 ± 43
Hemoglobin (g/dL)	15.3 ± 0.5	15.5 ± 0.5	15.6 ± 0.4	14.5[a]	15.8 ± 0.4	14.2 ± 0.7**
Hematocrit (%)	44.6 ± 1.9	45.6 ± 1.5	45.2 ± 1.2	40.5[a]	45.2 ± 1.1	41.1 ± 2.2**
MCV (fL)	54.9 ± 2.2	55.1 ± 1.6	54.1 ± 1.7	50.4[a]	52.1 ± 1.6	49.2 ± 2.1*
MCH (pg)	18.8 ± 0.5	18.8 ± 0.6	18.6 ± 0.3	18.1[a]	18.3 ± 0.5	17.0 ± 0.6**
MCHC (g/dL)	34.3 ± 0.5	34.0 ± 0.3	34.4 ± 0.6	35.8[a]	35.1 ± 0.5	34.6 ± 0.4
Reticulocytes (%)	9.79 ± 1.26	10.20 ± 1.14	8.67 ± 1.46	4.05[a]	3.00 ± 0.56	4.00 ± 0.90
Platelets (10^4/μL)	124.3 ± 11.6	144.5 ± 9.9*	136.7 ± 9.5	143.0[a]	112.8 ± 14.3	118.8 ± 35.4
White blood cells (10^2/μL)	116.5 ± 34.4	101.9 ± 37.0	102.0 ± 17.4	95.7[a]	82.7 ± 24.7	104.9 ± 31.8
Neutrophils (10^2/μL)	41.0 ± 28.6	24.6 ± 13.8	28.6 ± 7.8	14.2[a]	10.1 ± 4.0	17.5 ± 5.2*
Lymphocytes (10^2/μL)	67.8 ± 14.9	70.2 ± 21.5	64.6 ± 9.0	72.3[a]	68.5 ± 20.7	81.1 ± 32.2
Monocytes (10^2/μL)	5.60 ± 1.39	5.50 ± 2.09	6.86 ± 2.58	8.20[a]	2.82 ± 0.84	5.18 ± 2.28
Eosinophils (10^2/μL)	2.02 ± 0.66	1.54 ± 0.76	1.90 ± 0.32	0.90[a]	1.28 ± 0.38	1.16 ± 0.73
Basophils (10^2/μL)	0.04 ± 0.05	0.06 ± 0.05	0.04 ± 0.05	0.10[a]	0.00 ± 0.00	0.02 ± 0.04
PT (sec)	18.7 ± 1.0	18.0 ± 0.8	17.8 ± 1.1	17.2[a]	17.4 ± 0.9	18.9 ± 4.6
APTT (sec)	20.4 ± 1.0	20.2 ± 1.2	20.7 ± 1.9	21.8[a]	18.9 ± 1.6	25.3 ± 8.6

Values are given as the mean ± S.D.

*Significantly different from the control, $p \leq 0.05$.

**Significantly different from the control, $p \leq 0.01$.

[a]Data from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

TABLE III. Blood biochemical findings in male and female rats administered PFDoA

	Main Group				Recovery Group	
Dose (mg/kg/day)	0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES						
Number of animals examined	5	5	5	5	5	5
Total protein (g/dL)	5.62 ± 0.13	5.54 ± 0.24	5.50 ± 0.16	4.30 ± 0.43**	5.74 ± 0.11	4.70 ± 0.14**
Albumin (g/dL)	2.82 ± 0.12	2.85 ± 0.18	2.85 ± 0.06	2.32 ± 0.30**	2.78 ± 0.14	2.62 ± 0.12
A/G (ratio)	1.01 ± 0.09	1.06 ± 0.06	1.08 ± 0.08	1.17 ± 0.08*	0.95 ± 0.10	1.26 ± 0.12**
Protein fraction (%)						
Albumin	50.2 ± 2.2	51.4 ± 1.4	51.9 ± 1.8	53.8 ± 1.8*	48.5 ± 2.5	55.8 ± 2.5**
Globulin α_1	19.9 ± 3.9	19.9 ± 1.7	21.2 ± 2.3	14.3 ± 2.5*	23.7 ± 2.2	14.4 ± 2.3**
Globulin α_2	8.04 ± 0.80	7.14 ± 0.43	6.92 ± 0.40*	5.82 ± 0.86**	6.70 ± 0.20	6.60 ± 0.22
Globulin β	16.6 ± 1.9	16.3 ± 1.1	15.0 ± 0.8	16.9 ± 0.7	16.2 ± 0.8	16.4 ± 1.5
Globulin γ	5.28 ± 0.88	5.28 ± 0.96	4.92 ± 0.57	9.18 ± 2.40**	4.86 ± 0.59	6.90 ± 0.91**
AST (IU/L)	74.6 ± 11.1	83.0 ± 22.1	103.6 ± 84.8	125.4 ± 56.1	65.6 ± 12.0	69.0 ± 5.7
ALT (IU/L)	31.2 ± 3.1	34.0 ± 5.7	48.2 ± 41.0	53.2 ± 28.0	26.0 ± 6.2	28.0 ± 6.4
ALP (IU/L)	357.2 ± 28.6	366.6 ± 88.4	551.6 ± 95.2**	630.0 ± 72.0**	251.0 ± 30.2	534.4 ± 78.0**
γ-GTP (IU/L)	0.52 ± 0.25	0.60 ± 0.20	0.54 ± 0.15	2.56 ± 2.20	0.58 ± 0.19	1.08 ± 1.70
Total bilirubin (mg/dL)	0.066 ± 0.018	0.044 ± 0.011	0.052 ± 0.022	0.390 ± 0.260**	0.054 ± 0.009	0.080 ± 0.027
Glucose (mg/dL)	166.2 ± 6.9	173.2 ± 19.0	156.8 ± 3.4	122.0 ± 4.6**	180.0 ± 17.0	154.6 ± 28.0
Total cholesterol (mg/dL)	67.0 ± 9.7	44.6 ± 7.6*	40.6 ± 7.8*	53.4 ± 20.0	56.8 ± 8.6	58.2 ± 8.6
Triglycerides (mg/dL)	37.4 ± 14.3	45.2 ± 12.8	33.6 ± 12.0	27.2 ± 5.40	56.6 ± 21.7	19.0 ± 5.50**
BUN (mg/dL)	15.7 ± 0.8	15.6 ± 0.9	16.2 ± 3.5	21.9 ± 1.2**	14.9 ± 1.1	19.7 ± 2.3**
Crea (mg/dL)	0.57 ± 0.03	0.56 ± 0.03	0.54 ± 0.04	0.48 ± 0.05**	0.54 ± 0.04	0.49 ± 0.05
Na (mEq/L)	143.8 ± 0.8	143.8 ± 0.8	143.8 ± 1.3	144.4 ± 2.4	140.6 ± 0.5	140.2 ± 0.8
K (mEq/L)	4.68 ± 0.32	4.86 ± 0.28	4.98 ± 0.22	4.83 ± 0.75	4.74 ± 0.62	5.32 ± 0.38
CI (mEq/L)	109.0 ± 0.7	108.2 ± 1.1	108.8 ± 1.8	110.2 ± 0.8	103.6 ± 1.1	105.4 ± 1.7
Ca (mg/dL)	9.52 ± 0.38	9.62 ± 0.36	9.40 ± 0.32	8.38 ± 0.26**	10.0± 0.05	9.04 ± 0.37**
IP (mg/dL)	6.60 ± 0.27	7.06 ± 0.74	6.64 ± 0.74	7.28 ± 0.36	6.92 ± 0.59	8.12 ± 0.73*
FEMALES						
Number of animals examined	5	5	5	1	5	5
Total protein (g/dL)	6.20 ± 0.23	6.50 ± 0.31	6.16 ± 0.19	5.30[a]	6.20 ± 0.23	4.56 ± 0.77**
Albumin (g/dL)	2.96 ± 0.23	3.34 ± 0.25*	3.26 ± 0.13	2.38[a]	3.53 ± 0.26	2.68 ± 0.43**
A/G (ratio)	0.92 ± 0.09	1.05 ± 0.09	1.13 ± 0.08**	0.81[a]	1.32 ± 0.11	1.45 ± 0.12
Protein fraction (%)						
Albumin	47.7 ± 2.6	51.3 ± 2.2*	52.9 ± 1.7**	44.8[a]	56.9 ± 2.1	59.1 ± 1.9
Globulin α_1	19.9 ± 2.1	18.8 ± 1.6	17.6 ± 1.9	16.3[a]	15.7± 1.5	10.6 ± 3.5*
Globulin α_2	7.98 ± 0.61	6.82 ± 0.69*	7.22 ± 0.73	7.80[a]	5.72 ± 0.64	6.54 ± 1.41
Globulin β	19.0 ± 1.5	17.7± 1.6	16.9 ± 0.7	17.2[a]	14.7± 1.0	14.8 ± 2.2
Globulin γ	5.46 ± 1.30	5.32 ± 0.88	5.36 ± 0.89	13.90[a]	7.06 ± 1.60	9.00 ± 3.80
AST (IU/L)	79.4 ± 12.8	66.8 ± 4.7	87.0 ± 48.1	147.0[a]	66.2 ± 8.7	143.6 ± 104.0**
ALT (IU/L)	26.2 ± 4.0	24.4 ± 2.3	28.2 ± 6.2	32.0[a]	27.2 ± 7.5	53.4 ± 61.0
ALP (IU/L)	199.6± 21.6	180.0 ± 62.0	174.6 ± 40.7	499.0[a]	144.4± 44.7	657.4 ± 439.0**
y-GTP (IU/L)	0.62 ± 0.28	0.62 ± 0.36	0.54 ± 0.11	1.50[a]	0.50 ± 0.20	15.4 ± 17.0**
Total bilirubin (mg/dL)	0.048 ± 0.008	0.046 ± 0.009	0.038 ± 0.008	0.070[a]	0.074± 0.011	1.240 ± 2.300
Glucose (mg/dL)	145.0± 23.0	150.4 ± 8.7	160.8 ± 13.0	121.0[a]	140.4± 19.0	115.4 ± 25.0
Total cholesterol (mg/dL)	67.2 ± 19.0	55.6 ± 16.0	45.8 ± 12.0	54.0[a]	69.2 ± 15.0	55.2 ± 22.0
Triglycerides (mg/dL)	26.8 ± 8.9	67.4 ± 86.9	33.0 ± 15.2	19.0[a]	17.4 ± 4.5	21.2 ± 10.0
BUN (mg/dL)	26.52 ± 4.52	22.74 ± 3.84	23.34 ± 2.77	29.70[a]	15.98± 1.04	17.06 ± 4.14
Crea (mg/dL)	0.62 ± 0.03	0.60 ± 0.01	0.59 ± 0.03	0.48[a]	0.60 ± 0.02	0.52 ± 0.03**
Na (mEq/L)	139.0± 2.0	139.2 ± 1.9	138.2 ± 2.0	139.0[a]	140.2± 1.3	139.6 ± 0.9
K (mEq/L)	4.97 ± 0.17	5.23 ± 0.20	5.11 ± 0.28	5.44[a]	4.50 ± 0.56	4.97 ± 0.30
CI (mEq/L)	103.6± 1.3	104.4 ± 1.5	103.4 ± 1.1	106.0[a]	104.8± 0.8	104.8 ± 0.8
Ca (mg/dL)	10.5 ± 0.5	10.6 ± 0.5	10.4 ± 0.3	9.70[a]	10.3 ± 0.2	9.2 ± 0.8**
IP (mg/dL)	8.96 ± 0.71	8.42 ± 0.40	8.76 ± 0.86	7.80[a]	6.62 ± 0.51	7.82 ± 1.40

Values are given as the mean ± S.D.

*Significantly different from the control, $p \leq 0.05$.

**Significantly different from the control, $p \leq 0.01$.

[a]Data from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

significantly increased at 0.5 and 2.5 mg/kg/day in males. Significant increases in T-Bil and BUN and decreases in glucose, Crea, and Ca were noted in the 2.5 mg/kg/day group. Furthermore, T-Cho was significantly decreased at 0.1 and 0.5 mg/kg/day in males. Significant decreases in TP, the α_1-globulin fraction ratio, TG, and Ca, and significant increases in albumin and the γ-globulin fraction ratios, ALP, BUN, and IP were observed in males of the 2.5 mg/kg/day group at the end of recovery periods.

In females in the main group, many changes, including decreases in TP, albumin, and Crea, and increases in AST, ALP, γ-GTP, and T-Bil, were found in one female in the 2.5 mg/kg/day group (Table III). The albumin fraction ratio was significantly increased in females in the 0.1 and 0.5 mg/kg/day groups. Significant decreases in TP, albumin, α_1-globulin fraction ratio, Crea, and Ca, and significant increases in AST, ALP, and γ-GTP were observed in females in the recovery group at 2.5 mg/kg/day.

Necropsy Findings

In the main group, gross observations at necropsy revealed atrophy of the thymus in 3/7 males and 10/12 females, atrophy of the spleen in 3/7 males and 5/12 females, yellowish brown discoloration of the liver in 7/7 males and 6/12 females, black patches on the glandular stomach mucosa in 4/12 females, pancreas edema in 2/12 females, small-sized epididymis in 4/7 males, small-sized seminal vesicle in 4/7 males, pale yellow discoloration of the subcutis of general skin in 1/12 females, and atrophy of the lateral great muscle in 3/7 males at 2.5 mg/kg/day. In addition, opacity of the eye ball, granular surface on the forestomach mucosa, thickening of the forestomach mucosa, diverticulum of the ileum, yellow mass of the epididymis cauda, and yellow patches on the epididymis corpus were each observed in 1 male given 2.5 mg PFDoA/kg/day. Blood retention was identified in the uterus in five of seven females that died or were euthanized due to a moribund condition at the end of the gestation period in the 2.5 mg/kg/day group.

Yellowish brown or yellow discoloration in the liver (5/5 males and 4/5 females) and enlarged liver (4/5 males and 2/5 females), atrophy of the thymus (1/5 males and 1/5 females), spleen atrophy (1/5 females), edema of the submandibular gland and sublingual gland (1/5 females), atrophy of the lateral great muscle (1 female), pale yellow discoloration of the subcutis of the general skin (1 female), and small-sized seminal vesicle (1 male) were observed in the 2.5 mg/kg/day recovery group.

Organ Weight

The relative weight of the liver in males was significantly higher at 0.5 mg/kg/day and 2.5 mg/kg/day at the end of the administration period (Table IV). The absolute and relative weights of the thymus and absolute weights of the kidney, spleen, heart, pituitary gland, thyroid, adrenal gland, and epididymis in males given 2.5 mg PFDoA/kg/day were significantly decreased while the relative weights of the kidney and brain were significantly increased.

In females in the main group, many changes, including an increase in the relative liver weight and decreases in the absolute and relative weights of the spleen and thymus, were found in one surviving female given 2.5 mg/kg/day (Table IV). A significant increase in the relative liver weight and significant decreases in absolute weights of spleen, heart, pituitary gland, and thymus were observed at the end of the administration period in females given 0.5 mg PFDoA/kg/day.

Most changes observed in males given 2.5 mg PFDoA/kg/day at the end of the administration period remained after the 14-day recovery period (Table IV). In addition, the relative weights of testes and epididymides were significantly increased in the male recovery group. Significant increases in the relative weights of the brain, liver, and kidney and significant decreases in the absolute and relative weights of the ovary and absolute weights of the heart, pituitary gland, and adrenal gland were observed in females in the 2.5 mg/kg/day recovery group.

Histopathological Findings

Upon completion of the administration period, hepatic changes were observed in all males and females given 2.5 mg PFDoA/kg/day (Table V). They included diffuse hepatocyte hypertrophy, peribiliary inflammatory cellular infiltration, single cell necrosis of hepatocytes, focal necrosis, bilirubin deposition, and bile duct proliferation. The incidence of diffuse hepatocyte hypertrophy in both sexes and single cell necrosis of hepatocytes in females was significantly higher in the 2.5 mg/kg/day group. Focal necrosis was also detected in two females given 0.5 mg PFDoA/kg/day.

Histopathological changes were observed not only in the liver, but also in various organs in the 2.5 mg/kg/day group (Table V). A reduction in zymogen granules was seen in the pancreas in both sexes, and the frequency of the reduction in males was significantly increased. The incidence of edema of the interstitium in the pancreas was significantly higher in females in the 2.5 mg/kg/day group than in the controls. Atrophic changes were observed in the spleen, thymus, adrenal gland, muscle fibers, and male reproductive organs. The incidences of adrenal cortex atrophy in males and thymus cortex atrophy in females were significantly increased. Furthermore, a decrease in hematopoiesis in the bone marrow, ulcers in the glandular stomach, and erosion, hyperkeratosis, squamous cell hyperplasia, and inflammatory cellular infiltration, edema, and fibrosis of submucosa in the forestomach were noted.

As for male reproductive organs, atrophy of the glandular epithelium was observed in the prostate, seminal vesicle, and coagulating gland (Table V). In addition, cell debris at

TABLE IV. Organ weights of male and female rats administered PFDoA

		Main Group				Recovery Group	
Dose (mg/kg/day)		0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES							
Number of animals examined		5	5	5	5	5	5
Liver	(g)	12.0 ± 1.3	13.5 ±2.1	14.7 ±2.6	13.6 ±3.1	13.1 ± 1.2	15.2 ±2.8
	(%)[a]	2.51 ±0.14	2.67 ±0.21	3.00 ± 0.30*	4.30 ±0.27**	2.56 ±0.18	3.94 ±0.61**
Kidney[b]	(g)	3.01 ±0.27	3.28 ±0.22	3.26 ± 0.43	2.39 ±0.45*	3.15 ±0.14	2.76 ±0.21**
	(%)[a]	0.628 ±0.032	0.656 ±0.052	0.670 ± 0.058	0.760 ± 0.060**	0.614 ±0.029	0.718 ± 0.050**
Spleen	(g)	0.784 ±0.102	0.802 ±0.091	0.724 ± 0.123	0.488 ±0.155**	0.862 ±0.074	0.650 ± 0.025**
	(%)[a]	0.166 ±0.015	0.158 ±0.015	0.148 ± 0.013	0.152 ±0.029	0.170 ±0.019	0.170 ±0.010
Heart	(g)	1.480 ±0.180	1.520 ±0.150	1.390 ± 0.160	0.864 ±0.180**	1.480 ±0.094	1.110 ±0.110**
	(%)[a]	0.312 ±0.041	0.300 ±0.016	0.288 ± 0.029	0.274 ±0.013	0.292 ±0.023	0.286 ±0.011
Brain	(g)	2.21 ±0.13	2.14 ±0.10	2.16 ± 0.03	2.10 ±0.12	2.21 ±0.01	2.15 ±0.08
	(%)[a]	0.460 ±0.020	0.428 ±0.033	0.446 ± 0.034	0.688 ±0.160**	0.430 ±0.032	0.562 ±0.049**
Pituitary gland	(mg)	12.3 ±0.8	12.3 ±0.6	13.0 ±2.1	7.5 ± 1.6**	12.2 ±2.2	9.4 ± 1.5*
	(10^{-3}%)[a]	2.57 ±0.05	2.46 ±0.27	2.68 ± 0.51	2.37 ±0.30	2.40 ±0.43	2.44 ±0.33
Thymus	(mg)	325 ±97	442 ±75	328 ± 86	133 ±79**	382 ± 114	239 ±62*
	(10^{-3}%)[a]	67.6 ± 19.9	87.8 ± 12.7	67.0 ± 15.3	39.1 ± 18.9*	75.3 ±24.2	62.5 ± 19.0
Thyroid	(mg)	23.3 ±3.7	23.7 ± 1.5	22.3 ± 4.3	14.4 ±4.1**	22.4 ±3.7	16.2 ±3.8*
	(10^{-3}%)[a]	4.87 ±0.81	4.73 ±0.30	4.61 ± 1.00	4.53 ±0.77	4.38 ±0.61	4.22 ±0.96
Adrenal gland	(mg)	65.0 ±8.5	68.4 ±9.8	63.4 ± 17.0	38.4 ±5.9**	62.4 ±8.6	40.4 ±6.2**
	(10^{-3}%)[a]	13.5 ± 1.3	13.6 ± 1.9	13.2 ±3.9	12.5 ±2.9	12.3 ± 1.9	10.5 ± 1.5
Testis[b,c]	(g)	3.36 ±0.37	3.34 ±0.23	3.39 ± 0.30	2.81 ±0.65	3.43 ±0.28	3.39 ±0.42
	(%)[a]	0.671 ±0.054	0.647 ±0.049	0.677 ± 0.080	0.839 ±0.160	0.672 ±0.077	0.882 ±0.110**
Epididymis[b,c]	(g)	1.33 ±0.17	1.35 ±0.12	1.32 ± 0.10	0.90 ±0.31**	1.43 ±0.090	1.23 ±0.13*
	(%)[a]	0.267 ±0.033	0.263 ±0.024	0.263 ± 0.020	0.266 ±0.073	0.280 ±0.025	0.316 ±0.017*
FEMALES							
Number of animals examined		5	5	5	1	5	5
Liver	(g)	9.9 ±0.6	10.8 ±0.9	10.9 ± 0.9	10.9[d]	7.6 ±0.4	8.7 ± 1.8
	(%)[a]	3.23 ±0.19	3.43 ±0.11	3.70 ± 0.22**	4.52[d]	2.45 ±0.11	3.61 ±0.35**
Kidney[b]	(g)	2.19 ±0.17	2.06 ±0.22	1.97 ± 0.09	1.86[d]	2.02 ±0.08	1.99 ±0.29
	(%)[a]	0.720 ±0.093	0.656 ±0.038	0.668 ± 0.022	0.780[d]	0.656 ±0.022	0.836 ± 0.063**
Spleen	(g)	0.780 ±0.052	0.750 ±0.046	0.674 ± 0.081*	0.400[d]	0.560 ±0.100	0.522 ±0.110
	(%)[a]	0.256 ±0.040	0.240 ±0.019	0.232 ± 0.023	0.170[d]	0.182 ±0.035	0.218 ±0.035
Heart	(g)	1.040 ±0.090	1.080 ±0.054	0.928 ± 0.048*	0.770[d]	0.994 ±0.036	0.796 ±0.160*
	(%)[a]	0.342 ±0.022	0.346 ±0.018	0.316 ± 0.017	0.320[d]	0.324 ±0.011	0.334 ±0.034
Brain	(g)	2.02 ±0.11	2.00 ±0.06	2.00 ± 0.02	2.14[d]	2.10 ±0.09	1.99 ±0.09
	(%)[a]	0.662 ±0.057	0.640 ±0.041	0.680 ± 0.025	0.890[d]	0.682 ±0.028	0.852 ±0.150*
Pituitary gland	(mg)	17.3 ± 1.7	16.0 ±2.3	14.1 ± 1.9*	12.0[d]	17.7 ±2.2	10.4 ±4.5*
	(10^{-3}%)[a]	5.67 ±0.71	5.13 ±0.87	4.80 ± 0.60	5.00[d]	5.75 ±0.75	4.27 ± 1.3
Thymus	(mg)	310 ±27	297 ± 102	264 ± 16**	66.0[d]	298 ±70	241 ± 137
	(10^{-3}%)[a]	101.0 ± 11.2	93.7 ±27.8	89.5 ±3.8	27.5[d]	96.9 ±23.9	95.9 ±50.3
Thyroid	(mg)	17.8 ±4.7	18.8 ±3.7	18.3 ±4.2	11.0[d]	15.5 ± 1.5	13.1 ±5.6
	(10^{-3}%)[a]	5.74 ± 1.10	6.01 ± 1.20	6.21 ± 1.30	4.58[d]	5.02 ±0.47	5.32 ± 1.70
Adrenal gland	(mg)	84.2 ± 13.0	77.2 ± 8.6	79.4 ± 5.0	53.0[d]	70.8 ±9.2	47.4 ± 12.0**
	(10^{-3}%)[a]	27.5 ± 4.3	24.7 ± 3.3	27.0 ± 1.5	22.1[d]	23.0 ±3.1	19.9 ± 3.3
Ovary[b]	(mg)	115.4 ± 14.3	114.0 ± 21.1	109.6 ± 10.0	119.0[d]	100.4 ±17.4	56.0 ± 7.3**
	(10^{-3}%)[a]	37.6 ± 3.3	36.2 ± 4.7	37.2 ± 3.1	49.6[d]	32.6 ±6.0	23.7 ± 3.1*

Values are given as the mean ± S.D.

*Significantly different from the control, at $p \leq 0.05$.

**Significantly different from the control, at $p \leq 0.01$.

[a]Ratio of absolute organ weight to body weight on the necropsy day (relative organ weight).

[b]Values are represented as the total weights of the organs on both sides.

[c]Organ weight was measured for all animals (number of examined animals: 7 at 0 and 2.5 mg/kg/day and 12 at 0.1 and 0.5 mg/kg/day in the main group, and 5 at 0 and 2.5 mg/kg/day in the recovery group).

[d]Data from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

TABLE V. Histopathological findings in male and female rats administered PFDoA

		Main Group				Recovery Group	
Dose (mg/kg/day)	Grade	0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES							
Number of animals examined		7	12	12	7	5	5
Forestomach							
Erosion	+	0	0	0	1	0	0
Hyperkeratosis	+	0	0	0	2	0	0
Hyperplasia in squamous cells	+	0	0	0	2	0	0
Infiltration of inflammatory cells in the submucosa	+	0	0	0	1	0	0
Fibrosis of the submucosa	+	0	0	0	2	0	0
Pancreas							
Decrease in zymogen granules	+	0	0	0	5]**	0	1
	++	0	0	0	1]	0	0
Liver							
Deposition of bilirubin	+	0	0	0	1	0	2
Peribiliary infiltration of inflammatory cells	+	0	0	0	4	0	4*
Single cell necrosis of hepatocytes	+	0	0	0	1	0	0
	++	0	0	0	1	0	0
Focal necrosis	+	0	0	0	3	0	0
	++	0	0	0	1	?0	0
Diffuse hepatocyte hypertrophy	+	0	0	0	5*	0	3
Centrolobular hepatocyte hypertrophy	+	0	0	0	0	0	2
Periportal fatty changes	+	0	0	0	1	0	0
Fatty changes in midzonal	+	0	0	0	1	0	0
Fatty changes in diffuse	+	0	0	0	1	0	0
Testis							
Cell debris (Stage VII–VIII)	+	0	0	0	1	0	0
Decrease in elongated spermatids (Stage XII–XIV)	+	0	0	0	2	0	0
Epididymis							
Decrease in spermatozoa	+	0	0	0	2	0	1
	++	0	0	0	1	0	0
	+++	0	0	0	1	0	0
Cell debris in the lumen	+	0	0	0	2	0	0
	++	0	0	0	1	0	0
Spermatic granuloma	+	0	0	0	2	0	0
Prostate							
Glandular epithelium atrophy	+	0	0	0	3	0	1
Fibrosis in the interstitium	+	0	0	0	1	0	0
Seminal vesicles							
Glandular epithelium atrophy	+	0	0	0	3	0	1
	++	0	0	0	1	0	0
Coagulating gland							
Glandular epithelium atrophy	+	0	0	0	3	0	1
	++	0	0	0	1	0	0
Spleen							
White pulp atrophy	+	0	0	0	1	0	0
Red pulp atrophy	+	0	0	0	3	0	0
Thymus							
Atrophy of the cortex	+	0	0	0	0	0	1
	++	0	0	0	1	0	0
	+++	0	0	0	2	0	0
Bone marrow							
Decrease in hematopoiesis	+	0	0	0	2	0	0
	++	0	0	0	2	0	0
Adrenal glands							