最 終 報 告 書

C.I.ピグメントブルー29 のラットを用いる
反復経口投与毒性・生殖発生毒性併合試験
（試験番号：03-306）

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要　約

C.I.ピグメントブルー29 の 100，300 および 1000 mg/kg/day を，1 群 12 匹の SD ラットに，交配開始 14 日前から，雄は 42 日間，雌は分娩後哺育 4 日まで経口投与し，本物質の反復投与毒性および生殖発生毒性について検討した。

1．反復投与毒性

動物の臨床観察，感覚反射機能検査，着地開脚幅，握力，自発運動量，体重，摂餌量，尿検査，血液学検査および血液生化学検査において，被験物質の投与による影響は認められなかった。

病理学検査において，組織学的に胃の境界縁付近扁平上皮の軽度過形成が 1000 mg/kg 群の雌雄に認められた。また，雌で前胃の粘膜下織の水腫を伴う例も認められた。この胃の変化は，短期間で回復する可逆的変化であった。

以上の結果から，C.I.ピグメントブルー29 のラットへの反復投与により，胃に対する毒性影響が認められた。無影響量は（NOEL）は雌雄とも 300 mg/kg と結論された。

2．生殖発生毒性

親動物の性周期（雌），交尾成立期間，交尾率，受胎率，妊娠期間，黄体数，着床率，出産率，分娩率，分娩および哺育状態に変化は認められなかった。児動物に対しても，総出産児数，新生児数，性比，出生率，体重，形態および哺育 4 日生存率に，被験物質の投与に起因する変化は認められなかった。

したがって，雌雄親動物の生殖能および児動物の発生に対する無影響量は，いずれも 1000 mg/kg/day と結論された。

緒　言

C.I.ピグメントブルー29は，印刷インキ，塗料，絵具，クレヨン，ゴムおよび合成樹脂の着色剤，その他多くの材料の着色等に広く用いられている化学物質である[1]。本物質の毒性について，ラットおよびマウスの経口投与におけるLD_{50}値は10000 mg/kg以上[2]，またラットにおいては2000mg/kgを越える[3]との報告があり，急性毒性は弱いことが知られている。しかしながら，反復投与毒性や生殖・発生毒性などに関する報告は見当たらない。本試験は，OECDの高生産量既存化学物質安全点検事業の一環として実施したものである。

目　的

C.I.ピグメントブルー29をラットに反復経口投与し，本物質の反復経口投与毒性および生殖発生毒性を検討する。

材料および方法

1．被験物質

被験物質であるC.I.ピグメントブルー29（CAS No. 57455-37-5）は，水および有機溶媒に不溶，臭素水に可溶な青色粉末で，試験には，

から提供されたロット番号　　（組成 SiO_2：39.60%，Al_2O_3：23.76%，Fe_2O_3：0.45%，S：12.08%，Na_2O：22.59%）を冷暗所（2～6℃）密栓下で保管し，使用した。用いた被験物質は投与終了後に分析し，使用期間中安定であったことを確認した（Appendix 1）。本物質の特性は，Appendix 1に示す。

C.I.ピグメントブルー29は水および食物油に不溶であったことから，投与液は1w/v%メチルセルロース水溶液（メチルセルロース100cp，ロット番号KLP3708，和光純薬工業株式会社；局方精製水，ロット番号181382，共栄製薬株式会社）を溶媒とし，所定の投与用量となる濃度の懸濁液に調製した。調製した投与液は，1日の使用量ごとに小分けし，使用時まで冷所（2～6℃）遮光下で密栓して保管した。冷所遮光下で保管した投与液中の被験物質は，少なくとも9日間は安定であることが確認された（Appendix 2）ので，調製後9日以内に使用した。初回に調製された投与液について，所定の濃度で調製されていることを確認した（Appendix 3）。

2．動物および飼育条件

動物は，SD 系［Crj：CD(SD)IGS］ラットを用いた。ラットは，日本チャールス・リバー株式会社 厚木飼育センター（神奈川県厚木市下古沢 795）から 8 週齢のものを搬入（雄 57 匹，雌 67 匹）し，13 日間試験環境に馴化させた。馴化期間中に検疫および雌については 10 日間の性周期観察も併せて行い，発育および一般健康状態が良好で，雌では性周期に異常の認められなかったものについて，投与開始前日に体重を測定し，体重分布の中央値に近い雄は 48 匹，雌は 58 匹を選び，10 週齢で試験に用いた。1 群の動物数は雌雄各 12 匹とし，雌についてはさらに対照群と最高用量群の回復群として各 5 匹からなる 2 群の衛星群を設け，無作為抽出法により群分けを行った。なお，雌の回復群については交配を行わなかった。雄の回復群については，投与 42 日に対照群と最高用量群の中から無作為抽出法によりそれぞれ 5 匹を選別し，回復群とした。投与開始時の平均体重（体重範囲）は，雄 382（354～427）g，雌 238（208～272）g であった。ラットは，温度 22±3℃，湿度 55±10%，換気回数 10 回以上／時（オールフレッシュエアー方式），照明 12 時間／日（午前 7 時点灯，午後 7 時消灯）に設定したバリアーシステム動物室（第 1 室）で，個体別にステンレス製金網ケージ〔260W×380D×180H(mm)〕に収容し，これをステンレス製 5 段のラックに配置して飼育した。ただし，交尾の成立した雌は，巣作り材料（ホワイトフレーク，日本チャールス・リバー株式会社，ロット番号 16.3.10）を入れたポリカーボネート製ケージ〔265W×426D×200H(mm)〕に収容し，分娩後は児動物と同居させた。飼料（固型飼料ラボ MR ストック，日本農産工業株式会社，ロット番号 040462-4，040576-4，040861）および飲料水（孔径 1μm のカートリッジフィルターで濾過後紫外線照射した殺菌水道水）は，それぞれ給餌器および自動給水装置，または給水瓶（ポリカーボネートケージの場合）により，自由に摂取させた。

動物の個体識別は，ラックおよびケージへの標識札の貼付，並びに耳パンチ法により行った。

飼育期間中，動物室の温度は 21.6～24.4℃，湿度は 45～59%の範囲で推移（Appendix 4）し，また飼料，飲料水および巣作り材料の汚染物質の分析結果（Appendices 5，6，7）は，いずれも当研究所で設定した許容範囲内にあることが確認された。したがって，動物の飼育期間を通じて，試験成績の信頼性に影響を及ぼすと思われる環境要因の変化

は，なかったものと判断された。なお，本試験は「動物の愛護及び管理に関する法律」および「実験動物の飼養及び保管等に関する基準」に準拠して実施した。

3．投与量の設定，試験群の構成および投与方法

投与量は，3 日間および 14 日間反復経口投与による投与量設定試験の結果に基づいて設定した。雌雄各 2 匹のラットに本被験物質の 2000 mg/kg を 3 日間投与し，一般状態および体重への影響を調べた結果，雌雄ともに青色着色便が認められた以外に，変化は認められなかった。次に，1 群雌雄各 4 匹のラットに本被験物質を 0，30，100，300 および 1000 mg/kg/day で 14 日間反復投与し，一般状態の観察，体重および摂餌量の測定，血液学および血液生化学検査，剖検並びに器官重量の測定を行った結果，投与に起因する毒性影響は認められなかった。

したがって，本試験における投与量については，試験法ガイドラインにおける上限量の 1000 mg/kg/day を最高用量とし，以下，300 および 100 mg/kg/day の計 3 用量を設定した。

試験群の構成は，①溶媒投与群（以下，対照群），②被験物質の 100 mg/kg/day 投与群（100 mg/kg 群），③同 300 mg/kg/day 投与群（300 mg/kg 群），④同 1000 mg/kg/day 投与群（1000mg/kg 群）の 4 群とした。

投与方法は，投与液量を体重 1kg 当たり 10 mL とし，テフロン製胃ゾンデを装着した注射筒を用いて，投与液を胃内に投与した。対照群には，媒体として用いた 1w/v% メチルセルロース水溶液を同様に投与した。各個体の投与液量は，至近日の測定体重を基に算出した。投与期間は，雌雄とも交配開始 14 日前から，雄は 42 日間，雌は交配および妊娠期間を経て分娩後の哺育 4 日まで，最短 42 日～最長 46 日間，1 日 1 回，午前中（9：03～12：00）に投与した。ただし，雌の回復群は，雄と同様に 42 日間投与した。

4．観察および検査

1) 親動物に関する項目

親動物について，次の項目を観察あるいは検査した。なお，感覚反射機能検査，着地開脚幅，握力，自発運動量，尿検査，血液学検査，血液生化学検査，器官重量および病

理組織学検査については，各群から無作為抽出法により雌雄各5匹を選び，検査の対象とした。

(1)一般状態観察

投与期間中毎日，動物の生死，外観，行動等について観察した。観察時期について，投与量設定試験で変化が認められなかったことから，投与後約1時間に観察を行った。さらに，朝夕2回は，動物の生死や瀕死状態の有無について確認した。また，妊娠，出産，哺育の状態については，注意深く観察した。

(2)詳細な臨床観察

投与開始前日およびその後は週1回，動物をケージから取り出す時およびケージ外のアルミ製オープンフィールド（370W×560D×40Hmm）で，ケージからの出し易さ，ケージから出す時の扱い易さ，体躯緊張（弛緩～強直），皮膚（色），毛並み，立毛，眼分泌物，眼瞼閉鎖状態，眼球突出，流涙，口鼻分泌物（汚れ），流涎，下腹部被毛の尿による汚れ，肛門周囲の便による汚れ，発声，呼吸，姿勢，痙攣，振戦，探索行動（覚醒度），警戒性，自発運動（活動性），歩行（よろめき），異常行動（自咬，後ろ向き歩行等），常同（過度の毛繕い，反復旋回運動等），意識不全（混迷，カタレプシー，昏睡），四肢筋緊張度，排尿および排糞について観察し，認められた変化を評点（Appendix 8）で記録した。動物には無作為化法で観察番号を付け，観察者以外の者が群や動物番号を表示したケージの標識札を観察番号のみ表示した標識札に替え，観察者は観察番号順に観察を行うことにより，投与内容が不明な状態で観察した。

(3)感覚反射機能検査

雄は最終投与日，雌は哺育期間中に1回，また回復群の雌雄は最終投与日および回復期間終了日に，聴覚反応（ピンセットで軽くケージを叩く音に対する驚愕反応），視覚反応（顔面に棒を近づけた場合の接近反応），触覚反応（腰部に触れた場合の反応），耳介反射（耳介に触れた場合の耳介の反射），痛覚反応（尾根部をピンセットで摘んだ場合の逃避，発声等の反応），瞳孔反射（暗所から急に明るい場所に移した時の瞳孔の反射），同側屈筋反応（後肢の足趾をピンセットで摘んだ場合の屈筋の反応），眼瞼反射（眼瞼に接触した場合の眼瞼の反応）および正向反射（面上で動物を背臥位にした場合の正常姿勢にもどる反射）を調べ，認められた反応を評点（Appendix 9）で記録した。

(4)着地開脚幅，握力および自発運動量

雄は投与 41 日，雌は哺育期間中に 1 回，また回復群の雌雄は最終投与日および回復 13 日に，雄は 30 分間および 60 分間，雌は 30 分間の自発運動量（自発運動量測定装置，SUPERMEX，室町機械株式会社，動物が発する遠赤外線をセンサーが感知し，測定装置内の区画間の間における移動回数を測定），前肢および後肢の握力（ラット・マウス用握力測定装置，MK-380R/FR，室町機械株式会社）並びに着地開脚幅（足趾に墨を塗り，30 cmの高さから落とした時の足跡の幅）を測定した。回復群の雌の自発運動量は，60 分間測定した。

(5)体重および摂餌量

体重は，雄については投与 1（投与開始日，投与直前），7，14，21，28，35 および 42 日並びに回復 7 および 14 日，雌は投与 1，7 および 14 日，妊娠 0，7，14 および 20 日並びに哺育 0 および 4 日に，また，衛星群として設けた雌の回復群については交配を行わないため，雄と同じ日に測定した。さらに，雌雄とも屠殺日に測定した。摂餌量は,体重測定日に合わせて,ケージごとに翌日までの 24 時間飼料消費量を測定した。ただし，摂餌量の最終測定日は，雄では投与 41 日，雌では哺育 3 日，回復群では回復 13 日とした。交配期間中の投与 21 および 28 日には，交尾の成立していない雌雄の摂餌量は測定しなかった。

(6)雌の性周期検査

雌について，馴化・検疫期間に引き続き，交配前の 2 週間を経て交配期間の交尾が確認されるまで，Giemsa 染色による膣垢塗抹標本を作製し，鏡検により性周期段階の判定を行った。

(7)交配および分娩状態観察

交配前 2 週間の投与を終了（投与 15 日の午後）した雌雄を同一群内で 1 対 1 の組み合わせを作り，2 週間を限度として交尾が確認されるまで連続同居させた。交配期間中は毎朝一定時刻（9：30 頃）に交尾の確認を行い，交尾率(%) [(交尾動物数／同居動物数)×100]を算出した。交尾は，膣栓形成あるは膣垢中の精子の有無により確認し，確認された日を妊娠 0 日とした。分娩状態の観察も同じ時刻に行い，分娩の完了が確認された日を哺育 0 日とした。交配および分娩の観察結果から，各群について，受胎率(%) [(受胎雌数／交尾成立雌数)×100]，妊娠期間（妊娠 0 日から分娩の完了が確認された日

までの日数）および出産率(%)[(生児出産雌数／妊娠雌数)×100]を算出した。

(8)雄の尿検査

雄について，投与 40 日および回復群については回復 12 日に，動物を約 3 時間代謝ケージに収容し，得られた尿について，外観の観察，試験紙法（マルティスティックス，バイエル・三共株式会社）による pH，潜血，タンパク，糖，ケトン体，ビリルビンおよびウロビリノーゲンの定性的検査並びに沈渣の検査（URI-CELL 液，ケンブリッジケミカルプロダクト社，で染色して鏡検）を行った。さらに，18 時間収容して得られた尿について，尿量，比重（屈折計，エルマ光学株式会社）並びにナトリウムおよびカリウム（電解質自動分析装置，NAKL-132，東亜電波工業株式会社）を測定した。

(9)血液学検査

最終投与の翌日あるいは回復期間終了の翌日の解剖直前に，エーテル麻酔下で開腹して腹大動脈より採血した。動物は前日の午後 5 時より除餌し，水のみを給与した。採取した血液は 3 分割し，その一部は EDTA-2K で凝固阻止処理し，多項目自動血球計数装置（E-4000，シスメックス株式会社）により，赤血球数（電気抵抗検出方式），血色素量（ラウリル硫酸ナトリウム-ヘモグロビン法），ヘマトクリット値（パルス検出方式），平均赤血球容積，平均赤血球血色素量，平均赤血球血色素濃度（以上，計算値），白血球数および血小板数（以上，電気抵抗検出方式）を，また，塗抹標本を作製して網状赤血球数（Brilliant cresyl blue 染色して鏡検）および白血球百分率（May-Giemsa 染色して鏡検）を測定した。また，血液の一部を 3.8%クエン酸ナトリウム液で凝固阻止処理して血漿を得，血液凝固自動測定装置（KC-10A，米国アメルング社）により，プロトロンビン時間（Quick 一段法）および活性化部分トロンボプラスチン時間（エラヂン酸活性化法）を測定した。

(10)血液生化学検査

採取した血液の一部から血清を分離し，生化学自動分析装置（JCA-BM8 型クリナライザー，日本電子株式会社）により，総タンパク（ビューレット法），アルブミン（BCG 法），A/G 比（計算値），血糖（GluK[1]-G-6-PDH[2] 法），総コレステロール（酵素法，CES[3]-COD[4]-POD[5] 系），トリグリセライド（酵素法，LPL[6]-GK[7]-GPO[8]-POD[5] 系），総ビリルビン（ジアゾ法），尿素窒素（ウレアーゼ・UV 法），クレアチニン（Jaffe 法），AST，ALT，ALP（以上，JSCC[9] 法），γ-GTP（SSCC 法[10]），LDH（SFBC 法

[11])，ChE(BTC[12]-DTNB[13]法)，カルシウム（OCPC 法）および無機リン（酵素法，PNP[14]-XOD[15]-POD[5]系）を，また電解質自動分析装置（NAKL-132，東亜電波工業株式会社）により，ナトリウム，カリウムおよび塩素（以上，イオン電極法）を測定した。

1) グルコキナーゼ，2) グルコース-6-リン酸脱水素酵素，3) コレステロールエステラーゼ，4) コレステロールオキシダーゼ，5) ペルオキシダーゼ，6) リポプロテインリパーゼ，7) グリセロールキナーゼ，8) L-α-グリセロリン酸オキシダーゼ，9) 日本臨床化学会，10) スカンジナビア臨床化学会，11) フランス臨床生物学会，12) ブチリルチオコリン，13) 5，5'-ジチオビス-2-ニトロ安息香酸，14) プリンヌクレオシドホスフォリラーゼ，15) キサンチンオキシダーゼ

(11)剖検および器官重量

雄の計画屠殺動物は投与 42 日の翌日，雌は哺育 5 日，また，回復群については回復 14 日の翌日に，それぞれエーテル麻酔下で放血屠殺し，体表，開口部粘膜および内部諸器官を肉眼的に観察した。また，各群雌雄各 5 匹の肝臓，腎臓，副腎，胸腺，脾臓，脳，心臓，下垂体，甲状腺および精嚢並びに全ての雄の精巣および精巣上体を秤量（絶対重量）し，屠殺日の体重に基づいて対体重比（相対重量）を算出した。なお，対器官は左右を一括して，下垂体および甲状腺は固定後に秤量した。雌については，卵巣の黄体数および子宮の着床数を調べ，着床率(%) [(着床数／黄体数)×100]を算出した。

(12)病理組織学検査

全例について下記器官を採取し，10%中性リン酸緩衝ホルマリン液（精巣，精巣上体はブアン液で前固定）で固定し，保存した。

（脳，下垂体，甲状腺，胸腺，気管・肺（固定液を注入後浸漬），胃，腸，心臓，肝臓，脾臓，腎臓，副腎，膀胱，精巣，精巣上体，前立腺，精嚢，卵巣，子宮，脊髄（頸部，胸部，腰部），坐骨神経，骨髄（大腿骨），リンパ節（頸部リンパ節，腸間膜リンパ節），乳腺，その他肉眼的異常部位）

病理組織学検査は，対照群および 1000 mg/kg 群の雌雄各 5 匹のこれら保存器官について実施した。精巣については，精子形成サイクル検査（ステージII・III，V，VIIおよびXII）も行った。その結果，被験物質の投与による影響が胃に認められたので，回復群を含むその他の群については，雌雄各 5 匹の胃について検査した。各群の肉眼的異常

部位は全例について検査した。検査は，常法に従ってパラフィン切片を作製し，H·E 染色を施して鏡検した。また，精子形成サイクル検査のために精巣の PAS 染色標本も作製した。

2) 新生児に関する項目

(1)産児数, 性比および外表観察

分娩完了の確認後，各腹の産児数（生産児と死亡児の合計）を調べ，分娩率(%) [(総出産児数／着床数)×100]を，また肛門と生殖突起の長短により性別を判定し，群ごとの性比を算出した。さらに，新生児について，口腔内を含む外表の異常を観察した。

(2)一般状態観察

毎日，一般状態および生死を確認し，出生率(%) [(出産確認時生児数／総出産児数)×100]および哺育4日生存率(%) [(哺育4日の生児数／出産確認時生児数)×100]を算出した。

(3)体重

新生児について，哺育0および4日に，雌雄別に腹ごとの体重を測定し，1匹当たりの平均体重を算出した。

(4)病理学検査

死亡例はその都度，生存例は哺育4日にエーテル麻酔下で放血死させ，胸部および腹部における主要器官について，肉眼的に観察した。

5．統計解析

得られた平均値あるいは頻度について，対照群との有意差（危険率5%以下）を，次の方法で検定した。なお，出産児に関するデータは，1腹の平均を1標本とした。

(1)　パラメトリックデータ

多群間の比較については，Bartlett の分散検定を行った。分散が一様な場合は一元配置の分散分析を行い，その結果有意差を認めた場合，Dunnett 法により対照群に対する各群の比較検定を行った。分散が一様でない場合は，ノンパラメトリックデータに用いる検定法に従った（体重，体重増加量，摂餌量，着地開脚幅，握力，自発運動量，尿検査における定量的データ，血液学検査データ，血液生化学検査データ，器官重量，黄体数，着床数，交尾成立期間，妊娠期間，産児数，生児数，死亡児数等)。2 群間の比較

については，F 検定を行い，その結果分散が一様な場合は Student の t 検定を，分散が一様でない場合は Aspin-Welch の t 検定を行った。

(2)　ノンパラメトリックデータ

多群間の比較については，Kruskal-Wallis の順位検定を行い，その結果有意差を認めた場合，Dunnett 型の検定法により対照群と各群を比較した（白血球百分率，尿検査における定性的データ，着床率，出生率，分娩率，性周期，新生児生存率等）。2 群間の比較については，Mann-Whitney の U 検定を行った。

(3)　カテゴリカルデータ

Fisher の直接確率法を用いた（一般状態の観察，詳細な臨床観察，感覚反射機能検査，剖検および病理組織学検査における異常例の発現率，交尾率，受胎率，出産率，児動物の性比等）。

結　果

1．反復投与毒性

1) 一般状態および死亡（Tables 1～4，Appendices 10～13）

被験物質と同色の青色着色便が，被験物質投与の全ての群で，投与2日以降，最終投与日まで認められ，青色の程度は用量相関的に増強する傾向にあった。死亡は，認められず，健康状態の異常が伺われる一般状態の変化も認められなかった。回復期間では，便の色調変化は認められなかった。

2) 詳細な臨床観察（Tables 5，6，Appendices 14，15）

投与期間中および回復期間中の観察とも，被験物質の投与に起因する変化は認められなかった。投与2週の検査で，ハンドリング時に一時的な発声を示す個体数の有意な高値が100mg/kg群の雌に認められたが，変化に用量相関性は認められず，また，他の検査時点ではこのような変化は認められなかったことから，被験物質の投与とは無関係な偶発的変化と判断された。

3) 感覚反射機能検査（Tables 7，8，Appendices 16，17）

投与期間中および回復期間中の検査において，変化は認められなかった。

4) 着地開脚幅，握力および自発運動量（Tables 9，10，Appendices 18，19）

投与期間終了時屠殺動物では投与期間中の検査で，着地開脚幅，握力および自発運動量ともに，変化は認められなかった。回復期間終了時屠殺動物においては，投与期間中の検査で，雄の測定開始30分間の自発運動量に有意な高値が認められたが，60分間の自発運動量には有意差は認められなかった。また，回復期間終了時屠殺動物の回復期間中の測定で，雌雄とも前肢握力に有意な高値が認められた。

5) 体重（Tables 11，12，Appendices 20，21）

投与期間中および回復期間中，体重および体重増加量に有意な変化は認められなかった。

6) 摂餌量（Tables 13，14，Appendices 22，23）

被験物質の投与に起因する変化は認められなかった。100mg/kg 群の雌の妊娠 20 日並びに 300 mg/kg 群の雌の投与 14 日および妊娠 20 日の摂餌量は対照群と比べてやや高値で有意差が認められたが，変化に用量相関性は認められなかった。

7) 雄の尿検査（Table 15，Appendix 24）

投与期間中および回復期間中の検査において，各検査項目に有意な変化は認められなかった。

8) 血液学検査（Tables 16，17，Appendices 25，26，背景データ： Appendices 42，43）

投与期間終了時屠殺動物の検査において，被験物質投与各群の雄の血色素量，ヘマトクリット値および白血球数は対照群と比べて全般的にやや低値を示し，血色素量および白血球数には全ての群で有意差が認められた。また，血色素量やヘマトクリット値の低値に伴い，100mg/kg 群の平均赤血球容積および平均赤血球血色素量並びに 1000mg/kg 群の平均赤血球血色素量は有意な低値を示した。しかしながら，これら各群の平均値はいずれも当研究所の背景データにおける正常範囲の値（Appendices 42 参照）であり，また，用量相関的に変化する傾向も認められなかった。雌においては，各検査項目に有意な変化は認められなかった。回復期間終了時屠殺動物においては，雄で血色素量の有意な高値および白血球百分率における好酸球の比率の有意な低値が認められた。雌では，変化は認められなかった。

9) 血液生化学検査（Tables 18，19，Appendices 27，28，背景データ： Appendices 42，43）

投与期間終了時屠殺動物の検査において，被験物質の投与に起因する変化は認められなかった。100 mg/kg 群の雄のナトリウムは有意な低値を示したが，正常範囲内の値（Appendices 42 参照）であり，変化に用量相関性も認められなかった。回復期間終了時屠殺動物の検査においては，雌のアルブミンおよびカリウムが有意な低値を示したが，これらも正常範囲内の値（Appendices 43 参照）であった。

10) 剖検（Tables 20，21，Appendices 29～31）

被験物質の投与に起因する変化は認められなかった。被験物質の投与とは無関係に，散発的に認められた所見としては，投与期間終了時屠殺動物の検査において，雄で精巣上体の結節（片側性）が 100 mg/kg 群の 1 匹および 300 mg/kg 群の 1 匹に，雌で胸腺の赤色域が 1000 mg/kg 群の 1 匹に，また，回復期間終了時屠殺動物においては，雄で精巣の小型化（両側性）が 1000 mg/kg 群の 1 匹に認められた。

11) 器官重量（Tables 22，23，Appendices 32～35）

投与期間終了時屠殺動物および回復期間終了時屠殺動物とも，各器官の絶対重量および相対重量に有意な変化は認められなかった。

12) 病理組織学検査（Tables 24～26，Appendices 29～31， Photos 1～11）

被験物質の投与に起因する変化として，投与期間終了時屠殺動物で，胃の境界縁付近扁平上皮の軽度過形成が 1000 mg/kg 群の雄 5 匹および雌 4 匹に認められ，雌雄の発現率とも対照群と比べて有意差が認められた。また，雌の 1 匹では前胃粘膜下織の水腫も認められた。回復期間終了時屠殺動物では，胃の境界縁付近扁平上皮過形成が雌の 1 匹にのみ認められ，明らかな回復傾向が認められた。

被験物質の投与とは無関係な変化としては，肺の動脈壁鉱質沈着および泡沫細胞集簇，心臓の心筋変性・線維化，肝臓の巣状壊死，肝細胞脂肪変性（び慢性）および微小肉芽腫，腎臓の皮質リンパ球浸潤，孤立性嚢胞，好塩基性尿細管，硝子円柱および皮質尿細管鉱質沈着，前立腺の間質リンパ球浸潤が対照群と 1000 mg/kg 群あるいは対照群にのみ，低い発現率で認められた。また，雄の腎臓には近位尿細管上皮の硝子滴並びに雌雄の脾臓には髄外造血および褐色色素沈着が高い発現率で認められたが，1000 mg/kg 群における発現率や変化の程度に対照群との差は認められなかった。剖検で，被験物質の投与とは無関係に認められた精巣上体の結節部には精子肉芽腫，小型化した精巣には精細管萎縮および間質細胞過形成が認められた。

2．生殖発生毒性

1）親動物に及ぼす影響

(1) 性周期検査（Table 27，Appendices 36，37）

群分けの翌日から交配前までにおいて，全例が 4～5 日で発情を回帰し，性周期に有意な変化は認められなかった。

(2) 交尾率および受胎率（Table 27，Appendix 38）

交尾不成立および妊娠不成立の例は認められず，交尾成立までに要する日数，交尾率および受胎率に有意な変化は認められなかった。

(3) 黄体数，着床数および着床率（Table 27，Appendix 38）

黄体数，着床数および着床率に有意な変化は認められなかった。

(4) 出産率および妊娠期間（Table 27，Appendix 38）

出産率および妊娠期間に有意な変化は認められなかった。

(5) 分娩および哺育状態（Table 27，Appendix 38）

分娩および哺育状態に異常は認められなかった。

2）新生児に及ぼす影響

(1) 生存性および体重（Table 28，Appendix 39）

一腹当たりの総出産児数，分娩率，哺育 0 日および 4 日の新生児数および体重，出生率，性比並びに哺育 4 日の生存率に有意な変化は認められず，新生児の一般状態にも異常は認められなかった。

(2) 形態（Table 29，30，Appendices 40，41）

外表および内臓異常を有する児動物は認められなかった。内臓変異については，胸腺の頸部残留が対照群で 3 例（発現率 1.6%），1000 mg/kg 群で 4 例（2.4%），左臍動脈遺残が対照群で 1 例（0.5%），1000 mg/kg 群で 1 例（0.6%），および蛇行尿管が 100 mg/kg 群で 1 例(0.6%)認められた。これらの内臓変異を有する児動物は，対照群で 4 匹（2.2%），100 mg/kg 群で 1 匹（0.6%），300 mg/kg 群で 0 匹（0%），および 1000 mg/kg 群で 5 匹（3.1%）であった。以上の内臓変異の種類別発現率および内臓変異を有する児動物の発現率には，対照群と被験物質投与群との間に有意差はなく，また，用量と相関した増加傾向も認められなかった。

考　察

1．反復投与毒性について

動物の臨床観察，感覚反射機能検査，着地開脚幅，握力，自発運動量，体重，摂餌量，尿検査，血液学検査および血液生化学検査において，被験物質の投与のよる有害な影響は認められなかった。

投与期間中に認められた青色の便は毒性影響によるものではなく，経口投与した被験物質の着色によるものであった。

回復期間終了時屠殺動物の雄の投与期間中の検査で認められた測定開始後 30 分間の自発運動量の有意な高値については，その平均値（8901counts）が背景データの正常範囲（4406～9305 counts）値であり，60 分間の運動量には有意差は認められず，さらに投与期間終了時屠殺動物の運動量には有意な変化は認められていないことから，被験物質の投与とは無関係な偶発的変化と判断された。

また，血液学検査および血液生化学検査においては有意差を認める項目があったが，いずれも変化に用量相関性は認められず，しかも背景データにおける正常範囲の変化であったことから，被験物質の毒性影響を示唆する変化ではないと判断された。

病理学検査においては，組織学的に胃の境界縁付近扁平上皮の軽度過形成が 1000 mg/kg 群の雌雄に認められ，被験物質の投与による影響と考えられた。また，前胃の粘膜下織の水腫を伴う例も認められ，被験物質の軽度な局所刺激性を示唆する変化と考えられる。器官重量を含め，その他の器官には被験物質の投与による影響は認められなかった。

このように，被験物質の反復投与により認められた毒性影響は，病理組織学検査で 1000mg/kg 群の雌雄に認められた胃の扁平上皮過形成であった。

この胃の変化は，回復群では雌の 1 匹を除いて認められず，短期間で回復する可逆的変化であることが確認された。

なお，回復群では，雌雄に前肢握力の高値，雄に血色素量の高値および白血球百分率における好酸球比の低値，雌に血清アルブミンおよびカリウムの低値が認められた。しかしながら，前肢握力の高値はむしろ対照群がやや低値であったためであり，その他の変化も投与期間終了時の検査では認められておらず，しかも正常範囲の値（Appendix 42, 43 参照）であったことから，被験物質の投与による遅発的影響を示唆するものでは

なく，偶発的変化と考えられる。

2．生殖発生毒性について

親動物に対して，観察した各指標とも対照群と比べて有意な変化は認められず，生殖器官にも被験物質投与に起因する病理学的変化は認められなかった。

児動物に対しては，発生に関する各指標において被験物質の投与による影響は認められなかった。対照群を含む各投与群で少数例の内臓変異がみられたが，いずれも自然発生的に認められるもので[4]，それらの発現率は当研究所の背景データにおける正常範囲（胸腺の頸部残留：0～4.93%，左臍動脈遺残：0～2.33%，蛇行尿管：0～1.72%）内の値であり，発現率に有意差および用量相関性もないことから催奇形性を示唆する変化ではないと判断された。

以上の結果から，C.I.ピグメントブルー29 のラットへの反復投与による毒性影響は胃に認められ，親動物への反復投与による無影響量（NOEL）は雌雄ともに 300mg/kg/day と結論された。また，雌雄親動物の生殖能および児動物の発生に対する無影響量については 1000 mg/kg/day と結論された。

文　献

1）「12394 の化学商品」，p．1068，化学工業日報社，東京，1994．

2）第一化成工業株式会社 MSDS．

3）古橋忠和ら，C. I. ピグメントブルー29 のラットを用いる単回投与毒性試験，2005，日本バイオリサーチセンター 社内資料．

4）Morita，H.，et al，（1987）. Spontaneous malformations in laboratory animals，Frequency of external，internal and skeletal malformations in rats，rabbits and mice，*Cong. Anom.*，27，147-206．

C.I.ピグメントブルー29のラットを用いる

反復経口投与毒性・生殖発生毒性併合試験

（試験番号：03－306）

最終報告書　添付資料A

（図・群別平均値表）

財団法人　畜産生物科学安全研究所

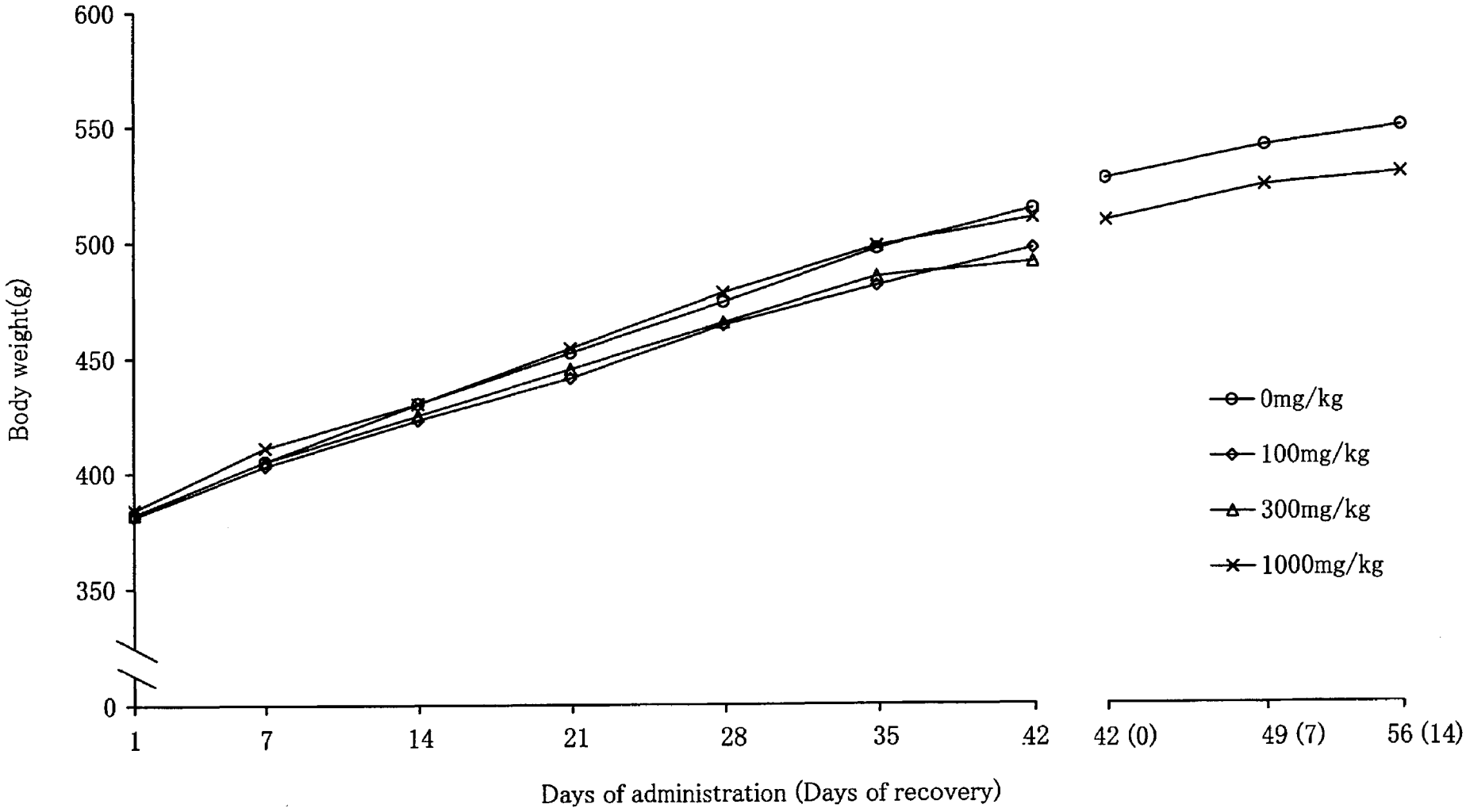

Fig. 1 Body weight changes of male rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

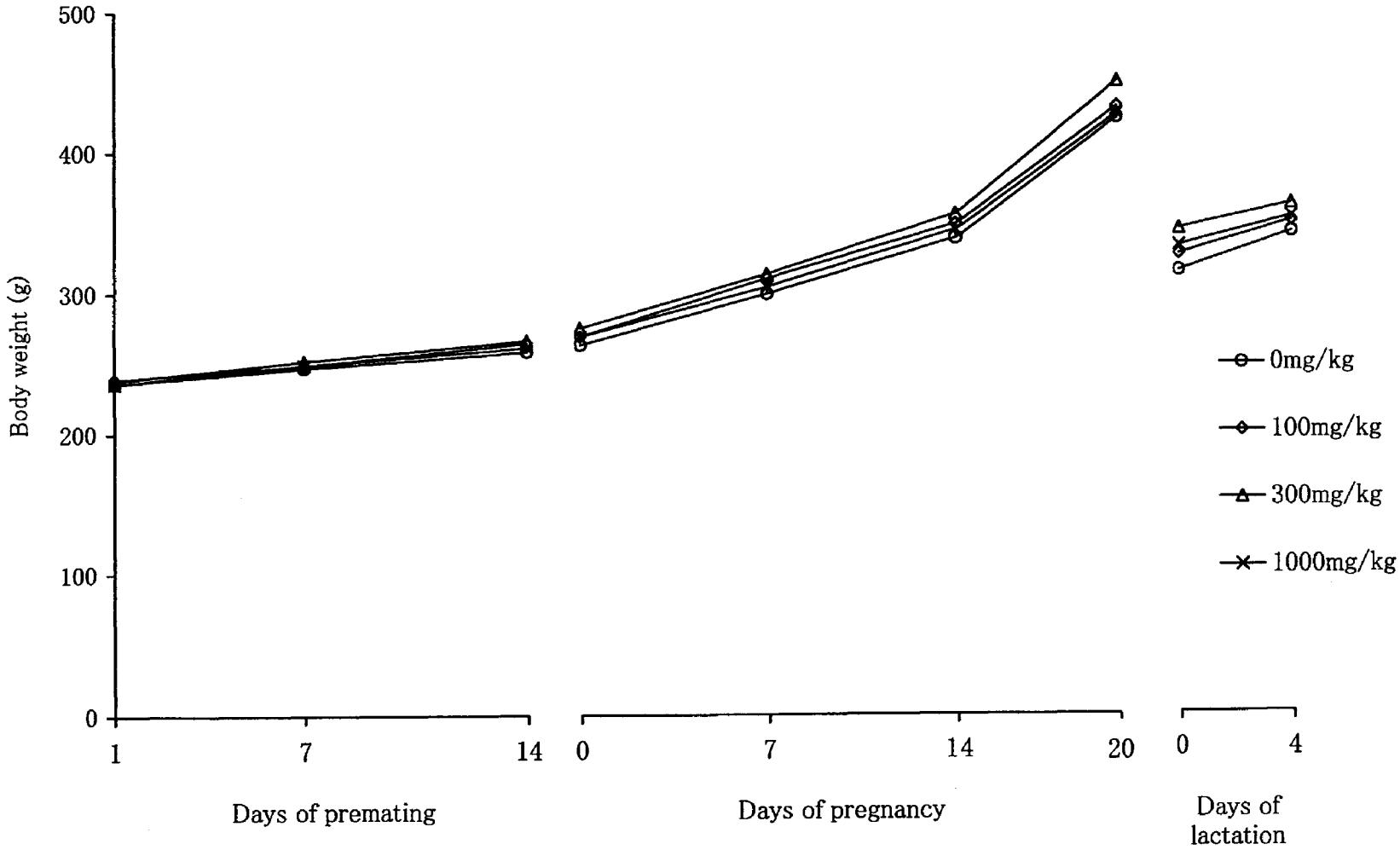

Fig. 2-1 Body weight change in female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

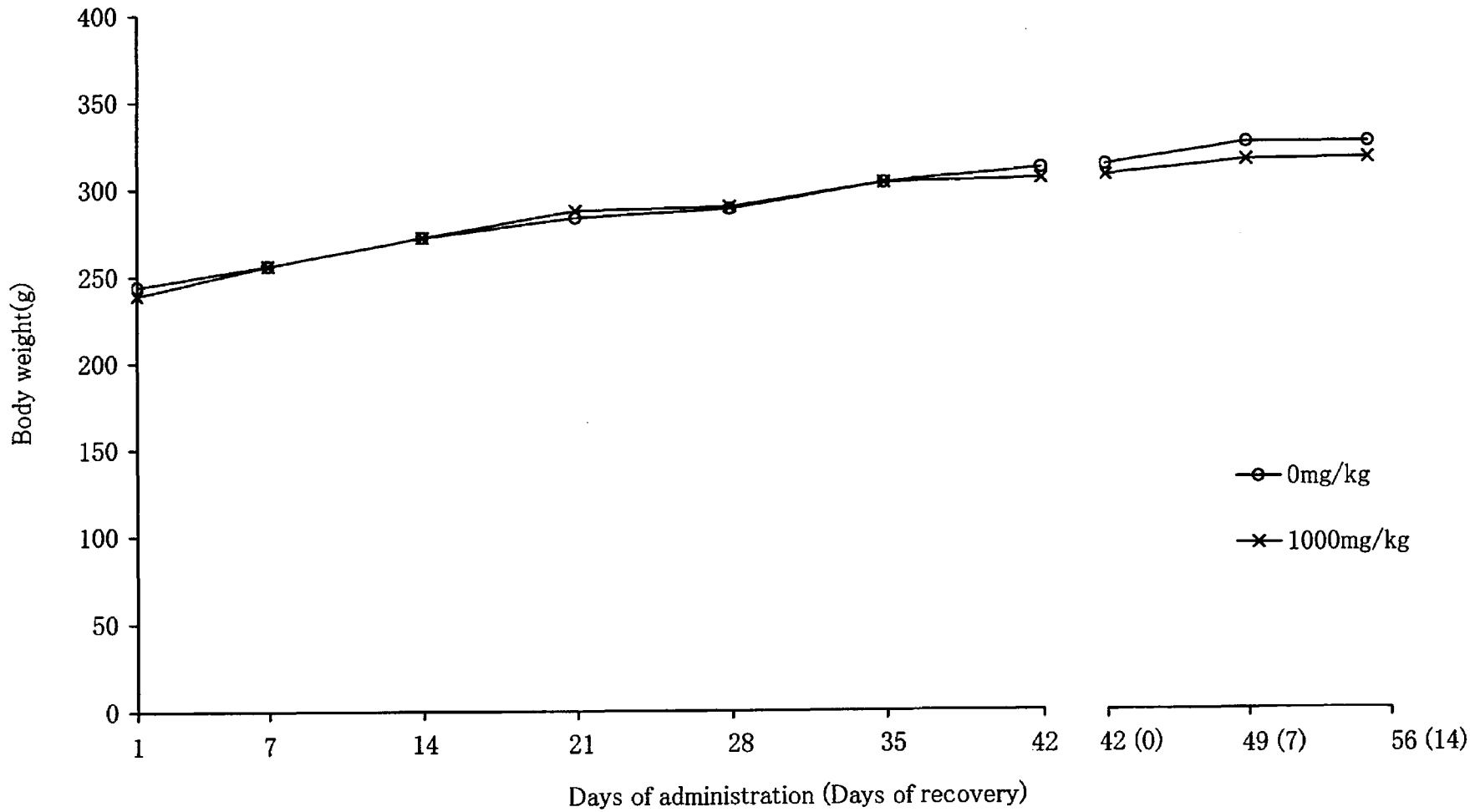

Fig. 2-2 Body weight changes of female rats of the satellite group treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

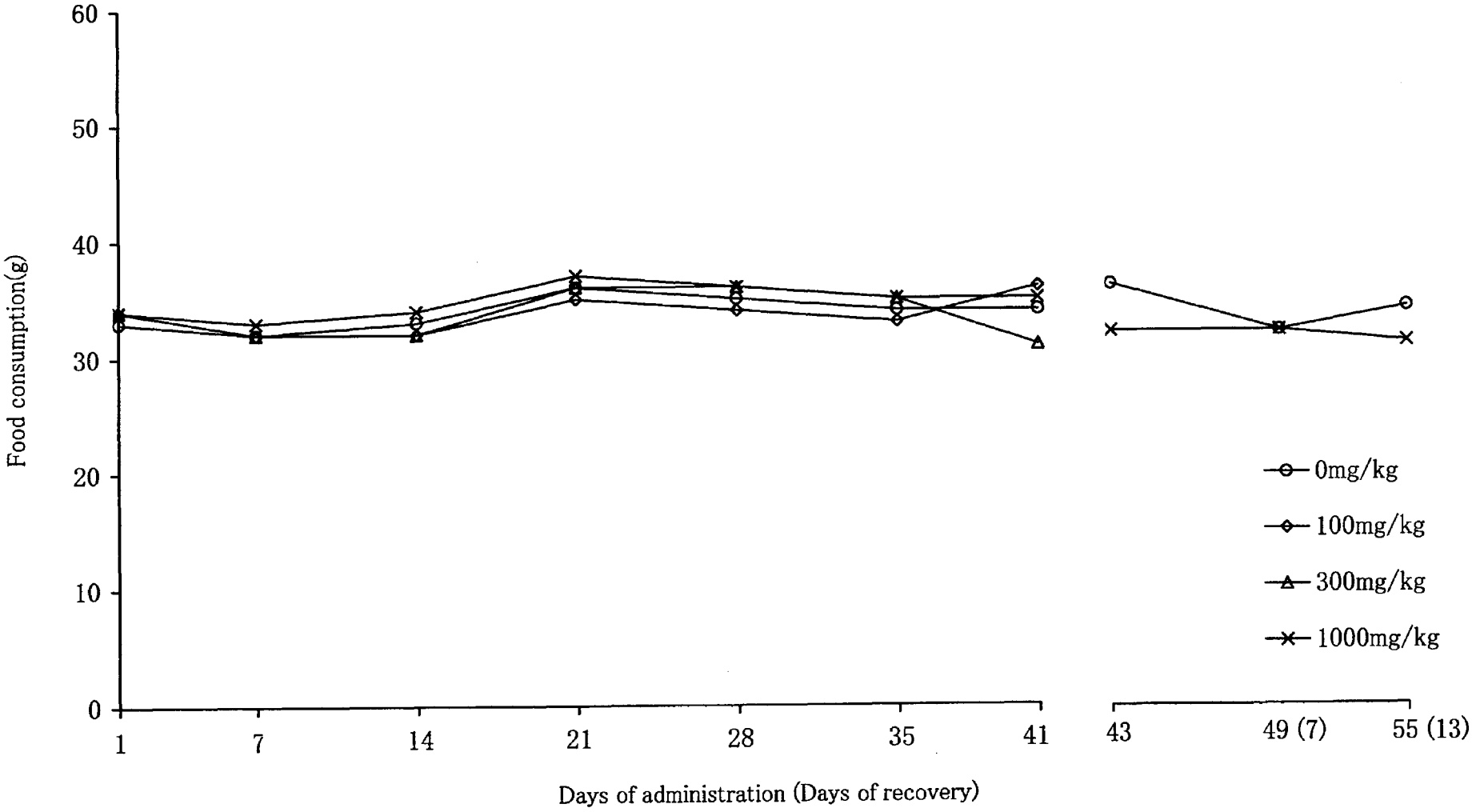

Fig.3 Food consumption changes of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

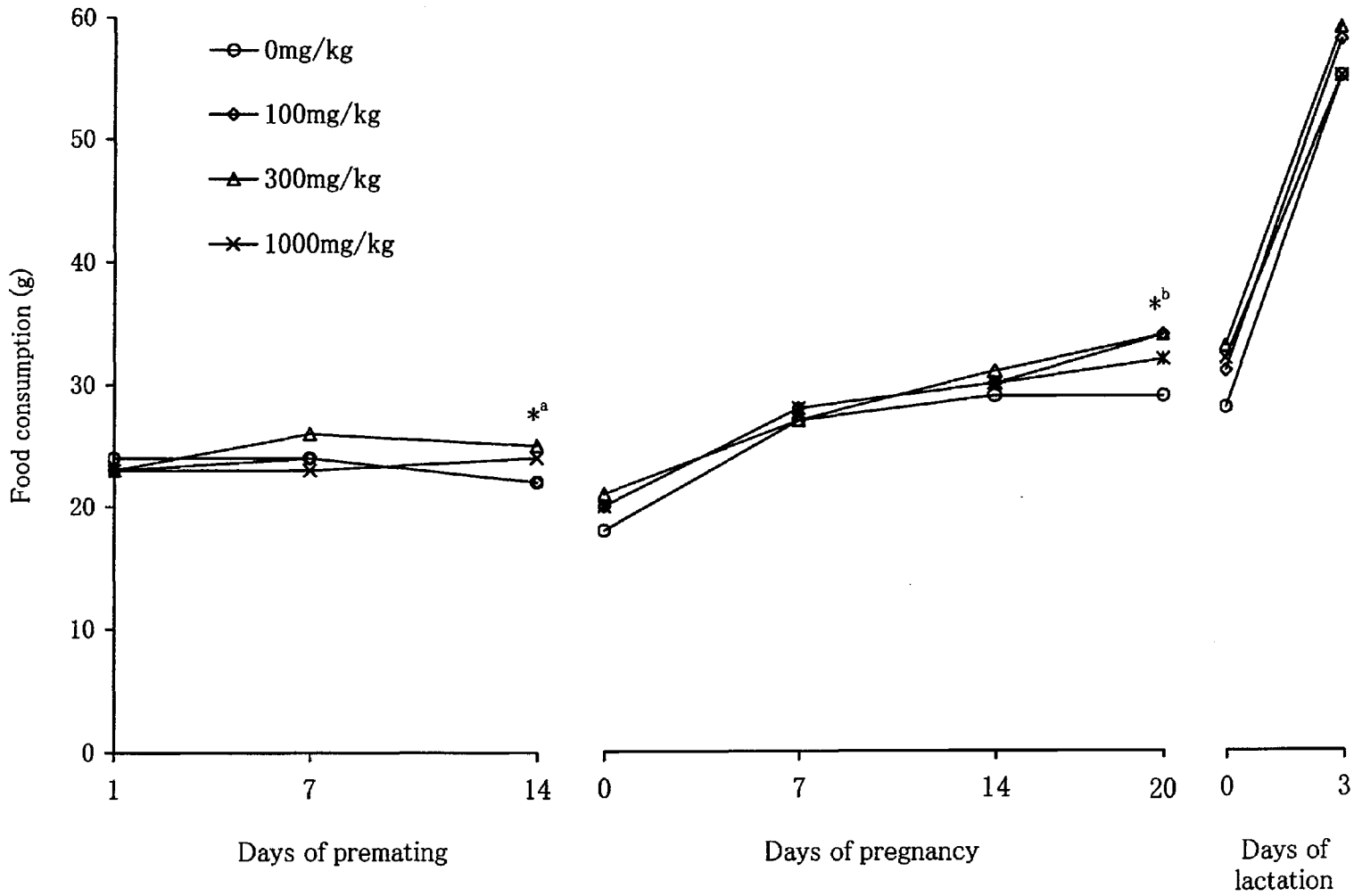

Fig. 4-1 Food consumption change in female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

*a : Significantly different from control at 5% level of probability (300mg/kg).
*b : Significantly different from control at 5% level of probability (100 and 300mg/kg).

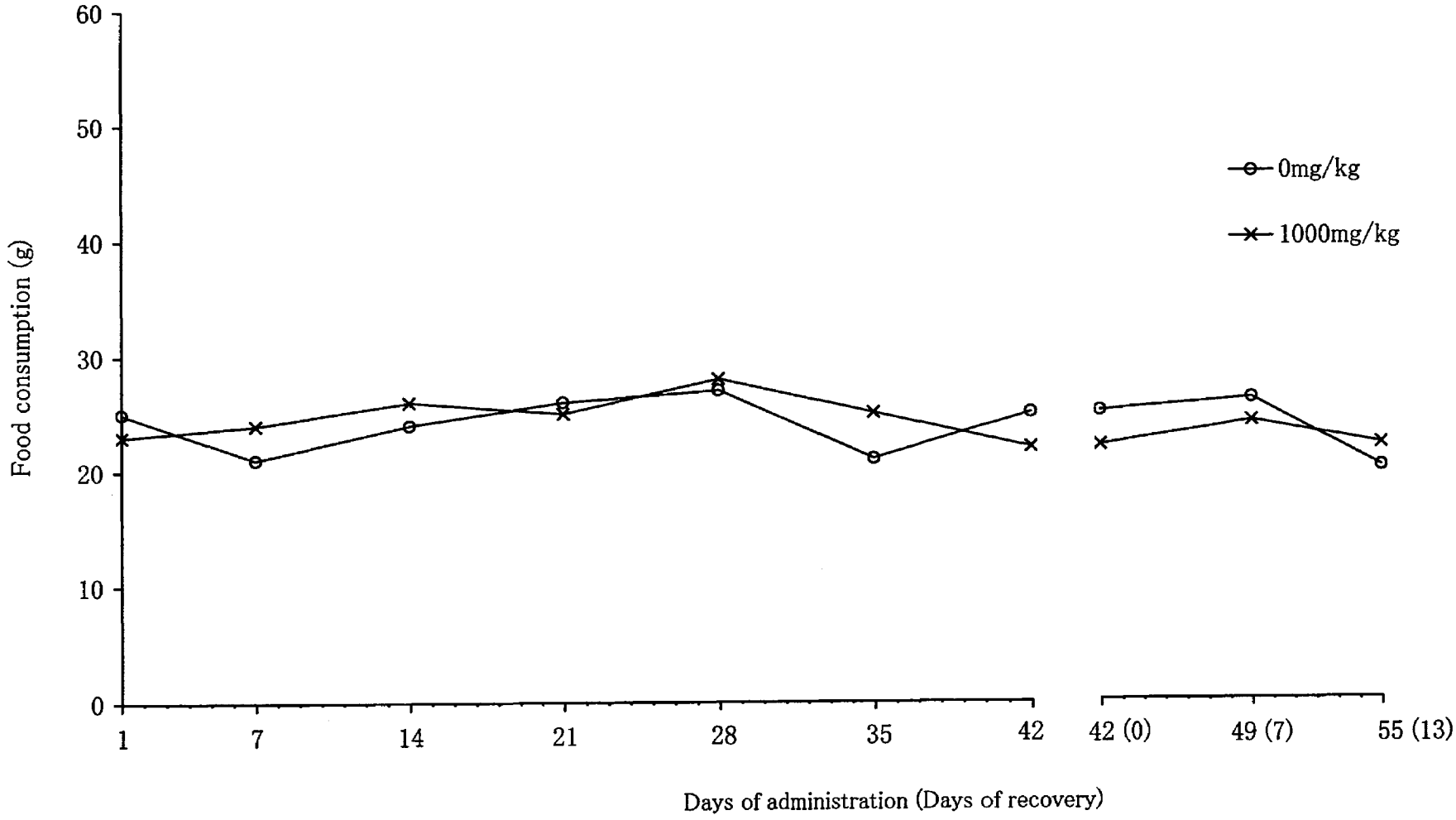

Fig. 4-2 Food consumption change in female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Table 1 Mortality rate of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose (mg/kg)	0	100	300	1000
No. of animals examined	12	12	12	12
No. of animals that died	0	0	0	0
Mortality (%)	0	0	0	0

Table 2 Mortality rate of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose (mg/kg)	0	100	300	1000
No. of animals examined	17	12	12	17
No. of animals that died	0	0	0	0
Mortality (%)	0	0	0	0

Table 3 Clinical signs of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			Administration period				Recovery period	
		Dose (mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Clinical signs	Grade	No. of animals	12 [a]	12	12	12 [a]	5	5
Colored stool(Blue)	−		12	0	0	0	5	5
	+		0	12	0	0	0	0
	++		0 (0)	0 (12)**	12 (12)**	0 (12)**	0 (0)	0 (0)
	+++		0	0	0	12	0	0

TK : Terminal killing.
KR : Killed by design after 14-day recovery period.
− : Negative. + : Slight. ++ : Moderate. +++ : Severe.
a : Include 5 rats of recovery group.
** : Significantly different from control at 1% level of probability.

Table 4-1 Clinical signs of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

		Dose (mg/kg)	0		100		300		1000	
		Fate	TK		TK		TK		TK	
Clinical signs	Grade	No. of animals	12		12		12		12	
Colored stool(Blue)	−		12		12		12		12	
	+		0		12		0		0	
	++		0	(0)	0	(12)**	12	(12)**	0	(12)**
	+++		0		0		0		12	

TK : Terminal kill on day 5 after perturition.
− : Negative. + : Slight. ++ : Moderate. +++ : Severe.
** : Significantly different from control at 1% level of probability.

Table 4-2 Clinical signs of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			Administration period		Recovery period	
		Dose (mg/kg)	0	1000	0	1000
		Fate	KR	KR	KR	KR
Clinical signs	Grade	No. of animals	5	5	5	5
Colored stool(Blue)	-		5	0	5	5
	+++		0	5 **	0	0

KR : Killed by design after 14-day recovery period.
- : Negative. +++ : Severe.
** : Significantly different from control at 1% level of probability.

Table 5-1 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< Before administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	11	11	11	10
	Temporally in handling	1	1	1	2
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	11	12	12	12
	2 or more	1	0	0	0
	Color: Pale yellow	2/2	2/2	2/2	-
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	1/1	-	1/1	1/1

Table 5-2 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 1 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	12	12	10
	Temporally in handling	0	0	0	2
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color:Pale yellow	4/4	1/1	2/2	1/1
Defecation	Not detected or 1	12	12	12	11
	2 or more	0	0	0	1
	Appearance:Normal	1/1	1/1	1/1	2/2

Table 5-3 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	12	12	11
	Temporally in handling	0	0	0	1
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	4/4	3/3	2/2	1/1
Defecation	Not detected or 1	11	12	12	12
	2 or more	1	0	0	0
	Appearance:Normal	1/1	4/4	1/1	1/1

Table 5-4 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 3 of administration period >

Items		Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal		12	12	12	12
Reactivity on handling	Normal		12	12	12	12
Muscle tone	Normal		12	12	12	12
Skin	Normal		12	12	12	12
Fur	Normal		12	12	12	12
Piloerection	Not detected		12	12	12	12
Eye discharge	Not detected		12	12	12	12
Palpebral closure	Not detected		12	12	12	12
Exophthalmos	Not detected		12	12	12	12
Lacrimation	Not detected		12	12	12	12
Smudge around mouth-nose	Not detected		12	12	12	12
Salivation	Not detected		12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected		12	12	12	12
Blotted fur around anus with feces	Not detected		12	12	12	12
Vocalization	Not detected		11	11	11	11
	Temporally in handling		1	1	1	1
Breathing	Normal		12	12	12	12
Body position	Normal		12	12	12	12
Convulsion	Not detected		12	12	12	12
Tremor	Not detected		12	12	12	12
Exploration	Normal		12	12	12	12
Alertness	Normal		12	12	12	12
Locomotor activity	Normal		12	12	12	12
Walk	Normal		12	12	12	12
Abnormal behavior	Normal		12	12	12	12
Stereotypy	Not detected		12	12	12	12
Failure of consciousness	Not detected		12	12	12	12
Limb tone	Normal		12	12	12	12
Urination	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Color: Pale yellow		3/3	2/2	-	-
Defecation	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Appearance:Normal		1/1	2/2	2/2	3/3

Table 5-5 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 4 of administration period >

Items		Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal		12	12	12	12
Reactivity on handling	Normal		12	12	12	12
Muscle tone	Normal		12	12	12	12
Skin	Normal		12	12	12	12
Fur	Normal		12	12	12	12
Piloerection	Not detected		12	12	12	12
Eye discharge	Not detected		12	12	12	12
Palpebral closure	Not detected		12	12	12	12
Exophthalmos	Not detected		12	12	12	12
Lacrimation	Not detected		12	12	12	12
Smudge around mouth-nose	Not detected		12	12	12	12
Salivation	Not detected		12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected		12	12	12	12
Blotted fur around anus with feces	Not detected		12	12	12	12
Vocalization	Not detected		11	12	12	9
	Temporally in handling		1	0	0	3
Breathing	Normal		12	12	12	12
Body position	Normal		12	12	12	12
Convulsion	Not detected		12	12	12	12
Tremor	Not detected		12	12	12	12
Exploration	Normal		12	12	12	12
Alertness	Normal		12	12	12	12
Locomotor activity	Normal		12	12	12	12
Walk	Normal		12	12	12	12
Abnormal behavior	Normal		12	12	12	12
Stereotypy	Not detected		12	12	12	12
Failure of consciousness	Not detected		12	12	12	12
Limb tone	Normal		12	12	12	12
Urination	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Color: Pale yellow		2/2	-	1/1	1/1
Defecation	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Appearance:Normal		-	-	-	-

Table 5-6 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 5 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	11	12	11	8
	Temporally in handling	1	0	1	4
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	3/3	-	1/1	2/2
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	1/1	1/1	1/1	-

Table 5-7 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 6 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	12	11	10
	Temporally in handling	0	0	1	2
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	2/2	-	2/2	1/1
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	2/2	1/1	-	-

Table 5-8 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 1 of recovery period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	4	4
	Temporally in handling	1	1
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	2/2	1/1
Defecation	Not detected or 1	4	5
	2 or more	1	0
	Appearance:Normal	1/1	-

Table 5-9 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of recovery period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	4	4
	Temporally in handling	1	1
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	4	5
	2 or more	1	0
	Color: Pale yellow	2/2	1/1
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	1/1	-

Table 6-1 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< Before administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	10	12	11
	Temporally in handling	0	2	0	1
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	1/1	1/1	5/5	1/1
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-2 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 1 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	12	12	9
	Temporally in handling	0	0	0	3
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	2/2	1/1	2/2	2/2
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-3 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of administration period >

Items		Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal		12	12	12	12
Reactivity on handling	Normal		12	12	12	12
Muscle tone	Normal		12	12	12	12
Skin	Normal		12	12	12	12
Fur	Normal		12	12	12	12
Piloerection	Not detected		12	12	12	12
Eye discharge	Not detected		12	12	12	12
Palpebral closure	Not detected		12	12	12	12
Exophthalmos	Not detected		12	12	12	12
Lacrimation	Not detected		12	12	12	12
Smudge around mouth-nose	Not detected		12	12	12	12
Salivation	Not detected		12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected		12	12	12	12
Blotted fur around anus with feces	Not detected		12	12	12	12
Vocalization	Not detected		12	6	10	11
	Temporally in handling		0	6 **	2	1
Breathing	Normal		12	12	12	12
Body position	Normal		12	12	12	12
Convulsion	Not detected		12	12	12	12
Tremor	Not detected		12	12	12	12
Exploration	Normal		12	12	12	12
Alertness	Normal		12	12	12	12
Locomotor activity	Normal		12	12	12	12
Walk	Normal		12	12	12	12
Abnormal behavior	Normal		12	12	12	12
Stereotypy	Not detected		12	12	12	12
Failure of consciousness	Not detected		12	12	12	12
Limb tone	Normal		12	12	12	12
Urination	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Color: Pale yellow		1/1	1/1	1/1	1/1
Defecation	Not detected or 1		12	12	12	12
	2 or more		0	0	0	0
	Appearance:Normal		-	-	-	-

** : Significantly different from control at 1% level of probability.

Table 6-4 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 3 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	11	10	12	11
	Temporally in handling	1	2	0	1
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	1/1	2/2	2/2	2/2
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-5 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 4 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	12	10	11	9
	Temporally in handling	0	2	1	3
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color:Pale yellow	2/2	1/1	1/1	3/3
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-6 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 5 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	11	11	12	12
	Temporally in handling	1	1	0	0
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	4/4	3/3	1/1	-
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-7 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 6 of administration period >

Items	Dose(mg/kg) No. of animals	0 12	100 12	300 12	1000 12
Reactivity on removal from the cage	Normal	12	12	12	12
Reactivity on handling	Normal	12	12	12	12
Muscle tone	Normal	12	12	12	12
Skin	Normal	12	12	12	12
Fur	Normal	12	12	12	12
Piloerection	Not detected	12	12	12	12
Eye discharge	Not detected	12	12	12	12
Palpebral closure	Not detected	12	12	12	12
Exophthalmos	Not detected	12	12	12	12
Lacrimation	Not detected	12	12	12	12
Smudge around mouth-nose	Not detected	12	12	12	12
Salivation	Not detected	12	12	12	12
Blotted fur in the lower abdomen with urine	Not detected	12	12	12	12
Blotted fur around anus with feces	Not detected	12	12	12	12
Vocalization	Not detected	11	11	12	12
	Temporally in handling	1	1	0	0
Breathing	Normal	12	12	12	12
Body position	Normal	12	12	12	12
Convulsion	Not detected	12	12	12	12
Tremor	Not detected	12	12	12	12
Exploration	Normal	12	12	12	12
Alertness	Normal	12	12	12	12
Locomotor activity	Normal	12	12	12	12
Walk	Normal	12	12	12	12
Abnormal behavior	Normal	12	12	12	12
Stereotypy	Not detected	12	12	12	12
Failure of consciousness	Not detected	12	12	12	12
Limb tone	Normal	12	12	12	12
Urination	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Color: Pale yellow	2/2	2/2	1/1	-
Defecation	Not detected or 1	12	12	12	12
	2 or more	0	0	0	0
	Appearance:Normal	-	-	-	-

Table 6-8 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< Before administration period >

Items		Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal		5	5
Reactivity on handling	Normal		5	5
Muscle tone	Normal		5	5
Skin	Normal		5	5
Fur	Normal		5	5
Piloerection	Not detected		5	5
Eye discharge	Not detected		5	5
Palpebral closure	Not detected		5	5
Exophthalmos	Not detected		5	5
Lacrimation	Not detected		5	5
Smudge around mouth-nose	Not detected		5	5
Salivation	Not detected		5	5
Blotted fur in the lower abdomen with urine	Not detected		5	5
Blotted fur around anus with feces	Not detected		5	5
Vocalization	Not detected		5	5
Breathing	Normal		5	5
Body position	Normal		5	5
Convulsion	Not detected		5	5
Tremor	Not detected		5	5
Exploration	Normal		5	5
Alertness	Normal		5	5
Locomotor activity	Normal		5	5
Walk	Normal		5	5
Abnormal behavior	Normal		5	5
Stereotypy	Not detected		5	5
Failure of consciousness	Not detected		5	5
Limb tone	Normal		5	5
Urination	Not detected or 1		5	5
	2 or more		0	0
	Color: Pale yellow		1/1	-
Defecation	Not detected or 1		5	5
	2 or more		0	0
	Appearance:Normal		-	-

Table 6-9 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 1 of administration period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	4	5
	Temporally in handling	1	0
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	1/1	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 6-10 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of administration period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	5	5
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	-	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 6-11 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 3 of administration period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	5	5
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	-	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance: Normal	-	-

Table 6-12 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 4 of administration period >

Items		Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal		5	5
Reactivity on handling	Normal		5	5
Muscle tone	Normal		5	5
Skin	Normal		5	5
Fur	Normal		5	5
Piloerection	Not detected		5	5
Eye discharge	Not detected		5	5
Palpebral closure	Not detected		5	5
Exophthalmos	Not detected		5	5
Lacrimation	Not detected		5	5
Smudge around mouth-nose	Not detected		5	5
Salivation	Not detected		5	5
Blotted fur in the lower abdomen with urine	Not detected		5	5
Blotted fur around anus with feces	Not detected		5	5
Vocalization	Not detected		5	5
Breathing	Normal		5	5
Body position	Normal		5	5
Convulsion	Not detected		5	5
Tremor	Not detected		5	5
Exploration	Normal		5	5
Alertness	Normal		5	5
Locomotor activity	Normal		5	5
Walk	Normal		5	5
Abnormal behavior	Normal		5	5
Stereotypy	Not detected		5	5
Failure of consciousness	Not detected		5	5
Limb tone	Normal		5	5
Urination	Not detected or 1		5	5
	2 or more		0	0
	Color: Pale yellow		1/1	-
Defecation	Not detected or 1		5	5
	2 or more		0	0
	Appearance:Normal		-	-

Table 6-13 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 5 of administration period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	5	5
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	1/1	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 6-14 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 6 of administration period >

Items	Dose(mg/kg) No. of animals	1 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	4	5
	Temporally in handling	1	0
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color:Pale yellow	1/1	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 6-15 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 1 of recovery period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	5	5
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color:Pale yellow	1/1	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 6-16 Incidence of detailed clinical signs in the FOB (Functional Observation Batteries) of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of recovery period >

Items	Dose(mg/kg) No. of animals	0 5	1000 5
Reactivity on removal from the cage	Normal	5	5
Reactivity on handling	Normal	5	5
Muscle tone	Normal	5	5
Skin	Normal	5	5
Fur	Normal	5	5
Piloerection	Not detected	5	5
Eye discharge	Not detected	5	5
Palpebral closure	Not detected	5	5
Exophthalmos	Not detected	5	5
Lacrimation	Not detected	5	5
Smudge around mouth-nose	Not detected	5	5
Salivation	Not detected	5	5
Blotted fur in the lower abdomen with urine	Not detected	5	5
Blotted fur around anus with feces	Not detected	5	5
Vocalization	Not detected	5	5
Breathing	Normal	5	5
Body position	Normal	5	5
Convulsion	Not detected	5	5
Tremor	Not detected	5	5
Exploration	Normal	5	5
Alertness	Normal	5	5
Locomotor activity	Normal	5	5
Walk	Normal	5	5
Abnormal behavior	Normal	5	5
Stereotypy	Not detected	5	5
Failure of consciousness	Not detected	5	5
Limb tone	Normal	5	5
Urination	Not detected or 1	5	5
	2 or more	0	0
	Color: Pale yellow	-	-
Defecation	Not detected or 1	5	5
	2 or more	0	0
	Appearance:Normal	-	-

Table 7-1 Incidence of responses in the sensory/reflex function test of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<On week 6 of administration period>

Items	Dose(mg/kg)	0	100	300	1000
	No. of animals examined	5	5	5	5
Hearing reaction	Normal	5	5	5	5
Eye sight reaction	Normal	5	5	5	5
Sense of touch reaction	Normal	5	5	5	5
Pain reaction	Normal	5	5	5	5
Pupil reflex	Normal	5	5	5	5
Pinna reflex	Normal	5	5	5	5
Ipsilateral flexor reflex	Normal	5	5	5	5
Eyelid reflex	Normal	5	5	5	5
Righting reflex	Normal	5	5	5	5

Table 7-2 Incidence of responses in the sensory/reflex function test of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

		On week 6 of administration period		On week 2 recovery period	
	Dose(mg/kg)	0	1000	0	1000
Items	No. of animals examined	5	5	5	5
Hearing reaction	Normal	5	5	5	5
Eye sight reaction	Normal	5	5	5	5
Sense of touch reaction	Normal	5	5	5	5
Pain reaction	Normal	5	5	5	5
Pupil reflex	Normal	5	5	5	5
Pinna reflex	Normal	5	5	5	5
Ipsilateral flexor reflex	Normal	5	5	5	5
Eyelid reflex	Normal	5	5	5	5
Righting reflex	Normal	5	5	5	5

Table 8-1 Incidence of responses in the sensory/reflex function test of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<On week 6 of administration period>

Items	Dose(mg/kg)	0	100	300	1000
	No. of animals examined	5	5	5	5
Hearing reaction	Normal	5	5	5	5
Eye sight reaction	Normal	5	5	5	5
Sense of touch reaction	Normal	5	5	5	5
Pain reaction	Normal	5	5	5	5
Pupil reflex	Normal	5	5	5	5
Pinna reflex	Normal	5	5	5	5
Ipsilateral flexor reflex	Normal	5	5	5	5
Eyelid reflex	Normal	5	5	5	5
Righting reflex	Normal	5	5	5	5

Table 8-2 Incidence of responses in the sensory/reflex function test of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

		On week 6 of administration period		On week 2 recovery period	
	Dose(mg/kg)	0	1000	0	1000
Items	No. of animals examined	5	5	5	5
Hearing reaction	Normal	5	5	5	5
Eye sight reaction	Normal	5	5	5	5
Sense of touch reaction	Normal	5	5	5	5
Pain reaction	Normal	5	5	5	5
Pupil reflex	Normal	5	5	5	5
Pinna reflex	Normal	5	5	5	5
Ipsilateral flexor reflex	Normal	5	5	5	5
Eyelid reflex	Normal	5	5	5	5
Righting reflex	Normal	5	5	5	5

Table 9-1 Mean value of landing foot splay, grip strength and motor activity of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<On week 6 of administration period>

Dose (mg/kg)	No. of animals	Landing foot splay (cm)	Grip strength (g)		Motor activity (counts)	
			Forelimb	Hindlimb	0～30min.	0～60min.
0	5	10.6 ± 1.0	635 ± 85	356 ± 129	7321 ± 947	8056 ± 1225
100	5	9.6 ± 1.3	753 ± 127	358 ± 89	7747 ± 1755	11746 ± 3323
300	5	9.4 ± 2.8	623 ± 146	258 ± 62	7292 ± 2103	8455 ± 2283
1000	5	9.9 ± 1.6	725 ± 230	405 ± 100	6639 ± 1528	8842 ± 4044

Each value is exprssed as mean±S.D.

Table 9-2 Mean value of landing foot splay, grip strength and motor activity of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<A : On week 6 of administration period, B : On week 2 of recovery period>

	Dose (mg/kg)	No. of animals	Landing foot splay (cm)	Grip strength (g)		Motor activity (counts)	
				Forelimb	Hindlimb	0~30min.	0~60min.
A	0	5	10.4 ± 2.6	695 ± 235	328 ± 73	8248 ± 379	10437 ± 2172
	1000	5	10.4 ± 2.1	630 ± 247	378 ± 60	8901 * ± 434	12211 ± 1750
B	0	5	10.3 ± 1.9	446 ± 122	421 ± 155	6877 ± 1305	8522 ± 2499
	1000	5	9.9 ± 2.0	644 * ± 150	423 ± 55	7233 ± 1849	8133 ± 2270

Each value is exprssed as mean±S.D.

* : Significantly different from control at 5% level of probability.

Table 10-1 Mean value of landing foot splay, grip strength and motor activity of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<On week 6 of administration period>

Dose (mg/kg)	No. of animals	Landing foot splay (cm)	Grip strength (g)		Motor activity (counts)
			Forelimb	Hindlimb	0～30min.
0	5	6.6 ± 0.9	567 ± 146	344 ± 162	7641 ± 541
100	5	6.8 ± 1.6	580 ± 85	307 ± 69	7895 ± 1578
300	5	7.7 ± 1.3	538 ± 150	323 ± 104	8762 ± 739
1000	5	7.7 ± 2.8	558 ± 213	251 ± 101	7052 ± 2140

Each value is exprssed as mean±S.D.

Table 10-2 Mean value of landing foot splay, grip strength and motor activity of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

<A : On week 6 of administration period, B : On week 2 of recovery period>

	Dose (mg/kg)	No. of animals	Landing foot splay (cm)	Grip strength (g)		Motor activity (counts)	
				Forelimb	Hindlimb	0～30min.	0～60min.
A	0	5	8.1 ± 1.9	485 ± 155	217 ± 34	8769 ± 1060	12310 ± 3290
	1000	5	6.7 ± 2.4	663 ± 176	268 ± 78	8882 ± 942	13688 ± 2628
B	0	5	7.5 ± 2.6	410 ± 90	295 ± 88	7982 ± 878	10789 ± 3946
	1000	5	6.2 ± 1.3	631 ** ± 98	340 ± 49	7629 ± 637	10897 ± 2840

Each value is exprssed as mean±S.D.

** : Significantly different from control at 1% level of probability.

Table 11 Body weights of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

(g)

Dose (mg/kg)	Days of administration 1	7	14	21	28	35	42	Gain 1～42	(Days of recovery) 42 (0)	49 (7)	56 (14)	Gain 42～56
0	382 ± 16 (12)	405 ± 18 (12)	430 ± 22 (12)	452 ± 23 (12)	474 ± 30 (12)	497 ± 31 (12)	514 ± 36 (12)	131 ± 27 (12)	526 ± 28 (5)	540 ± 35 (5)	548 ± 32 (5)	22 ± 5 (5)
100	381 ± 17 (12)	403 ± 18 (12)	423 ± 23 (12)	441 ± 24 (12)	464 ± 27 (12)	481 ± 29 (12)	497 ± 32 (12)	116 ± 24 (12)				
300	382 ± 21 (12)	405 ± 22 (12)	425 ± 28 (12)	445 ± 30 (12)	465 ± 31 (12)	485 ± 33 (12)	491 ± 37 (12)	108 ± 24 (12)				
1000	384 ± 15 (12)	411 ± 18 (12)	430 ± 21 (12)	454 ± 23 (12)	478 ± 25 (12)	498 ± 27 (12)	510 ± 31 (12)	126 ± 22 (12)	508 ± 44 (5)	523 ± 46 (5)	528 ± 48 (5)	19 ± 13 (5)

Each value is expressed as mean±S.D.
(n): No. of animals.

Table 12-1 Body weights of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

(g)

Dose (mg/kg)	Days of premating 1	7	14	Gain 1～14	Days of pregnancy 0	7	14	20	Gain 0～20	Days of lactation 0	4	Gain 0～4
0	236 ± 17 (12)	247 ± 18 (12)	258 ± 21 (12)	22 ± 8 (12)	263 ± 25 (12)	299 ± 29 (12)	338 ± 30 (12)	423 ± 32 (12)	160 ± 10 (12)	313 ± 31 (12)	340 ± 34 (12)	28 ± 10 (12)
100	239 ± 14 (12)	249 ± 11 (12)	264 ± 17 (12)	25 ± 9 (12)	269 ± 19 (12)	310 ± 20 (12)	348 ± 23 (12)	431 ± 29 (12)	162 ± 16 (12)	325 ± 24 (12)	348 ± 28 (12)	24 ± 7 (12)
300	238 ± 15 (12)	252 ± 18 (12)	266 ± 21 (12)	28 ± 9 (12)	275 ± 23 (12)	313 ± 24 (12)	355 ± 27 (12)	449 ± 35 (12)	174 ± 15 (12)	343 ± 27 (12)	360 ± 35 (12)	17 ± 15 (12)
1000	236 ± 13 (12)	249 ± 16 (12)	261 ± 19 (12)	25 ± 10 (12)	269 ± 20 (12)	304 ± 20 (12)	344 ± 25 (12)	426 ± 33 (12)	158 ± 20 (12)	331 ± 29 (12)	351 ± 32 (12)	20 ± 7 (12)

Each value is expressed as mean±S.D.
(n): No. of animals.

Table 12-2 Body weights of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated study dose toxicity with the reproduction/developmental toxicity screening test

(g)

Dose (mg/kg)	Days of administration 1	7	14	21	28	35	42	Gain 1～42	(Days of recovery) 42 (0)	49 (7)	56 (14)	Gain 42～56
0	244 ± 16 (5)	256 ± 13 (5)	272 ± 12 (5)	283 ± 15 (5)	288 ± 19 (5)	303 ± 14 (5)	311 ± 15 (5)	67 ± 11 (5)	311 ± 15 (5)	323 ± 18 (5)	323 ± 17 (5)	12 ± 4 (5)
1000	239 ± 10 (5)	256 ± 22 (5)	272 ± 20 (5)	287 ± 26 (5)	299 ± 37 (5)	303 ± 33 (5)	305 ± 29 (5)	66 ± 22 (5)	305 ± 29 (5)	313 ± 28 (5)	314 ± 32 (5)	9 ± 7 (5)

Each value is expressed as mean±S.D.
(n): No. of animals.

Table 13 Food consumption of male rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

(g/rat/day)

Dose (mg/kg)	Days of administration 1	7	14	21	28	35	41	(Days of recovery) 43 (1)	49 (7)	55 (13)
0	33 ± 4 (12)	32 ± 3 (12)	33 ± 2 (12)	36 ± 4 (12)	35 ± 3 (12)	34 ± 2 (12)	34 ± 3 (12)	36 ± 3 (5)	32 ± 1 (5)	34 ± 2 (5)
100	34 ± 2 (12)	32 ± 4 (12)	32 ± 3 (12)	35 ± 4 (12)	34 ± 3 (12)	33 ± 3 (12)	36 ± 4 (12)			
300	34 ± 3 (12)	32 ± 3 (12)	32 ± 2 (12)	36 ± 4 (12)	36 ± 2 (12)	35 ± 2 (12)	31 ± 11 (12)			
1000	34 ± 2 (12)	33 ± 4 (12)	34 ± 3 (12)	37 ± 4 (12)	36 ± 4 (12)	35 ± 4 (12)	35 ± 4 (12)	32 ± 6 (5)	32 ± 4 (5)	31 ± 5 (5)

Each value is expressed as mean±S.D.
(n) : No. of animals.

Table 14-1 Food consumption of female rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

(g/rat/day)

Dose	Days of premating			Days of pregnancy				Days of lactation	
(mg/kg)	1	7	14	0	7	14	20	0	3
0	24 ± 4 (12)	24 ± 3 (12)	22 ± 3 (12)	18 ± 5 (12)	27 ± 4 (12)	29 ± 3 (12)	29 ± 4 (12)	28 ± 7 (12)	55 ± 7 (12)
100	23 ± 3 (12)	24 ± 3 (12)	22 ± 2 (12)	20 ± 3 (12)	28 ± 4 (12)	30 ± 2 (12)	34 * ± 3 (12)	31 ± 5 (12)	58 ± 5 (12)
300	23 ± 3 (12)	26 ± 4 (12)	25 * ± 3 (12)	21 ± 4 (12)	27 ± 3 (12)	31 ± 4 (12)	34 * ± 3 (12)	33 ± 6 (12)	59 ± 6 (12)
1000	23 ± 4 (12)	23 ± 2 (12)	24 ± 3 (12)	20 ± 4 (12)	28 ± 9 (12)	30 ± 4 (12)	32 ± 4 (12)	32 ± 5 (12)	55 ± 8 (12)

Each value is expressed as mean±S.D.
(n) : No. of animals.
* : Significantly different from control at 5% level of probability.

Table 14-2 Food consumption of female rats of the satellite group treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

(g/rat/day)

Dose (mg/kg)	Days of administration						(Days of recovery)		
	1	7	14	21	28	35	42 (0)	49 (7)	55 (13)
0	25 ± 2 (5)	21 ± 5 (5)	24 ± 2 (5)	26 ± 2 (5)	27 ± 2 (5)	21 ± 4 (5)	25 ± 4 (5)	26 ± 3 (5)	20 ± 4 (5)
1000	23 ± 4 (5)	24 ± 3 (5)	26 ± 4 (5)	25 ± 5 (5)	28 ± 3 (5)	25 ± 5 (5)	22 ± 3 (5)	24 ± 5 (5)	22 ± 4 (5)

Each value is expressed as mean±S.D.
(n) : No. of animals.

Table 15-1 Urinary findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On day 40 of administration period >

Dose (mg/kg)	No. of animals	Color PY	Cloudy −	Cloudy +	Volume[a)] (mL/18hr)	Specific[a)] gravity	Na[a)] (mEq/L)	Na[a)] (mEq/18hr)	K[a)] (mEq/L)	K[a)] (mEq/18hr)	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5
0	5	5	5		15.9 ± 5.4	1.055 ±0.010	130 ± 13	2.03 ± 0.56	162 ± 57	2.38 ± 0.28					2	3
100	5	5	5		12.7 ± 4.2	1.059 ±0.013	149 ± 33	1.81 ± 0.28	214 ± 49	2.58 ± 0.36				1		4
300	5	5	5		12.0 ± 5.5	1.059 ±0.017	168 ± 46	1.85 ± 0.35	212 ± 65	2.26 ± 0.28					1	4
1000	5	5	5		11.4 ± 3.5	1.062 ±0.008	130 ± 16	1.46 ± 0.41	215 ± 44	2.33 ± 0.15					2	3

Dose (mg/kg)	No. of animals	Protein −	Protein ±	Protein +	Protein ++	Protein +++	Glucose −	Glucose ±	Glucose +	Ketone body −	Ketone body ±	Ketone body +	Ketone body ++	Occult blood −	Occult blood ±	Occult blood +	Occult blood ++	Occult blood +++	Urobilinogen 0.1	Urobilinogen 1	Urobilinogen 2	Bilirubin −	Bilirubin +	Bilirubin ++
0	5		2	2	1		5			3	1	1		4				1	5			5		
100	5			4	1		5				5			5					5			5		
300	5			5			5				3	2		5					5			5		
1000	5		1	3	1		5			2	3			5					5			5		

a): Mean±S.D.
Color : PY(pale yellow).
Cloudy : −(negligible), +(cloudy).
Protein : −(negligible), ±(15~30mg/dL), +(30mg/dL), ++(100mg/dL), +++(300mg/dL).
Glucose : −(negligible), ±(0.1g/dL), +(0.25g/dL).
Ketone body : −(negligible), ±(5mg/dL), +(15mg/dL), ++(40mg/dL).
Occult blood : −(negligible), ±(trace), +(slight), ++(moderate), +++(marked).
Urobilinogen : Ehrlich unit/dL.
Bilirubin : −(negligible), +(slight), ++(moderate).

Table 15-2 Urinary findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On day 40 of administration period >

Dose (mg/kg)	No. of animals	Erythrocytes				Leukocytes				Crystals: Mg				Crystals: Ca				Crystals: Ams			
		−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
0	5	4			1	5				2	1	2		5				5			
100	5	5				5				1	2	2		5				5			
300	5	5				5				1	2	1	1	5				5			
1000	5	5				5				2		3		5				5			

Dose (mg/kg)	No. of animals	Epithelial cells: Sq				Epithelial cells: R			Epithelial cells: S			Casts: G		Casts: H		Casts: W		Fat globules		
		−	+	++	+++	−	+	++	−	+	++	−	+	−	+	−	+	−	+	++
0	5	2	3			5			5			5		5		5		5		
100	5		5			5			5			5		5		5		5		
300	5	4	1			5			5			5		5		5		5		
1000	5	3	2			5			5			5		5		5		5		

− : Not observed, + : A few in some fields, ++ : A few in all fields, +++ : Many in all fields.

Crystals .
- Mg(ammonium magnesium phosphate).
- Ca(calcium carbonate).
- Ams(amorphous).

Epithelial cells.
- Sq(squamous).
- R(round).
- S(spindle).

Casts.
- G(granule).
- H(hyaline).
- W(waxy).

Table 15-3 Urinary findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< On week 2 of recovery period >

Dose (mg/kg)	No. of animals	Color PY	Color Y	Cloudy −	Cloudy +	Volume[a)] (mL/18hr)	Specific[a)] gravity	Na[a)] (mEq/L)	Na[a)] (mEq/18hr)	K[a)] (mEq/L)	K[a)] (mEq/18hr)	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5
0	5	2	3	5		10.4 ± 3.0	1.062 ±0.007	134 ± 19	1.37 ± 0.34	234 ± 31	2.37 ± 0.40						5
1000	5	2	3	5		9.8 ± 3.4	1.064 ±0.006	160 ± 21	1.56 ± 0.60	237 ± 19	2.27 ± 0.58						5

Dose (mg/kg)	No. of animals	Protein −	Protein ±	Protein +	Protein ++	Protein +++	Glucose −	Glucose ±	Glucose +	Ketone body −	Ketone body ±	Ketone body +	Ketone body ++	Occult blood −	Occult blood ±	Occult blood +	Occult blood ++	Occult blood ++++	Urobilinogen 0.1	Urobilinogen 1	Urobilinogen 2	Bilirubin −	Bilirubin +	Bilirubin ++
0	5			3	2		5				4	1		5					5			5		
1000	5			2	3		5				4	1		5					5			5		

a): Mean±S.D.
Color : PY(pale yellow).
Cloudy : −(negligible), +(cloudy).
Protein : −(negligible), ±(15～30mg/dL), +(30mg/dL), ++(100mg/dL), +++(300mg/dL).
Glucose : −(negligible), ±(0.1g/dL), +(0.25g/dL).
Ketone body : −(negligible), ±(5mg/dL), +(15mg/dL), ++(40mg/dL).
Occult blood : −(negligible), ±(trace), +(slight), ++(moderate), +++(marked).
Urobilinogen : Ehrlich unit/dL.
Bilirubin : −(negligible), +(slight), ++(moderate).

Table 15-4 Urinary findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity with the reproduction/developmental toxicity screening test

< On week 2 of recovery period >

Dose (mg/kg)	No. of animals	Erythrocytes				Leukocytes				Crystals: Mg				Crystals: Ca				Crystals: Ams			
		−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
0	5	5				5						2	3	5				5			
1000	5	5				5				1		4		5				5			

Dose (mg/kg)	No. of animals	Epithelial cells: Sq				Epithelial cells: R			Epithelial cells: S			Casts: G		Casts: H		Casts: W		Fat globules		
		−	+	++	+++	−	+	++	−	+	++	−	+	−	+	−	+	−	+	++
0	5	2	3			5			5			5		5		5		5		
1000	5	3	2			5			5			5		5		5		5		

− : Not observed, + : A few in some fields, ++ : A few in all fields, +++ : Many in all fields.

Crystals .
- Mg(ammonium magnesium phosphate).
- Ca(calcium carbonate).
- Ams(amorphous).

Epithelial cells.
- Sq(squamous).
- R(round).
- S(spindle).

Casts.
- G(granule).
- H(hyaline).
- W(waxy).

Table 16-1 Hematological findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After administration period >

Dose (mg/kg)	No. of animals	RBC ($10^4/\mu L$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (%)	Ret. (‰)	PT (sec)	APTT (sec)
0	5	846 ± 52	15.9 ± 0.5	46.0 ± 1.8	55 ± 2	18.8 ± 0.7	34.6 ± 0.4	29 ± 8	13.3 ± 0.5	23.0 ± 1.8
100	5	834 ± 44	14.5 ** ± 0.8	42.9 ± 1.9	52 * ± 2	17.4 ** ± 0.7	33.8 ± 0.8	28 ± 4	13.2 ± 0.5	22.0 ± 1.5
300	5	820 ± 46	14.8 * ± 0.8	43.4 ± 1.7	53 ± 1	18.0 ± 0.3	34.1 ± 0.8	26 ± 6	13.7 ± 0.7	22.8 ± 0.8
1000	5	835 ± 25	14.8 * ± 0.3	43.8 ± 1.4	53 ± 2	17.7 * ± 0.3	33.8 ± 1.0	28 ± 5	13.1 ± 0.5	22.1 ± 1.6

Dose (mg/kg)	No. of animals	WBC ($10^2/\mu L$)	Differential leukocyte counts (%)							Plat. ($10^4/\mu L$)
			Baso.	Eosin.	Neutro. Stab.	Neutro. Seg.	Lymph.	Mono.	Other	
0	5	92 ± 26	0 ± 0	1 ± 1	0 ± 0	16 ± 6	80 ± 5	3 ± 1	0 ± 0	121 ± 14
100	5	59 * ± 10	0 ± 0	1 ± 1	0 ± 0	26 ± 13	70 ± 15	3 ± 2	0 ± 0	115 ± 12
300	5	58 * ± 13	0 ± 0	2 ± 2	0 ± 0	19 ± 7	75 ± 8	4 ± 3	0 ± 0	139 ± 20
1000	5	60 * ± 10	0 ± 0	1 ± 0	0 ± 0	14 ± 2	82 ± 2	3 ± 1	0 ± 0	140 ± 13

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

** : Significantly different from control at 1% level of probability.

Table 16-2 Hematological findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After recovery period >

Dose (mg/kg)	No. of animals	RBC ($10^4/\mu L$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (%)	Ret. (‰)	PT (sec)	APTT (sec)
0	5	880 ± 31	15.4 ± 0.3	45.8 ± 1.2	52 ± 1	17.5 ± 0.4	33.7 ± 0.7	24 ± 3	13.3 ± 0.8	24.1 ± 1.1
1000	5	915 ± 20	16.1 * ± 0.3	47.0 ± 1.6	51 ± 1	17.6 ± 0.2	34.2 ± 0.6	26 ± 4	13.3 ± 0.5	24.7 ± 1.0

Dose (mg/kg)	No. of animals	WBC ($10^2/\mu L$)	Differential leukocyte counts (%)							Plat. ($10^4/\mu L$)
			Baso.	Eosin.	Neutro. Stab.	Neutro. Seg.	Lymph.	Mono.	Other	
0	5	84 ± 12	0 ± 0	2 ± 1	0 ± 0	22 ± 7	72 ± 5	4 ± 2	0 ± 0	131 ± 19
1000	5	84 ± 25	0 ± 0	0 * ± 1	0 ± 0	17 ± 4	79 ± 3	4 ± 1	0 ± 0	136 ± 11

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

Table 17-1 Hematological findings of female rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After administration period >

Dose (mg/kg)	No. of animals	RBC ($10^4/\mu L$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (%)	Ret. (‰)	PT (sec)	APTT (sec)
0	5	698 ± 46	13.4 ± 0.8	39.9 ± 2.1	57 ± 1	19.2 ± 0.4	33.5 ± 0.7	65 ± 7	13.9 ± 0.3	18.9 ± 1.2
100	5	710 ± 33	13.6 ± 0.2	39.9 ± 1.0	56 ± 2	19.2 ± 0.8	34.1 ± 0.7	67 ± 8	13.6 ± 0.8	19.2 ± 1.9
300	5	681 ± 44	12.9 ± 0.6	38.3 ± 1.5	56 ± 2	18.9 ± 0.6	33.6 ± 0.7	59 ± 3	13.7 ± 0.5	17.9 ± 2.7
1000	5	693 ± 45	12.6 ± 1.2	37.1 ± 3.1	54 ± 4	18.2 ± 1.3	33.9 ± 0.6	58 ± 9	13.7 ± 0.4	18.7 ± 0.9

Dose (mg/kg)	No. of animals	WBC ($10^2/\mu L$)	Differential leukocyte counts (%)							Plat. ($10^4/\mu L$)
			Baso.	Eosin.	Neutro. Stab.	Neutro. Seg.	Lymph.	Mono.	Other	
0	5	86 ± 8	0 ± 0	0 ± 0	0 ± 0	26 ± 7	71 ± 6	3 ± 2	0 ± 0	173 ± 21
100	5	79 ± 22	0 ± 0	0 ± 1	0 ± 0	26 ± 15	70 ± 14	3 ± 3	0 ± 0	169 ± 18
300	5	71 ± 14	0 ± 0	1 ± 1	0 ± 0	21 ± 10	76 ± 11	1 ± 2	0 ± 0	171 ± 41
1000	5	65 ± 7	0 ± 0	1 ± 2	0 ± 0	20 ± 5	76 ± 8	3 ± 2	0 ± 0	183 ± 40

Each value is expressed as mean±S.D.

Table 17-2 Hematological findings of female rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After recovery period >

Dose (mg/kg)	No. of animals	RBC ($10^4/\mu L$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (%)	Ret. (‰)	PT (sec)	APTT (sec)
0	5	821 ± 30	15.6 ± 0.5	45.0 ± 1.5	55 ± 1	19.1 ± 0.7	34.8 ± 1.0	25 ± 6	12.9 ± 0.2	19.1 ± 1.9
1000	5	850 ± 17	15.9 ± 0.5	45.8 ± 1.6	54 ± 2	18.7 ± 0.6	34.8 ± 0.5	23 ± 5	12.9 ± 0.4	18.1 ± 0.9

Dose (mg/kg)	No. of animals	WBC ($10^2/\mu L$)	Differential leukocyte counts (%)							Plat. ($10^4/\mu L$)
			Baso.	Eosin.	Neutro. Stab.	Neutro. Seg.	Lymph.	Mono.	Other	
0	5	47 ± 23	0 ± 0	2 ± 2	0 ± 0	14 ± 3	82 ± 3	2 ± 1	0 ± 0	129 ± 9
1000	5	44 ± 14	0 ± 0	1 ± 1	0 ± 0	17 ± 6	80 ± 6	1 ± 1	0 ± 0	136 ± 6

Each value is expressed as mean±S.D.

Table 18-1 Blood biochemical findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After admonistration period >

Dose (mg/kg)	No. of animals	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	γ-GTP (IU/L)	ChE (IU/L)	T.P. (g/dL)	Alb. (g/dL)	A/G	T-Cho. (mg/dL)
0	5	420 ± 85	86 ± 9	45 ± 3	451 ± 97	0.51 ± 0.13	47 ± 14	6.08 ± 0.20	3.02 ± 0.03	0.99 ± 0.08	69 ± 11
100	5	435 ± 111	84 ± 9	45 ± 5	433 ± 90	0.58 ± 0.09	54 ± 14	5.75 ± 0.31	2.76 ± 0.23	0.92 ± 0.06	64 ± 6
300	5	489 ± 133	93 ± 18	46 ± 11	448 ± 88	0.58 ± 0.17	46 ± 8	5.71 ± 0.52	2.73 ± 0.24	0.92 ± 0.04	72 ± 16
1000	5	515 ± 171	85 ± 8	40 ± 5	483 ± 112	0.66 ± 0.28	54 ± 18	5.86 ± 0.15	2.94 ± 0.15	1.01 ± 0.06	64 ± 8

Dose (mg/kg)	No. of animals	T.G. (mg/dL)	Glu. (mg/dL)	BUN (mg/dL)	Crea. (mg/dL)	T-Bil. (mg/dL)	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
0	5	54 ± 27	152 ± 12	14.6 ± 1.3	0.47 ± 0.04	0.30 ± 0.06	9.7 ± 0.2	6.9 ± 0.5	145 ± 0	4.54 ± 0.12	107 ± 0
100	5	53 ± 22	158 ± 10	14.8 ± 1.3	0.43 ± 0.05	0.29 ± 0.04	9.4 ± 0.2	7.0 ± 0.2	144 * ± 1	4.58 ± 0.29	106 ± 1
300	5	42 ± 24	149 ± 10	15.1 ± 1.2	0.43 ± 0.02	0.28 ± 0.04	9.3 ± 0.4	6.5 ± 0.3	144 ± 1	4.76 ± 0.25	107 ± 1
1000	5	41 ± 3	146 ± 11	14.1 ± 1.6	0.44 ± 0.02	0.29 ± 0.03	9.5 ± 0.4	7.0 ± 0.6	145 ± 0	4.77 ± 0.36	106 ± 1

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

Table 18-2 Blood biochemical findings of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After recovery period >

Dose (mg/kg)	No. of animals	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	γ-GTP (IU/L)	ChE (IU/L)	T.P. (g/dL)	Alb. (g/dL)	A/G	T-Cho. (mg/dL)
0	5	319 ± 185	74 ± 11	37 ± 3	362 ± 68	0.75 ± 0.36	41 ± 9	6.14 ± 0.26	2.98 ± 0.11	0.95 ± 0.05	60 ± 13
1000	5	301 ± 116	80 ± 9	42 ± 5	292 ± 48	0.72 ± 0.53	54 ± 16	6.19 ± 0.29	2.95 ± 0.19	0.91 ± 0.06	59 ± 17

Dose (mg/kg)	No. of animals	T.G. (mg/dL)	Glu. (mg/dL)	BUN (mg/dL)	Crea. (mg/dL)	T-Bil. (mg/dL)	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
0	5	39 ± 22	165 ± 13	14.5 ± 1.7	0.44 ± 0.04	0.30 ± 0.04	10.0 ± 0.3	6.4 ± 0.3	146 ± 1	4.83 ± 0.26	105 ± 1
1000	5	49 ± 27	161 ± 28	15.5 ± 1.3	0.44 ± 0.01	0.27 ± 0.07	9.7 ± 0.3	6.6 ± 0.4	146 ± 2	4.94 ± 0.40	105 ± 2

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

Table 19-1 Blood biochemical findings of female rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After admonistration period >

Dose (mg/kg)	No. of animals	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	γ-GTP (IU/L)	ChE (IU/L)	T.P. (g/dL)	Alb. (g/dL)	A/G	T-Cho. (mg/dL)
0	5	353 ± 168	85 ± 13	65 ± 14	346 ± 145	0.82 ± 0.24	286 ± 65	6.19 ± 0.44	3.20 ± 0.40	1.07 ± 0.14	77 ± 4
100	5	342 ± 145	92 ± 13	69 ± 8	318 ± 130	0.75 ± 0.27	317 ± 99	6.15 ± 0.13	3.29 ± 0.16	1.15 ± 0.08	80 ± 19
300	5	327 ± 124	88 ± 12	63 ± 14	431 ± 255	0.73 ± 0.18	226 ± 45	5.87 ± 0.09	2.97 ± 0.08	1.02 ± 0.03	72 ± 12
1000	5	459 ± 131	96 ± 30	63 ± 19	276 ± 67	0.64 ± 0.11	282 ± 81	6.07 ± 0.22	3.11 ± 0.24	1.05 ± 0.12	74 ± 12

Dose (mg/kg)	No. of animals	T.G. (mg/dL)	Glu. (mg/dL)	BUN (mg/dL)	Crea. (mg/dL)	T-Bil. (mg/dL)	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
0	5	105 ± 52	147 ± 13	23.6 ± 4.5	0.50 ± 0.05	0.25 ± 0.04	10.3 ± 0.4	8.1 ± 0.8	146 ± 2	5.04 ± 0.17	103 ± 2
100	5	131 ± 28	136 ± 15	24.1 ± 4.1	0.48 ± 0.03	0.24 ± 0.02	10.2 ± 0.4	7.9 ± 0.8	146 ± 2	4.79 ± 0.15	103 ± 2
300	5	95 ± 30	146 ± 14	23.6 ± 2.5	0.51 ± 0.03	0.24 ± 0.02	10.2 ± 0.2	8.8 ± 0.5	145 ± 2	5.12 ± 0.33	104 ± 2
1000	5	126 ± 57	132 ± 8	23.8 ± 2.2	0.50 ± 0.01	0.24 ± 0.05	9.9 ± 0.2	7.5 ± 1.1	146 ± 1	4.90 ± 0.30	105 ± 2

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

** : Significantly different from control at 1% level of probability.

Table 19-2 Blood biochemical findings of female rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

< After recovery period >

Dose (mg/kg)	No. of animals	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	γ-GTP (IU/L)	ChE (IU/L)	T.P. (g/dL)	Alb. (g/dL)	A/G	T-Cho. (mg/dL)
0	5	394 ± 282	151 ± 165	56 ± 32	164 ± 47	1.11 ± 0.53	520 ± 117	6.77 ± 0.23	3.88 ± 0.22	1.35 ± 0.11	101 ± 13
1000	5	396 ± 142	99 ± 60	51 ± 44	165 ± 22	0.94 ± 0.24	525 ± 114	6.49 ± 0.25	3.53 * ± 0.22	1.19 ± 0.12	89 ± 26

Dose (mg/kg)	No. of animals	T.G. (mg/dL)	Glu. (mg/dL)	BUN (mg/dL)	Crea. (mg/dL)	T-Bil. (mg/dL)	Ca (mg/dL)	P (mg/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
0	5	24 ± 3	125 ± 11	15.9 ± 2.4	0.47 ± 0.04	0.26 ± 0.03	10.1 ± 0.4	5.7 ± 0.3	146 ± 1	5.06 ± 0.46	106 ± 1
1000	5	19 ± 4	126 ± 12	16.1 ± 2.5	0.48 ± 0.02	0.25 ± 0.03	9.7 ± 0.1	5.7 ± 0.4	147 ± 1	4.49 * ± 0.29	108 ± 1

Each value is expressed as mean±S.D.

* : Significantly different from control at 5% level of probability.

Table 20 Incidence of necropsy findings of male rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose(mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	7	12	12	7	5	5
Epididyms	: Nodule	-	7	11	11	7	5	5
		+	0	1	1	0	0	0
Testis	: Small	-	7	12	12	7	5	4
		+	0	0	0	0	0	1

- : Negtive. + : Slight.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.

Table 21 Incidence of necropsy findings of female rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose(mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	12	12	12	12	5	5
Thymus	: Reddish area	-	12	12	12	11	5	5
		+	0	0	0	1	0	0

- : Negtive. + : Slight.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.

Table 22-1 Absolute and relative organ weights of male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test
< After administration period >

	Dose (mg/kg)	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyroid (mg)	Pituitary (mg)	Adrenal (mg)	Testis (g)	Seminal vesicle (g)	Epididymis (g)
Absolute	0	468 ±34 (7)	2.09 ±0.08 (5)	11.86 ±1.43 (5)	3.02 ±0.13 (5)	0.86 ±0.10 (5)	1.51 ±0.10 (5)	0.36 ±0.14 (5)	32.6 ±3.8 (5)	14.4 ±0.9 (5)	73.7 ±21.5 (5)	3.60 ±0.14 (7)	2.22 ±0.14 (5)	1.34 ±0.08 (7)
	100	464 ±28 (12)	2.01 ±0.05 (5)	11.55 ±0.98 (5)	3.14 ±0.33 (5)	0.86 ±0.15 (5)	1.49 ±0.19 (5)	0.27 ±0.07 (5)	30.5 ±2.1 (5)	12.7 ±1.2 (5)	59.2 ±3.3 (5)	3.31 ±0.39 (12)	2.25 ±0.23 (5)	1.31 ±0.12 (12)
	300	461 ±33 (12)	2.04 ±0.05 (5)	11.39 ±1.46 (5)	3.21 ±0.35 (5)	0.84 ±0.12 (5)	1.38 ±0.06 (5)	0.28 ±0.06 (5)	31.1 ±3.5 (5)	14.7 ±3.1 (5)	58.3 ±6.6 (5)	3.53 ±0.35 (12)	2.23 ±0.38 (5)	1.37 ±0.11 (12)
	1000	475 ±16 (7)	2.05 ±0.05 (5)	11.76 ±0.94 (5)	3.21 ±0.21 (5)	0.75 ±0.13 (5)	1.58 ±0.08 (5)	0.33 ±0.04 (5)	36.6 ±4.1 (5)	14.0 ±1.6 (5)	59.2 ±7.1 (5)	3.60 ±0.13 (7)	2.42 ±0.29 (5)	1.38 ±0.08 (7)
Relative @	0	468 ±34 (7)	0.45 ±0.02 (5)	2.54 ±0.19 (5)	0.65 ±0.06 (5)	0.19 ±0.02 (5)	0.32 ±0.01 (5)	0.08 ±0.02 (5)	7.0 ±0.9 (5)	3.1 ±0.2 (5)	15.7 ±4.5 (5)	0.77 ±0.07 (7)	0.48 ±0.05 (5)	0.29 ±0.02 (7)
	100	464 ±28 (12)	0.44 ±0.02 (5)	2.51 ±0.09 (5)	0.68 ±0.07 (5)	0.19 ±0.03 (5)	0.32 ±0.02 (5)	0.06 ±0.01 (5)	6.7 ±0.8 (5)	2.8 ±0.3 (5)	12.9 ±1.1 (5)	0.72 ±0.09 (12)	0.49 ±0.07 (5)	0.28 ±0.02 (12)
	300	461 ±33 (12)	0.45 ±0.03 (5)	2.47 ±0.10 (5)	0.70 ±0.05 (5)	0.18 ±0.02 (5)	0.30 ±0.03 (5)	0.06 ±0.01 (5)	6.8 ±0.9 (5)	3.2 ±0.4 (5)	12.8 ±1.9 (5)	0.77 ±0.07 (12)	0.49 ±0.08 (5)	0.30 ±0.02 (12)
	1000	475 ±16 (7)	0.43 ±0.02 (5)	2.48 ±0.15 (5)	0.68 ±0.04 (5)	0.16 ±0.03 (5)	0.33 ±0.01 (5)	0.07 ±0.01 (5)	7.7 ±0.8 (5)	2.9 ±0.3 (5)	12.5 ±1.5 (5)	0.76 ±0.03 (7)	0.51 ±0.06 (5)	0.29 ±0.03 (7)

Each value is expressed as mean ± S.D.
(n) : No. of animals.
@ : Relative organ weight per 100g body weight.

Table 22-2 Absolute and relative organ weights of male rats treated orally with C.I.pigment blue in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test
< After recovery period >

	Dose (mg/kg)	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyroid (mg)	Pituitary (mg)	Adrenal (mg)	Testis (g)	Seminal vesicle (g)	Epididymis (g)
Absolute	0	508 ±28 (5)	2.05 ±0.10 (5)	12.03 ±1.08 (5)	3.37 ±0.16 (5)	0.78 ±0.09 (5)	1.48 ±0.05 (5)	0.37 ±0.08 (5)	32.6 ±3.5 (5)	14.3 ±0.4 (5)	57.5 ±6.4 (5)	3.52 ±0.21 (5)	2.57 ±0.36 (5)	1.43 ±0.07 (5)
	1000	492 ±41 (5)	2.06 ±0.09 (5)	11.89 ±1.70 (5)	3.23 ±0.29 (5)	0.75 ±0.13 (5)	1.41 ±0.05 (5)	0.27 ±0.06 (5)	30.1 ±3.6 (5)	13.8 ±1.8 (5)	62.0 ±10.9 (5)	3.35 ±0.57 (5)	2.65 ±0.49 (5)	1.44 ±0.25 (5)
Relative @	0	508 ±28 (5)	0.41 ±0.03 (5)	2.37 ±0.16 (5)	0.66 ±0.04 (5)	0.15 ±0.01 (5)	0.29 ±0.02 (5)	0.07 ±0.02 (5)	6.4 ±0.8 (5)	2.8 ±0.2 (5)	11.3 ±1.4 (5)	0.69 ±0.05 (5)	0.51 ±0.06 (5)	0.28 ±0.02 (5)
	1000	492 ±41 (5)	0.42 ±0.03 (5)	2.41 ±0.18 (5)	0.66 ±0.04 (5)	0.15 ±0.02 (5)	0.29 ±0.02 (5)	0.06 ±0.01 (5)	6.1 ±0.4 (5)	2.8 ±0.4 (5)	12.6 ±2.1 (5)	0.68 ±0.12 (5)	0.54 ±0.08 (5)	0.29 ±0.04 (5)

Each value is expressed as mean ± S.D.
(n) : No. of animals.
@ : Relative organ weight per 100g body weight.

Table 23-1 Absolute and relative organ weights of female rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test
< After administration period >

	Dose (mg/kg)	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyroid (mg)	Pituitary (mg)	Adrenal (mg)
Absolute	0	310 ±39 (5)	1.92 ±0.09 (5)	10.84 ±1.09 (5)	2.01 ±0.50 (5)	0.70 ±0.14 (5)	1.03 ±0.12 (5)	0.28 ±0.05 (5)	26.7 ±2.5 (5)	20.3 ±3.2 (5)	80.1 ±13.2 (5)
	100	326 ±36 (5)	1.94 ±0.06 (5)	11.09 ±1.12 (5)	2.15 ±0.23 (5)	0.67 ±0.10 (5)	1.05 ±0.06 (5)	0.27 ±0.04 (5)	26.6 ±2.6 (5)	18.7 ±2.0 (5)	75.3 ±17.5 (5)
	300	319 ±16 (5)	1.91 ±0.07 (5)	10.64 ±1.17 (5)	2.03 ±0.15 (5)	0.71 ±0.11 (5)	1.04 ±0.08 (5)	0.30 ±0.10 (5)	25.6 ±1.7 (5)	19.5 ±2.0 (5)	79.4 ±12.3 (5)
	1000	311 ±25 (5)	1.88 ±0.09 (5)	10.03 ±0.62 (5)	1.95 ±0.19 (5)	0.62 ±0.15 (5)	1.04 ±0.10 (5)	0.32 ±0.10 (5)	24.0 ±2.4 (5)	18.8 ±1.6 (5)	69.8 ±11.9 (5)
Relative @	0	310 ±39 (5)	0.62 ±0.07 (5)	3.51 ±0.17 (5)	0.64 ±0.08 (5)	0.22 ±0.03 (5)	0.33 ±0.02 (5)	0.09 ±0.01 (5)	8.7 ±0.9 (5)	6.5 ±0.3 (5)	25.9 ±3.9 (5)
	100	326 ±36 (5)	0.60 ±0.06 (5)	3.40 ±0.18 (5)	0.66 ±0.06 (5)	0.21 ±0.03 (5)	0.32 ±0.03 (5)	0.08 ±0.02 (5)	8.2 ±0.7 (5)	5.8 ±0.4 (5)	23.8 ±9.0 (5)
	300	319 ±16 (5)	0.60 ±0.03 (5)	3.33 ±0.27 (5)	0.64 ±0.04 (5)	0.22 ±0.04 (5)	0.33 ±0.03 (5)	0.09 ±0.03 (5)	8.0 ±0.7 (5)	6.1 ±0.5 (5)	24.9 ±4.1 (5)
	1000	311 ±25 (5)	0.61 ±0.05 (5)	3.23 ±0.22 (5)	0.62 ±0.02 (5)	0.20 ±0.05 (5)	0.34 ±0.02 (5)	0.10 ±0.03 (5)	7.7 ±0.5 (5)	6.1 ±0.9 (5)	22.7 ±5.1 (5)

Each value is expressed as mean ± S.D.
(n) : No. of animals.
@ : Relative organ weight per 100g body weight.

Table 23-2 Absolute and relative organ weights of female rats of satellite group treated orally C.I.pigment blue in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test < After recovery period >

	Dose (mg/kg)	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyroid (mg)	Pituitary (mg)	Adrenal (mg)
Absolute	0	297 ±17 (5)	1.92 ±0.05 (5)	7.49 ±0.45 (5)	1.95 ±0.23 (5)	0.55 ±0.04 (5)	0.94 ±0.11 (5)	0.33 ±0.06 (5)	25.6 ±3.7 (5)	18.7 ±2.3 (5)	81.6 ±8.4 (5)
	1000	291 ±30 (5)	1.86 ±0.06 (5)	6.97 ±0.78 (5)	1.76 ±0.20 (5)	0.53 ±0.12 (5)	0.94 ±0.09 (5)	0.30 ±0.08 (5)	24.6 ±3.3 (5)	18.2 ±3.5 (5)	70.7 ±13.0 (5)
Relative @	0	297 ±17 (5)	0.65 ±0.03 (5)	2.53 ±0.09 (5)	0.66 ±0.05 (5)	0.19 ±0.004 (5)	0.32 ±0.02 (5)	0.11 ±0.02 (5)	8.7 ±1.1 (5)	6.3 ±0.7 (5)	27.5 ±2.1 (5)
	1000	291 ±30 (5)	0.65 ±0.07 (5)	2.40 ±0.26 (5)	0.61 ±0.06 (5)	0.18 ±0.05 (5)	0.32 ±0.03 (5)	0.10 ±0.02 (5)	8.5 ±1.1 (5)	6.2 ±0.7 (5)	24.5 ±5.4 (5)

Each value is expressed as mean ± S.D.
(n) : No. of animals.
@ : Relative organ weight per 100g body weight.

Table 24-1 Incidence histopathological findings of male rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose (mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	5	5	5	5	5	5
Lung	: Mineralization, artery	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
	Accumulation, foam cell	-	4	#	#	4	#	#
		+	1	#	#	1	#	#
Heart	: Myocardial degeneration/fibrosis	-	4	#	#	4	#	#
		+	1	#	#	1	#	#
Liver	: Microgranuloma	-	4	#	#	4	#	#
		+	1	#	#	1	#	#
	Necrosis, focal	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
	Degeneration, fatty, hepatocyte, diffuse	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Stomach	: Squamous hyperplasia, limiting ridge	-	5	5	5	0	5	5
		+	0	0	0	5 **	0	0

- : Negtive. + : Slight. #: Not examined.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.
** : Significantly different from control at 1% level of probability.
No abnormalities were detected in the brain, pituitary, thyroid, parathyroid, trachea, intestine, adrenal, urinary bladder, seminal vesicle, spinal cord, sciatic nerve, bone marrow, mammary gland and lymph node from animals of control and 1000 mg/kg groups.

Table 24-2 Incidence histopathological findings of male rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose (mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	5	5	5	5	5	5
Kidney	: Cellular infiltration, lymphocyte, cortex	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
	Hyaline droplet, proximal tubular epithelium	-	0	#	#	0	#	#
		+	5	#	#	5	#	#
	Cyst, solitary	-	4	#	#	4	#	#
		+	1	#	#	1	#	#
	Basophilic tubules	-	3	#	#	2	#	#
		+	2	#	#	3	#	#
	Mineralization, tubular, cortex	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Thymus	: Hemorrhage	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Spleen	: Hematopoiesis, extramedullary	-	0	#	#	0	#	#
		+	5	#	#	5	#	#
	Deposit, pigment, brown	-	0	#	#	0	#	#
		+	5	#	#	5	#	#

- : Negtive. + : Slight. #: Not examined.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.
No abnormalities were detected in the brain, pituitary, thyroid, parathyroid, trachea, intestine, adrenal, urinary bladder, seminal vesicle, spinal cord, sciatic nerve, bone marrow, mammary gland and lymph node from animals of control and 1000 mg/kg groups.

Table 24-3 Incidence histopathological findings of male rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose (mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	5	5	5	5	5	5
Testis	: Atrophy, seminiferous tubule	-	5	#	#	5	#	0/1[a]
		++	0	#	#	0	#	1/1
	Hyperplasia, interstitial cell	-	5	#	#	5	#	0/1[a]
		+	0	#	#	0	#	1/1
Epididymis	: Granuloma, spermatic	-	5	0/1[a]	0/1[a]	5	#	#
		+	0	1/1	0/1	0	#	#
		++	0	0/1	1/1	0	#	#
Prostate	: Cellular infiltration, lymphocyte, interstitium	-	3	#	#	5	#	#
		+	2	#	#	0	#	#

- : Negtive. + : Slight. ++ : Moderate. #: Not examined.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.
a : Examined the organ with macroscopical abnormality.
No abnormalities were detected in the brain, pituitary, thyroid, parathyroid, trachea, intestine, adrenal, urinary bladder, seminal vesicle, spinal cord, sciatic nerve, bone marrow, mammary gland and lymph node from animals of control and 1000 mg/kg groups.

Table 25 Incidence histopathological findings of female rats treated with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

			After administration period				After recovery period	
		Dose(mg/kg)	0	100	300	1000	0	1000
		Fate	TK	TK	TK	TK	KR	KR
Organs	: Findings	No. of animals	5	5	5	5	5	5
Lung	: Mineralization, artery	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Liver	: Necrosis, focal	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Stomach	: Squamous hyperplasia, limiting ridge	-	5	5	5	1	5	4
		+	0	0	0	4 *	0	1
	Edema, submucosa, forestomach	-	5	5	5	4	5	5
		+	0	0	0	1	0	0
Kidney	: Cast, hyaline	-	4	#	#	4	#	#
		+	1	#	#	1	#	#
	Basophilic tubules	-	4	#	#	5	#	#
		+	1	#	#	0	#	#
Thymus	: Hemorrhage	-	5	#	#	4	#	#
		+	0	#	#	1	#	#
Spleen	: Hematopoiesis, extramedullary	-	0	#	#	0	#	#
		+	3	#	#	3	#	#
		++	2	#	#	2	#	#
	Deposit, pigment, brown	-	0	#	#	0	#	#
		+	5	#	#	5	#	#

- : Negtive. + : Slight. ++ : Moderate. #: Not examined.
TK : Terminal killing. KR : Killed by design after 14-day recovery period.
* : Significantly different from control at 5% level of probability.
No abnormalities were detected in the brain, pituitary, thyroid, parathyroid, trachea, intestine, adrenal, urinary bladder, ovary, uterus, spinal cord, sciatic nerve, bone marrow, mammary gland and lymph node from animals of control and 1000 mg/kg groups.

Table 26 The number of cells in seminiferous epithelia assessed by the individual examination in male rats treated orally with C.I.pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose (mg/kg)	No. of animals		Stage II-III			Stage V			Stage VII			Stage XII	
			G	P	T	G	P	T	G	R/P	T	G	Z/P
0	5	Mean	0.72	2.43	7.62	0.77	2.74	7.32	0.11	3.52	7.59	0.10	5.69
		S.D.	0.08	0.22	0.92	0.07	0.31	0.47	0.02	0.28	0.23	0.01	0.44
1000	5	Mean	0.68	2.23	7.14	0.72	2.50	7.25	0.09	3.73	7.64	0.08	5.26
		S.D.	0.08	0.23	0.42	0.06	0.29	0.68	0.01	0.38	0.37	0.01	0.22

G : spermatogonia.
P : pachytene spermatocyte.
R : preleptotene spermacyte.
Z : zygotene spermacyte.
T : round spermatid.

Table 27 Reproduction results of rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose (mg/kg)	0	100	300	1000
Estrous cycle (days, Mean ± S.D.)	4.1 ± 0.2	4.0 ± 0.1	4.1 ± 0.4	4.1 ± 0.2
No. of pairs mated	12	12	12	12
No. of pairs with successful copulation	12	12	12	12
Copulation index (%)	100	100	100	100
Pairing days until copulation (days, Mean ± S.D.)	2.4 ± 1.2	2.8 ± 1.3	2.3 ± 1.0	2.3 ± 1.0
No. of pregnant females	12	12	12	12
Fertility index (%)	100	100	100	100
No. of corpora lutea (Mean ± S.D.)	16.8 ± 1.7	17.3 ± 1.5	17.0 ± 1.8	15.6 ± 2.2
No. of implantation sites (Mean ± S.D.)	15.8 ± 1.6	15.9 ± 1.6	16.6 ± 1.8	14.7 ± 2.7
Implantation index (%, Mean ± S.D.)	94.8 ± 6.6	92.4 ± 7.4	97.7 ± 5.7	93.8 ± 8.7
No. of pregnant females with parturition	12	12	12	12
Gestation length (days, Mean ± S.D.)	22.3 ± 0.5	22.3 ± 0.5	22.4 ± 0.5	22.6 ± 0.5
No. of pregnant females with live pups	12	12	12	12
Gestation index (%)	100	100	100	100
No. of pregnant females with live pups on day 4	12	12	12	12

Copulation index = (No. of pairs with successful copulation/No. of pairs mated) ×100.
Fertility index = (No. of pregnant females/No. of pairs with successful copulation) ×100.
Gestation index = (No. of females with live pups/No. of pregnant females) ×100.

Table 28 Litter results of rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose(mg/kg)	0	100	300	1000
No. of pups born	15.0 ± 1.6	14.9 ± 1.2	15.7 ± 1.6	13.6 ± 3.1
Delivery Index (%)	94.9 ± 5.0	94.2 ± 7.8	94.9 ± 7.5	91.7 ± 11.0
No. of pups alive on day 0 of lactation				
Total	14.7 ± 1.7	14.9 ± 1.2	15.3 ± 1.5	13.3 ± 2.9
Male	6.8 ± 2.2	6.8 ± 2.7	7.9 ± 2.2	6.3 ± 2.5
Female	7.9 ± 3.1	8.1 ± 2.2	7.4 ± 2.5	7.0 ± 1.7
Live birth Index (%)	97.7 ± 3.5	100 ± 0	98.0 ± 5.1	98.5 ± 3.8
Sex ratio(Male/Female)	0.88	0.85	1.07	0.87
No. of pups alive on day 4 of lactation				
Total	14.6 ± 1.6	14.8 ± 1.1	15.3 ± 1.5	13.3 ± 2.9
Male	6.8 ± 2.2	6.8 ± 2.6	7.9 ± 2.2	6.3 ± 2.5
Female	7.8 ± 2.9	8.1 ± 2.2	7.3 ± 2.5	7.0 ± 1.7
Viability Index (%)	99.5 ± 1.7	99.5 ± 1.7	99.5 ± 1.8	100 ± 0
Body weight of live pups (g)				
on day 0				
Male	7.1 ± 0.6	7.0 ± 0.5	7.2 ± 0.7	7.4 ± 0.9
Female	6.7 ± 0.5	6.7 ± 0.4	6.8 ± 0.7	7.1 ± 0.8
on day 4				
Male	11.2 ± 1.1	11.3 ± 1.0	11.3 ± 1.2	12.1 ± 1.8
Female	10.9 ± 1.2	10.7 ± 1.0	10.6 ± 1.3	11.6 ± 1.8

Delivery index = (No. of pups born/No. of implantation sites)×100.
Live birth index = (No. of live pups on day 0/No. of pups born)×100.
Viability index = (No. of live pups on day 4/No. of live pups on day 0)×100.
Sex ratio = Total number of male pups/Total number of female pups.
Each value is expressed as Mean±S.D.,except sex retio.

Table 29 External findings of pups from pregnant rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose(mg/kg)	0	100	300	1000
No. of pups examined	180	179	188	162
No. of pups with external malformations#	0 (0)	0 (0)	0 (0)	0 (0)
External malformations#	0 (0)	0 (0)	0 (0)	0 (0)

: No. of pups (Mean±S.D. of individual litter percentages).

Table 30 Visceral findings of pups from pregnant rats treated orally with C.I. pigment blue 29 in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

Dose(mg/kg)	0	100	300	1000
No. of pups examined	180	179	188	162
No. of pups with visceral malformations	0 (0)	0 (0)	0 (0)	0 (0)
No. of pups with visceral variations#	4 (2.2±4.2)	1 (0.6±2.0)	0 (0)	5 (3.1±6.0)
Visceral variations#				
Thymic remnant in neck	3 (1.6±3.0)	0 (0)	0 (0)	4 (2.4±4.9)
Persistent left umbilical artery	1 (0.5±1.8)	0 (0)	0 (0)	1 (0.6±2.2)
Convoluted ureters	0 (0)	1 (0.6±2.0)	0 (0)	0 (0)

: No. of pups (Mean±S.D. of individual litter percentages).