DISCUSSION AND CONCLUSIONS

4,4′-Thiobis(6-*t*-butyl-*m*-cresol) (TBBC) is used in the rubber and plastics industries as an antioxidant and as a stabilizer in polyethylene and polyolefin food packaging materials. Because of concern regarding the elevated cancer risk of workers in the rubber industry, the National Cancer Institute nominated TBBC for toxicology and carcinogenesis studies as a representative of the sulfur-containing class of antioxidants used in rubber processing. Because food packaging appeared to represent the most widespread potential for human exposure, the oral route of administration was chosen for the 15-day, 13-week, and 2-year studies in F344/N rats and B6C3F$_1$ mice.

The principal toxic effects associated with the administration of TBBC in the present studies occurred in the liver and kidney of rats and mice. With the exception of the renal lesions observed in the 15-day and 13-week studies, these findings are in agreement with the few studies reported in the literature. Birnbaum *et al.* (1983) reported that the liver was the major site of metabolism of TBBC in rats and that the compound was excreted primarily in the bile. In a 30-day feed study in rats, 2,500 ppm TBBC produced increased liver weight and growth retardation; rats fed diets containing 500 ppm for 90 days displayed only reduced feed consumption and slight growth retardation (Lefaux, 1968). A dose-related increase in liver weight accompanied by a slight increase in the number of Kupffer cells was reported in females exposed to 200 mg/kg in a study in which mice were administered 10, 100, or 200 mg/kg daily by gavage for 14 days (Munson *et al.*, 1988). In the NTP 15-day studies in rats or mice receiving TBBC in feed at doses ranging from 1,000 to 25,000 ppm, liver toxicity was not observed in surviving animals. However, in the NTP 13-week studies in rats, absolute and relative liver weights were significantly greater in females receiving 5,000 ppm than in controls. Males and females in the 2,500 and 5,000 ppm groups exhibited Kupffer cell hypertrophy, hepatocyte necrosis, and bile duct hyperplasia. In addition, males and females exposed to 5,000 ppm TBBC also exhibited centrilobular hepatocyte hypertrophy. Consistent with these histopathologic findings in the 13-week rat studies, there were significant elevations in serum levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Increased levels of ALT are usually associated with damage to hepatocytes; increases in ALP are usually associated with biliary disease. Male and female rats receiving 5,000 ppm in these studies exhibited a significant increase in size and number of macrophages in the mesenteric lymph nodes; a lesser, but similar response occurred in 2,500 ppm rats.

The 13-week NTP study in mice also elicited hepatotoxicity in 2,500 ppm males and females as exhibited by slight but significant increases in absolute and relative liver weights and the presence of Kupffer cell hypertrophy and bile duct hyperplasia. The response in rats at the same exposure level (2,500 ppm) was similar, except that liver weights in 2,500 ppm rats were unaffected and necrosis and centrilobular hypertrophy were observed in rats but not in mice. Based on average daily feed consumption, 2,500 ppm rats ingested roughly one-third as much TBBC on a body weight basis as mice. Thus, the liver of rats may be more sensitive than that of mice to the effects of this chemical. Additionally, there was a mild increase in size and number of macrophages in mesenteric lymph nodes of male and female mice administered 2,500 ppm; this response was similar to that observed in 2,500 ppm rats.

In the 2-year rat study, the highest exposure level (2,500 ppm TBBC) produced liver toxicity. At this exposure level, males and females exhibited increases in liver weights, Kupffer cell hypertrophy, cytoplasmic vacuolization, and basophilic and mixed cell foci at the 15-month interim evaluation and at the end of the 2-year study. In addition, marked significant increases in serum ALT and sorbitol dehydrogenase activities (SDH) occurred in males and females at the 15-month evaluation; these cytoplasmic enzymes are released into the blood following hepatocellular injury. The mild but significant increases in ALP which occurred in males in various exposure groups at the 3-, 9-, and 15-month evaluations are indicative of disturbances involving the hepatobiliary system. This increase did not occur in females. Although certain liver responses occurred in males and females, liver weight increase was more pronounced in females, there was a strong significant increase in the incidence of cytoplasmic vacuolization

in females but not in males, and mixed cell foci occurred in twice as many 2,500 ppm females as 2,500 ppm males. Thus, the preponderance of these responses occurred in females.

The incidence of hepatocellular adenoma or carcinoma (combined) was slightly increased in male rats administered 2,500 ppm TBBC (0 ppm, 1/50; 500 ppm, 3/50; 1,000 ppm, 3/50; 2,500 ppm, 5/49), but the increased incidence was not significant and did not exceed the range of 0% to 10% in historical control male rats. Furthermore, the incidences of these neoplasms were not increased in females, despite the fact that females demonstrated a greater number of different nonneoplastic responses. Therefore, the incidence of hepatocellular adenoma or carcinoma (combined) in male rats is not considered a carcinogenic response to TBBC.

In contrast to the findings in the 13-week study at 2,500 ppm, liver weights of mice were unaffected and there were no microscopic findings of hepatotoxicity in mice exposed to 1,000 ppm TBBC in feed for 2 years. Since 1,000 ppm male and female mice actually had a greater average daily ingestion of TBBC on a mg/kg body weight basis than did rats exposed to 2,500 ppm TBBC, the lack of microscopic findings in mice may indicate (as appeared to be the case in the 13-week studies) a higher degree of liver sensitivity in rats. This conclusion is strengthened by the marked significant increase in ALT and SDH found in rats but not mice. Total bilirubin in 1,000 ppm male mice was slightly but significantly greater than that in controls at 9 and 15 months. This response did not occur in female mice or in rats. In addition, the serum activity of ALP was significantly higher in male and female mice at various exposure levels and time points; these increases were milder in degree but similar to those that occurred in the rats. Increases in serum levels of total bilirubin would be consistent with either cholestasis or a liver function disorder in which circulating bilirubin could not be removed by the liver for conjugation and excretion. Increases in both ALP activity and total bilirubin concentration would be consistent with cholestasis. However, increases in total bilirubin concentration related to cholestasis are usually accompanied by increases in direct bilirubin, which did not occur in the present studies. In males, liver lesions which occurred with a significant negative trend included fatty change, clear cell foci, and hepatocellular adenoma or carcinoma (combined). The significant negative trends were considered to be related to the administration of TBBC. In 1,000 ppm male mice, the incidence of hepatocellular adenoma or carcinoma (combined) was significantly lower than that of controls by pairwise comparison. This result may be due to the reduction in mean body weight, since a significant positive association has been found between liver neoplasm prevalence and body weight in male B6C3F$_1$ mice (Rao *et al.*, 1990).

Evidence of kidney toxicity was present in rats and mice in the NTP 15-day studies and in rats in the 13-week study. In 10,000 ppm rats in the 15-day study, necrosis of the papilla was observed in one female and two males and focal necrosis of the tubules was observed in four males and seven females. Eight male mice and three female mice receiving 5,000 ppm in the 15-day study had tubule necrosis. Following 13 weeks of exposure, pigmentation and degeneration of the renal cortical tubule epithelial cells were present in male and female rats receiving 2,500 or 5,000 ppm; mild to moderate cortical tubule necrosis was also found in 5,000 ppm males and females. These lesions appear to be related to the administration of TBBC. Kidney lesions were not reported in the feed studies summarized by Lefaux (1968) in which rats were exposed to 500 or 2,500 ppm for 30 days and 50 or 500 ppm for 90 days. In the present NTP 2-year rat study, chronic nephropathy common in aging rats was found in nearly all animals. However, the severity of nephropathy in 2,500 ppm females was significantly greater than that in the control group, and the increase was attributed to the administration of TBBC. In remaining female exposure groups and in all exposed males, the severity of nephropathy was similar to that of the controls.

In the 13-week NTP studies, TBBC again affected hematology parameters in rats and mice. Significant decreases in hemoglobin and hematocrit values occurred in male rats and male and female mice; mean erythrocyte volume values were significantly lower in rats and mice; erythrocyte counts were significantly lower in mice but not in rats; and neutrophil counts were significantly higher in rats but not in mice.

In the 2-year study, results of hematocrit and hemoglobin analyses performed on two sets of male rats evaluated at 15 months were conflicting. However,

the results in each set of females indicated significant decreases; male mice also had a significant decrease in these parameters and in erythrocyte counts.

The significant increases in platelets which occurred mainly in 2,500 ppm male and female rats in the 2-year study are consistent with a reactive thrombocytosis. This condition has been observed with inflammations, trauma, surgery, hyposplenic or asplenic states, malignancies, acute blood loss, and hyperadrenocorticism.

The neurotoxicity evaluation in the 13-week study demonstrated statistically significant increases in grip strength in exposed rats, which did not occur in the 2-year study. While these evaluations were performed on animals of the same strain and age using the same methodology, they were conducted at two different laboratories. Therefore, the toxicologic significance of the positive findings in the 13-week study is uncertain. Further, no significant effects of TBBC were found on motor nerve excitability or conduction, neuromuscular transmission, muscle contractility, or neuropathology.

Although the rate of survival was less than 50% in 1,000 ppm male rats (42%) and 2,500 ppm male rats (36%), the survival rate for the control group was only 36% and reduced survival does not appear to be chemical related. Further, 50% of the 2,500 ppm males survived until week 97 and 50% of the 1,000 ppm male rats survived until week 101, allowing adequate time for the possible development of neoplasms. Some degree of chemical-related toxicity in 2,500 ppm rats was observed; mean body weights of rats in this group were slightly but consistently reduced, despite the fact that feed consumption by this group was similar to that by the controls. The final mean body weight of 2,500 ppm males was 5% less than that of the controls; the mean body weight of females exposed to 2,500 ppm TBBC dropped to 14% below that of the controls at week 65 and was 6% lower than that of the controls at the end of the study. There was also enough evidence of liver toxicity in the 2,500 ppm male and female rats in the 2-year study to indicate that a greater exposure level would have compromised the sensitivity of the study to detect neoplasia. In addition, exposure to 5,000 ppm TBBC in the 13-week study resulted in a significant increase in absolute and relative liver weight in females, marked reductions in final mean body weights and feed consumption in both males and females, and liver and kidney toxicity in males and females, as mentioned earlier. These observations indicate that rats could not have tolerated an exposure level much higher than 2,500 ppm.

Although no overt organ toxicity was observed in mice in the highest exposure group in the 2-year study (1,000 ppm), the reductions in final mean body weights were indicative of a toxic response to TBBC. The final mean body weights of 1,000 ppm male and female mice were 8% and 18% lower than that of the controls, respectively; feed consumption by the 1,000 ppm males was similar to that by the controls. In the 13-week study, 2,500 ppm males had a final mean body weight 15% lower than that of the controls and the final mean body weight of 2,500 ppm females was 22% lower than that of the controls. This exposure level also produced Kupffer cell hypertrophy and bile duct hyperplasia in males and females. At 15 months, males had a significant increase in total bilirubin at all exposure levels and 500 and 1,000 ppm females had a significant elevation in ALP. It is probable that an exposure level greater than 1,000 ppm for 2 years would have caused severe weight loss and liver toxicity in mice.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4'-thiobis(6-*t*-butyl-*m*-cresol) in male or female F344/N rats administered 500, 1,000, or 2,500 ppm or in male or female B6C3F$_1$ mice administered 250, 500, or 1,000 ppm.

Nonneoplastic lesions associated with exposure to TBBC included: Kupffer cell hypertrophy, cytoplasmic vacuolization, and mixed cell foci in the liver of male and female rats, fatty change in the liver of female rats, and an increase in the severity of nephropathy in the kidney of female rats. In addition, decreased incidences of fibroadenoma, adenoma, or carcinoma (combined) were observed in the mammary gland of female rats. Decreases also occurred in the incidences of fatty change, clear cell foci, and adenoma or carcinoma (combined) in the liver of male mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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(トリフルオロメチル)ベンゼン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

Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test of Trifluoromethylbenzene by Oral Administration in Rats

要　約

(トリフルオロメチル)ベンゼンは，化学産業の分野で染料あるいは高重合体の原料として使用されている化学物質である．本被験物質の経口投与によるLD_{50}値は，マウスで10000 mg/kg，ラットで15000 mg/kgであることが報告されているが[1]，反復投与毒性あるいは生殖発生毒性についての報告は見当たらない．今回，OECD既存化学物質安全性点検に係わる毒性試験の一環として，(トリフルオロメチル)ベンゼンの，20，100および500 mg/kgをCrj：CD(SD系)ラットの雌雄(各12匹/群)に交配前14日間，雄ではその後交配期間を含む35日間，雌では交配期間，妊娠期間および分娩後3日まで経口投与し，親動物に対する反復投与毒性および生殖能力ならびに次世代児の発生・発育に及ぼす影響について検討した．

1.反復投与毒性

一般状態，体重推移，摂餌量および血液学検査では，被験物質投与の影響はみられなかった．血液生化学検査では，500 mg/kg群に総蛋白質，アルブミン，総コレステロール，トリグリセライドおよびリン脂質の増加ならびにグルコースの減少が認められた．剖検では，500 mg/kg群の雄で腎臓の肥大および退色がみられ，器官重量では雄で100 mg/kg以上の群に肝臓および腎臓重量の増加，雌では500 mg/kg群に肝臓重量の増加が認められた．病理組織学検査では，100 mg/kg以上の群の雌雄で小葉中心性の肝細胞の肥大がみられ，雄で近位尿細管上皮の硝子滴の出現，壊死および好塩基性変化ならびに近位尿細管の拡張が認められた．

2.生殖発生毒性

親動物の生殖に関しては，性周期，雌雄の交尾率，授(受)胎率，黄体数，着床数，妊娠期間，出産率および分娩状態に被験物質投与の影響は認められなかった．死産率，出生児数，出生率および出生児の性比に被験物質投与の影響はみられず，外表異常の発生もなかった．出生児体重の増加抑制が20 mg/kg以上の投与群でみられたが，剖検では被験物質投与の影響は認められなかった．

以上のことから，本試験条件下における反復投与毒性に関する無影響量は雌雄とも20 mg/kg，生殖発生毒性に関する無影響量は親動物では500 mg/kg，出生児では20 mg/kg未満と推察された．

方　法

1.被験物質および投与液の調製

(トリフルオロメチル)ベンゼン(純度99.7%，Lot No.KCM2054，和光純薬工業㈱提供)は，エタノールおよびエーテルに易溶，水に不溶の無色透明の液体である．入手後の被験物質は室温で保管し，投与期間終了後に供給源にて分析を行って試験期間中安定であったことを確認した．媒体にはコーンオイル(キシダ化学㈱，Lot No L51257F)を使用し，これに被験物質を0.4，2および10 w/v%濃度になるように懸濁して投与液を調製した．調製した投与液は冷蔵保存した．なお，投与開始週に，投与液の濃度を測定し，設定値の±10%以内にあることを確認した．また，投与開始前に，本調製法による0.1，1および10 w/v%懸濁液が低温遮光下で調製後8日間安定であることを確認した．

2.使用動物および飼育条件

9週齢のSprague-Dawley系ラット(Crj：CD，日本チャールス・リバー㈱)を雌雄各55匹購入し，7日間の検疫馴化を行ったのち，雌雄各48匹を選んで10週齢で試験に使用した．投与開始時の体重は雄で319.7～391.2 g，雌で224.7～265.0 gであった．動物は温度24±2℃，湿度55±10%，照明12時間(午前7時～午後7時)および換気回数13回/時に設定したバリアーシステム飼育室でステンレススチール製ハンガーケージに，投与期間中は1匹(雌雄別)，交配期間中は2匹(雌雄各1匹)，妊娠および哺育期間中は床敷(ホワイトフレーク，日本チャールス・リバー㈱)を入れたポリカーボネイト製ケージに1匹ずつ(哺育期間中は哺育児を含む)収容し，飼育した．飼料は，固型飼料(MF，オリエンタル酵母工業㈱)を，飲水は次亜塩素酸ナトリウムを添加(約2 ppm)した水をそれぞれ自由に摂取させた．

3.投与量，投与方法，試験群構成および群分け

投与量は，予備試験の結果より設定した．すなわち，本被験物質の0，100，500および1000 mg/kgを2週間反復投与した結果，500 mg/kg以上の群でGPT，総コレステロールおよびリン脂質の増加または増加傾向ならびに肝臓重量の増加がみられ，100 mg/kg群にもその傾向が窺われた．したがって，本試験では高用量を500 mg/kgとし，以下100および20 mg/kgを設定した．

投与経路は経口とし，雄では交配前14日間およびその後交配期間を含む35日間の合計49日間，雌では交配前14日間，交配期間(最長14日間)，妊娠期間および哺育3日までの期間，1日1回，胃管を用いて投与した．投与容量は5 ml/kgとし，雄ならびに交配前および交配期間中の雌については最新の体重を基に，交尾成立後の雌については妊娠0日の体重を基にそれぞれ算出した．

試験群は，上記3用量にコーンオイルのみを投与する対照を加え計4群とした．1群当たりの動物数は雌雄各12匹とし，群分けは，投与開始前日の体重を基に層別連続無作為化法で行った。

4.反復投与毒性に関する観察・検査

1)一般状態

雌雄とも，全例について一般状態の観察および死亡の有無を毎日観察した．

2)体重および摂餌量

体重については，雄は投与期間を通じて週2回測定した．雌は，交配前の投与期間および交配期間中は週2回，妊娠期間中は妊娠0，4，7，10，14，17および21日，哺育期間中は哺育0(分娩日)および4日に測定した．摂餌量については，交配期間を除き体重測定日に測定したが，妊娠および哺育0日は測定せず，翌日測定した．

3)血液学検査

雄全例について，投与期間終了後に，18時間以上絶食させたのち，ペントバルビタール・ナトリウム麻酔下に開腹し，腹部大静脈から採血を行った．採取した血液は EDTA-2K処理(EDTA-2K加血液)して多項目自動血球計数装置(Sysmex CC-780，東亜医用電子㈱)を用いて，白血球数(電気抵抗検出方式)，赤血球数(電気抵抗検出方式)，ヘモグロビン量(オキシヘモグロビン法)，ヘマトクリット値(血球パルス波高値検出方式)および血小板数(電気抵抗検出方式)を測定し，これらを基に平均赤血球容積(MCV)，平均赤血球血色素量(MCH)および平均赤血球血色素濃度(MCHC)を算出した．

4)血液生化学検査

血液学検査に引き続き採取した血液を室温で約60分間放置後，3000回転/分で10分間遠心分離し，得られた血清を用いて自動分析装置(736-10，㈱日立製作所)により，総蛋白質(ビウレット法)，アルブミン(BCG法)，A/G比(総蛋白質およびアルブミンより算出)，総ビリルビン(アルカリアゾビリルビン法)，GOT(Karmen法)，GPT(Wróblewski-La Due法)，γ-グルタミルトランスペプチダーゼ(L-γ-グルタミル-DBHA基質法)，アルカリ性フォスファターゼ(*p*-ニトロフェニルリン酸基質法)，総コレステロール(COD-DAOS法)，トリグリセライド(GPO-DAOS法・グリセリン消去法)，リン脂質(酵素法・DAOS発色法)，グルコース(グルコキナーゼ・G-6-PDH法)，尿素窒素(ウレアーゼ-GlDH法)，クレアチニン(Jaffé法)，無機リン(モリブデン酸直接法)およびカルシウム(OCPC法)を測定した．また，電解質分析装置(PVA-αⅢ，㈱アナリティカル・インスツルメンツ)によりナトリウム(電極法)，カリウム(電極法)およびクロール(電量滴定法)を測定した．

5)病理学検査

雄では投与期間終了後の採血を行ったのちに，雌では哺育4日にエーテル麻酔下で外側腸骨動脈を切断して放血死させ，解剖して諸器官および組織の肉眼的観察を行い，雌について黄体数および着床痕数を調べた．剖検後，脳，心臓，肺(気管支を含む)，胸腺，肝臓，脾臓，腎臓，副腎，精巣，精巣上体および卵巣を摘出して器官重量(絶対重量)を測定するとともに，剖検日の体重を基に体重比器官重量(相対重量)を算出した．重量測定器官に加え，肉眼的異常部位を採取して10%中性緩衝ホルマリン溶液(精巣および精巣上体はブアン液で前固定)で固定した．対照群および500 mg/kg群の脳，心臓，肺(気管支を含む)，胸腺，肝臓，脾臓，腎臓，副腎，精巣，精巣上体および卵巣については，常法に従ってパラフィン切片を作製し，ヘマトキシリン・エオジン(HE)染色を施し，光学顕微鏡下で観察した．さらに，肝臓，胸腺および腎臓については，被験物質投与に関連したと考えられる変化がみられたため，100 mg/kg以下の投与群のこれらの器官についても同様の検査を行った．また，一部の動物で肝臓および腎臓の脂肪染色を実施した．なお，肉眼的異常部位，交尾が確認されなかった雄の精巣，精巣上体および雌の卵巣については，すべて病理組織学検査を行った．

5.生殖発生毒性に関する観察・検査

1)生殖機能

雌について交配開始日の2週間前(投与開始日)から交尾確認日まで，毎日午前の一定時間に膣垢を採取し，性周期検査を行った．

交配は雌雄(12週齢)1対1で一晩同居させる方法で行い，翌朝膣垢中の精子または膣栓が確認されたものを交尾成立とし，その日を妊娠0日とした．また，交配は同一群内で行い，交配期間は最長2週間とした．なお，交配相手が死亡した雌については，同群内の交尾が確認された雄と同居させた．交配期間終了後，交尾所要日数，交尾率［(交尾動物数/同居動物数)×100］および授(受)胎率［(受胎動物数/交尾動物数)×100］を算出した．

2)分娩および哺育状態ならびに新生児の観察

交尾が確認された雌は全例を自然分娩させ，分娩徴候を含め分娩状態および授乳，営巣などの哺育状態を観察するとともに，妊娠期間，出産率［(生児出産雌数/妊娠雌数)×100］を算出した．午後12時の時点で分娩が終了している動物を当該日分娩とし，その日を哺育0日とした。出産児については，分娩時に出産児数，出生児数，死産児数，出生児の性別および外表異常を検査した．出生児については，出生日および哺育4日に体重を個体

ごとに測定するとともに出生率[(出生児数/着床痕数)×100]および4日生存率 [(生後4日の生児数/出生児数)×100] を算出した．生後4日に出生児の全例をエーテル麻酔下で放血致死させ，器官・組織の肉眼的観察を行った．

6.統計処理

体重，摂餌量，血液学検査，血液生化学検査，交尾所要日数，性周期検査値(発情回数，発情周期)，器官重量，妊娠期間，黄体数，着床痕数，総出産児数および出生児数については各群ごとに平均値と標準偏差を求め，Bartlett法により分散の均一性を検定した．分散が均一な場合は一元配置型の分散分析を行い，ここで群間に有意差が認められ，かつ，各群の例数が同じ場合はDunnett法により，異なる場合はScheffé法により対照群と各群の一対比較検定を行った．分散が均一でない場合はKruskal-Wallisによって順位検定を行い，群間に有意差が認められ，かつ，各群の例数が同一の場合はDunnett型の，異なる場合はScheffé型の一対比較検定を行った．上記分散分析あるいはKruskal-Wallis法で群間に有意差を認めない場合は各群の多重比較は行わなかった．また交尾率，受(授)胎率，出産率および出生児の性比についてはχ^2検定により，死産率，出生率および4日生存率についてはWilcoxonの順位和検定により対照群と各投与群間の比較を行った．いずれの場合も有意水準を5%とした．なお，出生児に関する測定値については一腹単位で処理した．

結　果

1.反復投与毒性

1) 一般状態

各投与群の雌雄とも投与期間を通して被験物質投与による一般状態の変化は認められなかった．なお，投与過誤により500 mg/kg群の雌雄各1例が死亡した．

2) 体重(Fig.1) および摂餌量

各投与群の雌雄とも投与期間を通して体重および摂餌量に対照群との間の差は認められなかった．

3) 血液学検査(Table 1)

500 mg/kg群でMCHにごく軽度の減少がみられたが，他の赤血球系パラメータに変動はみられず，毒性学的意義はないものと考えられた．

4) 血液生化学検査(Table 2)

GOTの低下が100および500 mg/kg群で認められた．さらに，500 mg/kg群で総蛋白質，アルブミン，総コレステロール，トリグリセライド，リン脂質およびカルシウムの増加ならびにグルコースの減少が認められた．

5) 器官重量(Table 3)

雄では，500 mg/kg群で肝臓および腎臓の絶対および相対重量の増加がみられ，100 mg/kg群においても肝臓の絶対重量の増加傾向および相対重量の増加，腎臓の絶対および相対重量の増加傾向が認められた．

雌では，500 mg/kg群で肝臓の絶対および相対重量の増加が認められた．

6) 剖検所見

投与期間終了後の雄の剖検では，500 mg/kg群で腎臓の肥大が2例，退色が3例，小陥凹が1例に認められた．そのほか，対照群の1例に肺の灰白色化がみられた．

哺育4日の雌の剖検では，脾臓と周囲脂肪組織との癒着が対照群の1例，肝臓の横隔膜面結節が20 mg/kg群の1例に認められた．

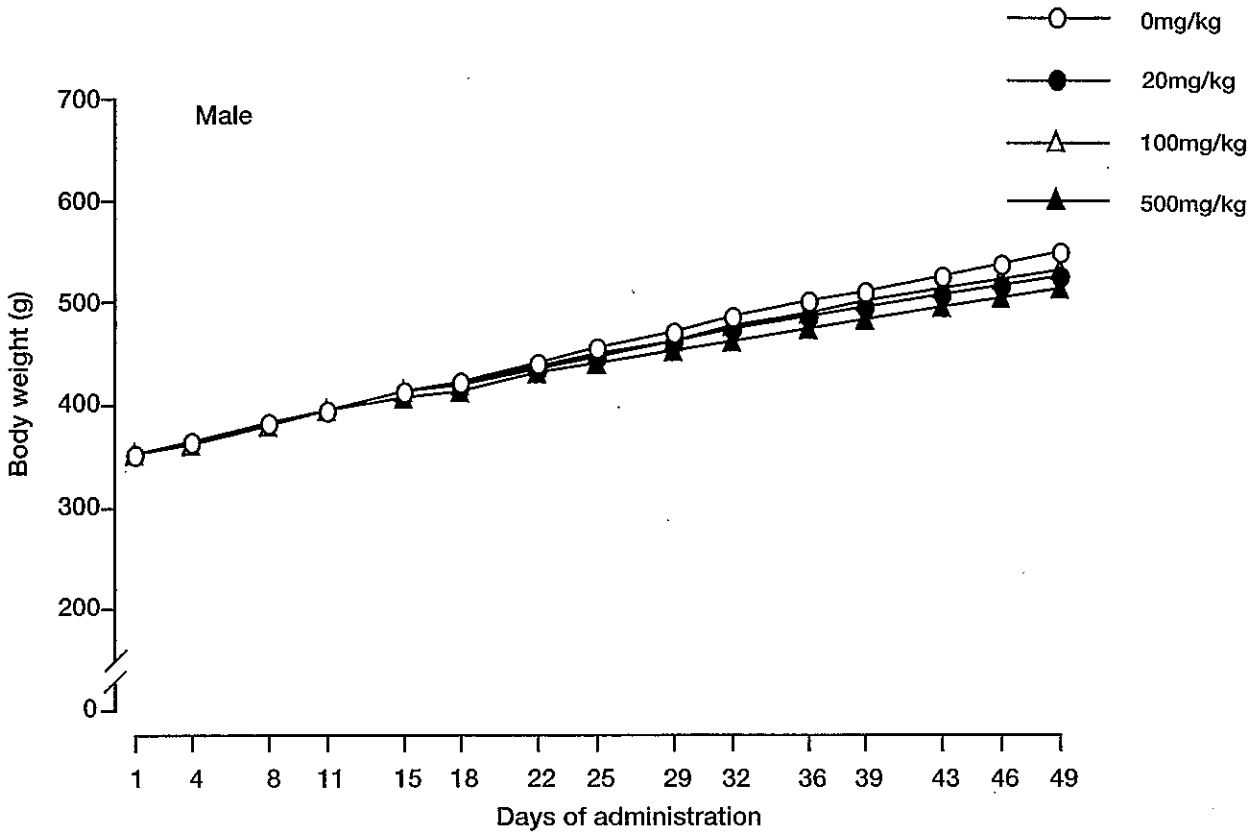
7) 病理組織学検査(Table 4)

雄では，小葉中心性の肝細胞の肥大が500 mg/kg群の11例，100 mg/kg群の10例に認められた．また，腎臓の近位尿細管上皮の硝子滴の出現および上皮の壊死が500 mg/kg群の11例，100 mg/kg群の12例，近位尿細管の拡張が500 mg/kg群の9例，100 mg/kg群の1例，近位尿細管上皮の好塩基性変化が500 mg/kg群の6例，100 mg/kg群の2例，腎臓の瘢痕が500 mg/kg群の1例に認められた．そのほか，偶発的変化として対照群では雄の1例に肺のマクロファージによる肺胞中隔あるいはリンパ球による血管周囲への細胞浸潤および動脈中膜の肥厚がみられた．

雌では，小葉中心性の肝細胞の肥大が500 mg/kg群の10例，100 mg/kg群の3例，胸腺皮質の萎縮が500 mg/kg群の2例に認められた．そのほか，偶発的変化として対照群および500 mg/kg群の各1例に脾臓の髄外造血の亢進，対照群の1例に脾臓と周囲脂肪組織との癒着が認められた．また，対照群の1例に肺のマクロファージによる肺胞中隔あるいはリンパ球による血管周囲への細胞浸潤および動脈中膜の肥厚がみられた．

500 mg/kg群の雌の死亡例では，生存例と同様の小葉中心性の肝細胞の肥大がみられたほか，腎臓の間質の毛細血管，小静脈および動脈ならびに糸球体毛細血管の血栓がみられ，さらに胸腺皮質のリンパ球の壊死および前胃の潰瘍が認められた．

100 mg/kg群の全児死亡例では，肝小葉辺縁部から中間帯にかけての脂肪化，近位尿細管上皮の脂肪化および壊死が認められた．



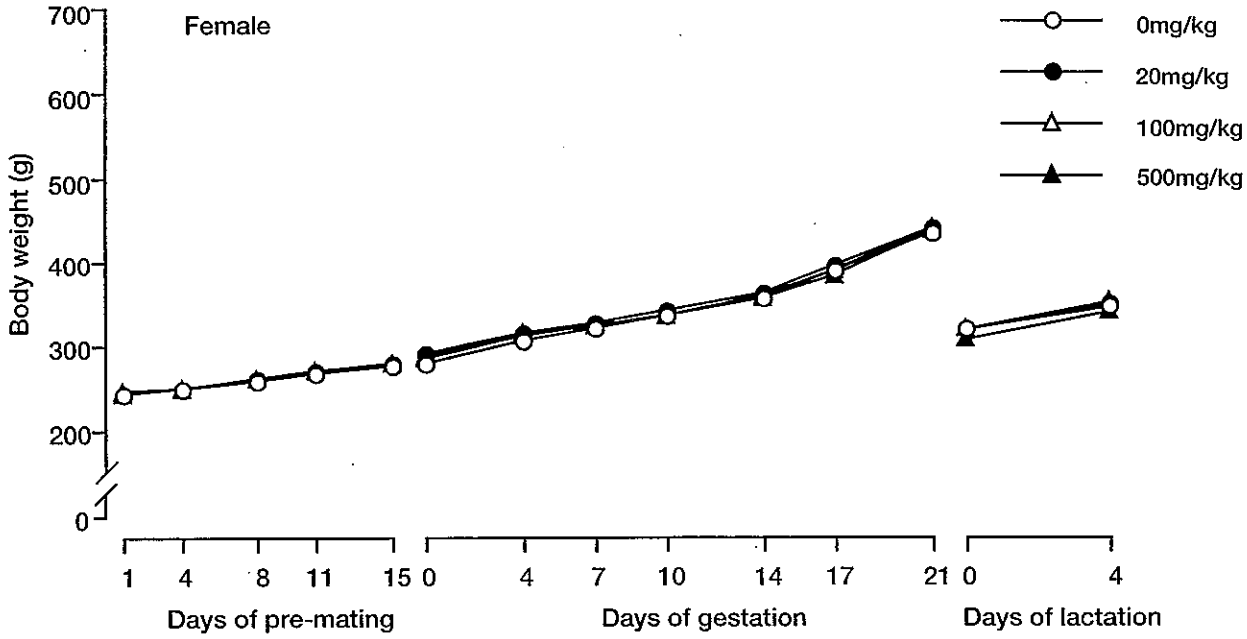


Fig. 1 Mean body weight changes of rats treated orally with trifluoromethylbenzene in the combined repeat dose and reproductive/developmental toxicity screening test