IARC (1987) concluded that the chlorophenoxy herbicides should be placed in group 2B because of limited evidence for carcinogenicity to humans and because no adequate published data were available on the carcinogenicity of MCPA to animals.

While PCOC is a breakdown product and possible contaminant of (impurity in) MCPA, implications of these finding for the effects of PCOC itself can remain only speculative.

4.1.2.8 Toxicity for reproduction

In a combined repeated dose/reproduction screening test carried out according to OECD draft guideline 422 (Hansen, 1996) groups of 10 male and 10 female rats were given 0, 50, 200, or 600 mg PCOC/kg in soybean oil by gavage for two weeks prior to mating and until day 20 of gestation. No toxic effects on any reproductive or developmental parameters were observed, resulting in a no effect level for these endpoints of 600 mg/kg.

In a recently conducted *in vitro* assay for estrogenic effects using human breast cancer cells (Körner et al, 1996; Körner et al, 1997), PCOC was found to express activity corresponding to $1x10^{-6}$ that of 17-β-Estradiol. It is difficult to evaluate what possible influence this might have on reproductive parameters.

Risk characterisation

4.1.2.9 General aspects

Major effects of possible concern are corrosivity, acute inhalation toxicity and repeat dose toxicity. Direct exposure is possible for production workers, and indirect exposure for workers, consumers and the general population.

The human risk assessment according to the pesticide scenario is not conducted based on a decision at EU Technical meeting on risk assessment of existing substances (TM III, Nov.1996) referring to this part of the risk assessment being conducted by DGVI working group on risk assessment of plant protection products.

4.1.2.10 Workers[4]

Production facility workers (see 4.1.1.2. for exposure levels). Realistic worst case exposure is likely to be of the order of 0.7 mg/kg/day according to information provided by one of the producers (A.H. Marks, 1997b).

[4] Herbicide application workers. (see 4.1.1.1. for exposure levels).
The exposure PCOC as a 1% impurity in MCPA can be in the order 0.28 mg/kg/day (agricultural) or 0.35 mg/kg/day (municipal weed control).

For the end-points irritation/corrosivity the concentration is below the level of concern. For repeat dose toxicity this should not present a major health problem, e.g. for repeat dose toxicity the margin of safety based on a NOAEL of 200 mg/kg/day is 200/0.35= 571.

The margin of safety for effects is in the order of 300-600, thus workplace exposure to PCOC does not seem to present a major risk.

Repeat dose toxicity is not likely to present a major health problem. The margin of safety based on a NOAEL of 200 mg/kg/day (slight effect on liver enzyme (ALAT), haemoglobin conc.) is 200/0.7 = 285.

Also the end-point irritation/corrosivity does not seem to cause any health concern. In situations with possible contact with the substance safety measures, such as wearing appropriate PPE, are prescribed in the existing productions. Further, health surveillance programmes including examination of the respiratory function have been undertaken for several years. According to the medical reports submitted by the producers no significant effects on the respiratory system have been observed.

It should be stressed that direct skin contact with PCOC can lead to burns and/or irritation, but that adequate warning of this effect is given by the manufacturers classification (R-35) and that the wearing of approriate PPE is compulsary when exposure at the workplace is possible (according to the UK Control Of Substances Hazardous to Health regulation - referred to in (Marks A H , 1997a)).

Conclusion of the risk assessment for workers:

( ) i) There is need for further information and/or testing
(X) ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already
( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

4.1.2.11 Consumers

For this group exposure may be in the order of 0.07 mg/kg for each event corresponding to a daily dose of $9.6x10^{-4}$ mg/kg/day (see 4.1.1.3. for further details). With a NOAEL for repeat dose toxicity of 200 mg/kg/day the margin of safety is at least 20,000 for each single event.

Conclusion of the risk assessment for consumers:

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

4.1.2.12 Man exposed indirectly via the environment

The exposure of man indirectly via the environment through herbicide use is likely to be $10x10^{-8}$ mg/kg/day via human intake media.

Regional exposure resulting from production of PCOC is estimated as being low ($1.3x10^{-5}$ mg/kg/day), while local indirect exposure estimates of $1.2x10^{-4}$ mg/kg/day does not give rise to immediate concern with regard to corrosivity or repeat dose toxicity.

Conclusions of the risk assessment for man exposed indirectly via the environment:

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

4.1.2.13 Combined exposure

On the basis of the conclusion made in 4.1.1.2. and 4.1.1.3. a consumer, who also works at a production site and sprays garden herbicides, will receive the highest dose of PCOC during work and during gardening activities of 1.05 mg/kg/day. The dose received indirectly via the environment is low compared to this, $1.2x10^{-4}$ mg/kg/day, but would occur regularly. A margin of safety of 190 (200 mg/kg /1.05 mg/kg) would not seem to present undue risk.

Conclusions of the risk assessment for man during combined exposure:
( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.1.3 Exposure assessment

The substance PCOC gives no reason for concern in relation to the following physical-chemical properties. The tests performed all gave or were expected to give negative results.

4.1.3.1 Occupational exposure

4.1.3.2 Consumer exposure

Indirect exposure via the environment
Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.3.3 Explosivity

Explosive properties have not been tested. No reports of explosive properties were found in the available literature, nor does the chemical structure contain any elements associated with explosivity.

4.1.3.4 Flammability

The substance does not burn according to methods used (EF 3.10 and EF 3.10 mod.), nor is it flammable in contact with water. (Quist Laboratory, 1983).

4.1.3.5 Oxidizing potential

The substance was classified as non oxidizing according to the test method from the working group PC II Annex V EEC/831/79, sixth amendment of Dir. 67/548/EEC. (Dantest, 1983).

4.1.4 Risk characterisation

It is not likely that any of the above mentioned adverse effects should occur under the conditions mentioned.

5 CONCLUSIONS

The documentation varies from original studies according to OECD test guidelines with GLP to literature references of varying quality. 4-Chloro-2-methylphenol (PCOC) is used in the industry as an intermediate in the synthesis of the phenoxy herbicides MCPA, MCPB and mecoprop (MCPP). From the industrial production, processing and formulation PCOC is emitted to air and waste water. The produced pesticides contain PCOC as impurity (normally < 1%, 0,5% estimated as realistic worst case). The use of the pesticides in the agriculture as herbicides results in exposure to soil of PCOC as an impurity and degradation product.

As MCPA is transformed to PCOC, and PCOC has a high vapour pressure, the atmosphere will receive a contribution from application of the above mentioned pesticides. PCOC has a low to medium adsorption and may be considered mobile in some soils.

PCOC is according to an experiment primarily degradable by photolysis in clean water with a half-life of 4 days. However, a re-estimation of photolysis to typical EU surface water resulted in an estimated photolytic degradation half-life of 300-700 days and therefore photolysis is considered negligible. The available biodegradation data are somewhat conflicting but based on a judgement of the balance of evidence the "realistic worst case" aerobic biodegradation half-life of PCOC in soil is estimated to be 21 days, whereas no biodegradation has been found under anaerobic conditions. The aerobic biodegradation half-life in surface waters is also estimated to be 21 days. The estimated half-life in biological waste water treatment plants is 0.7 hour resulting in an estimated removal of 88% which is in general accordance with simple mass balance estimations from one of the main manufacturers sites. The substance is therefore considered to be ready biodegradable (borderline).

PCOC has been found in water, soil, air and groundwater. In water occurs mainly around emission sources, in air near fields applied with MCPA or MCPP, and in soil and biota after the application of the herbicides. The findings in groundwater are assumed to be the result of mobility and reduced degradation under anaerobic conditions.

The exposure assessment is primarily based on monitoring data from the two main manufacturing sites where all production and all processing of PCOC takes place and where approximately 60% of the production volume is formulated. A worst case environmental exposure scenario for a separate formulation site is included in the risk assessment.

The emissions to surface water from production sites are local and the risk assessment based on monitoring data ($C_{STP + influent}$ and actual dilution in STPs) and TGD default environmental exposure assesment for formulation site where 10% of the production volume of phenoxy acids is formulated. Because only the STPs receiving waste water from one of the production sites and the formulation sites are using sludge application to soil, The sludge application is considered local.

PCOC is very toxic to aquatic organisms. The acute toxicity to fish LC_{50} (96h) was observed to be about 2.3-6.6 mg/l. The EC_{50} (48h) to daphnids were 0.29-1.0 mg/l. The EC_{50} (96h) to algae was 8.2 mg/l and the EC_{10} (96h) was 0.89 mg/l. The long term toxic effects were observed in fish to have a NOEC(28d) of 0.5 mg/l and the Daphnia reproduction NOEC(21d) was 0.55 mg/l.

The $PEC_{local(water)}/PNEC_{aquatic\ organisms}$ relationship is < 1. Model calculation using EUSES version 1.0 supports the assumption of no risks for adverse effects in the aquatic environment and for the microorganisms of STPs.

There are no data available on the terrestrial toxicity. The equilibrium partitioning method is applied as a conservative calculation, comparing $PEC_{soil,\ porewater}$ with $PNEC_{aquatic\ organisms}$: $PEC_{soil}/PNEC < 1$.

PEC_{air}: There are no effect data present and no relation PEC/PNEC can be calculated.

PCOC has a bioaccumulation potential based on log Kow 3.09, but BCF found in fish was low (≤ 30). The risk characterisation of secondary poisoning is therefore not performed.

The substance is considered to be of no concern to aquatic organisms and microorganisms of STPs, and no further information on environmental release from production and formulation facilities is required.

No current evidence was found for the use of PCOC as such in products, although it may formerly have been employed as a disinfectant. Direct exposure is therefore likely to be restricted to those involved in the manufacture and handling of PCOC, and in conjunction with its use in the manufacture of phenoxy herbicides. Based on limited information, exposures in the range of 0.02 - 0.7 mg/kg/day are estimated for these activities.

The main exposure of human beings to PCOC is likely to be via production, or use of phenoxy herbicides which may contain it as an impurity (< 1%), or as a breakdown product following exposure of herbicides to sunlight, or to their metabolic transformation to the substance. It is difficult to quantify exposure occurring through transformation, but this is assumed to be less than 1%. During production, a realistic worst case exposure of 0.7 mg/kg/day is indicated. In conjunction with agricultural application of herbicides, a worst-case estimate of exposure to PCOC of 0.28 mg/kg/day is obtained. Municipal gardeners may be exposed to higher levels with an estimate of 0.35 mg/kg/day suggested as a realistic worst case.

Similarly, some consumer exposure should also be expected, as the same herbicides can be used in lawn treatment and similar gardening activities. While no detailed information was found on such exposures, it may be amount to 0.07 mg/kg per event. Assuming a really worst case of five events per year, the total yearly dose of PCOC would be 0.35 mg/kg/year corresponding to 9.6×10^{-4} mg/kg/day.

Indirect exposure via the environment resulting from partitioning into air/water/soil and biomagnification in food sources is low at a regional level, combined secondary exposure estimate being in the range of 1.40×10^{-5} mg/kg/day of PCOC. Local indirect exposure estimates are about 1.2×10^{-4} mg/kg/day.

The acute toxicity of PCOC (LD_{50} oral rat 2650-3196 mg/kg, LD_{50} dermal rat 2240 mg/kg, LC_{50} inhal. rat 4h 0.9mg/l or >30 mg/l) does not give rise to immediate concern, particularly considering that the substance (crystalline needles) is unlikely to form aerosols or dusts, and that PPE is mandated during handling of the substance.

PCOC is corrosive in high concentrations, and has been assigned risk phrase R-35 by the manufacturers which should provide adequate warning to those handling it in industrial settings. No consumer exposure is expected at concentrations which could approach that required for corrosivity. No sensitization was observed in a Guinea pig maximization test and no case studies indicating sensitization of persons handling the substance were found.

There were no effects on reproduction according to OECD screening test 422 at doses of up to 600 mg/kg for a total of 40 days.

In 28-day repeat dose studies in rats, the best NOAEL appears to be 200 mg/kg, with a LOAEL of 800 mg/kg where salivation after dosing and ruffled fur was seen in some animals. At this dose, levels of serum alanine-aminotransferase were increased in males, and effects were seen on blood parameters (reduced thromboplastin times, reduction of leukocyte and erythrocyte counts). Liver weights in females were increased, but no histopathological changes were seen in this, or any other organs examined. Decreased adrenal weights were also seen in females at 200 mg/kg and above, but were unaccompanied by histopathological changes.

PCOC has not been investigated for carcinogenicity. Two older tests were positive for mutagenicity, one *in vivo* (mouse micronucleus test) and one *in vitro* in a single strain (TA97) of Salmonella in the Ames test (while showing no activity in other strains in a number of separate tests). Repeated testing with TA97 gave unequivocally negative results. A repeat of the micronucleus test according to current guidelines also gave clearly negative results. On the balance, it is not felt that there is evidence for PCOC being a mutagen.

The estimated human local indirect exposure of 1.2×10^{-4} mg/kg/day is well below the repeat dose toxicity (NOAEL 200 mg/kg/day).

For the population with the highest potential exposure (production workers assuming inhalation exposure at 5 mg/m^3 for eight hours) a margin of safety of 285 (200 mg/kg/0.7 mg/kg/day) is obtained with regard to the repeat dose NOAEL. For agricultural workers engaged in spraying phenoxy herbicides the ratio is 200 mg/kg /0.28 mg/kg, or 714. For municipal gardeners (0.35 mg/kg/day) a margin of safety of 571 is obtained. Consumers may be exposed to 0.07 mg/kg/day once, or a few times yearly. All other exposure scenarios result in much higher margins of safety.

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1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステルのラットを用いる単回経口投与毒性試験

Single Dose Oral Toxicity Test of Tris(2-ethylhexyl) 1,2,4-benzenetricarboxylate in Rats

要約

既存化学物質の安全性を評価するため，1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステルを雌雄のCrj:CD(SD)系ラットに単回経口投与し，急性毒性を検討した．なお，雌雄とも投与量は2000 mg/kgの1用量とし，対照として媒体(コーン油)投与群を設けた．

一般状態の観察では，コーン油の影響と考えられる軟便が2000 mg/kg群の雌雄全例に認められた．観察期間における死亡例は，2000 mg/kg群の雌雄いずれにも認められなかった．体重は，2000 mg/kg群の雌雄ともに観察期間終了時まで順調に増加した．剖検では，2000 mg/kg群の雌雄いずれにも異常は認められなかった．

方法

1. 被験物質

1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステル(CAS No.3319-31-1，大八化学工業㈱，Lot.No. N-60601，純度99.0%以上，分子量546.87，凝固点-30℃，沸点430℃)は淡黄色透明，油溶性の液体であり，使用時まで室温条件下で密閉遮光保管した．なお，投与液は調製後，冷蔵保存で7日間安定であることを確認した．

2. 供試動物

生後5週のCrj:CD(SD)系ラット(SPF)雌雄各15匹を日本チャールス・リバー(株)から購入した．8日間にわたり動物を検疫・馴化飼育した後，6週齢で試験に用いた．投与時の体重は，雄で149～163 g，雌で126～140 gであった．

3.飼　育

動物は，温度23±2℃，湿度55±10%，換気回数20回/時間，照度150～300 lux，照明時間12時間(午前7時点灯，午後7時消灯)に設定された飼育室で，㈱東京技研サービスの自動水洗式飼育機を使用し，ステンレス製網目飼育ケージに5匹ずつ収容して飼育した．飼育ケージおよび給餌器は週1回取り換えた．動物には，オリエンタル酵母工業㈱製造の固型飼料MFを自由に摂取させ，飲料水としては，水道水を自由に摂取させた．

4. 用量設定理由

200および2000 mg/kgの用量を雌雄各3匹のラットに投与した予備試験では，いずれの投与群にも死亡例は認められなかった．以上の結果を参考にして，本試験では雌雄ともに2000 mg/kgの1用量を設定し，さらにコーン油のみを投与する対照群を設けた．

5. 群分け

動物はあらかじめ体重によって層別化し，無作為抽出法により各試験群を構成するように群分けした．

6. 投与液の調製および投与方法

所定量の被験物質をコーン油(ナカライテスク㈱)に溶解し投与液を調製した．溶液の濃度は，2000 mg/kg群で40.0 w/v%であった．

投与経路は経口とし，16時間絶食させた動物に注射ポンプと胃ゾンデを用い，被験物質溶液を投与した．投与容量は体重100 gあたり0.5 mlとし，個体別に測定した体重に基づいて算出した．給餌は被験物質投与3時間後に行った．

7. 一般状態の観察

中毒症状および生死の観察は，投与後6時間までは1時間毎に，その後は1日2回(午前と午後，休日は午前のみ)の割合で，投与後14日まで実施した．

8. 体　重

体重は投与直前，投与後7および14日に測定した．

9. 病理学検査

観察期間終了時の生存例については，エーテル麻酔下で放血安楽死させ解剖した．肉眼的異常所見を記録した．

結果および考察

1. 死亡率およびLD_{50}値

2000 mg/kg群の雌雄いずれにも死亡例は認めらず，LD_{50}値は雌雄とも2000 mg/kg以上と推定された．

2. 一般状態

対照群および2000 mg/kg群の雌雄全例において，軟便が投与後1時間から認められたが，投与後4時間には消失した．軟便の発現および消失の時間については，対

照群と2000 mg/kg群の間で差は認められなかったことから，軟便は，媒体として用いたコーン油の投与によるものと考えられた．

3. 体重

対照群および2000 mg/kg群の雌雄全例において，投与後7および14日の測定で対照群とほぼ同様な増加が認められた．

4. 剖検所見

対照群および2000 mg/kg群の雌雄いずれにも，異常を示す所見は認められなかった．

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1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステルのラットを用いる28日間反復経口投与毒性試験

Twenty-eight-day Repeat Dose Oral Toxicity Test of Tris(2-ethylhexyl) 1,2,4-benzenetricarboxylate in Rats

要約

1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステルは，塩化ビニル用可塑剤として使用される化合物である．本化合物の毒性については，ほとんど報告がないため，今回，既存化学物質の安全点検に係わる毒性調査事業の一環として，SD系ラットを用いる強制経口投与による28日間反復投与毒性試験を実施した．

ラットは1群雌雄各5匹で4試験群，対照群および高用量群には雌雄各5匹の回復群を設け，計60匹を使用した．

1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステルは，コーンオイルに溶解し，0, 100, 300および1000 mg/kgを毎日1回，4週間連続経口投与し，一般状態の観察，体重測定，摂餌量測定，血液学検査，血液凝固検査，血液生化学検査，尿検査，器官重量測定および病理学検査を行った．なお，回復期間は2週間とし，投与終了時と同様な検査を実施した．

その結果は，次のとおりである．

一般状態の観察では，雌雄いずれの群にも異常動物は観察されず，死亡例もなかった．

体重，摂餌量，飼料効率，および血液生化学検査および器官重量には，雌雄とも被験物質投与に起因すると考えられる変化は認められなかった．

血液学検査の結果，雌雄とも被験物質投与に起因すると考えられる変化は認められなかった．

尿検査の結果，雌雄の1000 mg/kg群で尿量が増加した動物が認められたが，平均尿量および尿比重に有意差は認められなかった．

病理学検査の結果，肉眼および組織学的検索ともに，被験物質投与の影響が示唆される病変は観察されなかった．なお，肉眼所見において肺の有色斑／区域が，組織所見において腎臓の好酸性小体が，対照群に比べ雄の投与群に多く観察されたが，いずれも自然発生性病変が偶発的に増加したものと考えられた．

以上の結果，雌雄とも無影響量は1000 mg/kg/dayと判断された．

材料および方法

1. 被験物質

1,2,4-ベンゼントリカルボン酸トリス(2-エチルヘキシル)エステル (CAS No.3319-31-1，大八化学工業㈱提供)は淡黄色透明の油溶性液体で，分子式$C_{33}H_{54}O_6$，分子量546.87の化合物である．本試験に用いたロットN-60601の純度は99.0％以上であった．

2. 供試動物

供試したラット[Crj:CD(SD)系，SPF]は日本チャールス・リバー㈱(神奈川県)から4週齢で購入した．動物を検収後，試験環境に9日間馴化させた後，6週齢で投与を開始した．動物はあらかじめ体重によって層別化し，無作為抽出法により各試験群を構成するように群分けした．動物の識別は，個別飼育ケージに動物標識番号(Animal ID-No.)を付すことにより行った．投与開始時の体重は雄で130～151 g，雌で110～121 gであった．

3. 飼育条件

動物はバリアシステムの飼育室で飼育し，環境調節の目標値は温度23±2℃，相対湿度55±10％，換気回数20回／時，照明150～300 lux，12時間(午前7時点灯，午後7時消灯)とした．㈱東京技研サービスの水洗式飼育機を使用し，金属製前面・床網目飼育ケージに動物を1匹ずつ収容し，オリエンタル酵母工業㈱製造の放射線滅菌改良NIH公開ラット・マウス飼料および水道水を自由に摂取させた．飼育ケージは隔週1回，給餌器は週1回取り換えた．

なお，動物の馴化期間を含め，投与および回復期間中，データの信頼性に影響を及ぼしたと思われる環境要因の変化はなかった．

4. 試験群の構成

試験群は0，100，300および1000 mg/kgの4群とし，1群雌雄各5匹を用い，0および1000 mg/kg群に雌雄各5匹の回復群を設け，計60匹を使用した．

〔用量設定理由〕

本試験に先立って用量設定のための2週間投与試験(投与量：0，200，600および1800 mg/kg)を実施した．その結果，一般状態，体重，摂餌量，血液学検査，血液生化学検査，器官重量および病理学検査において被験物質投与に起因すると考えられる変化は認められなかった．従って，28日間反復投与試験の高用量は，1000 mg/kgとし，以下公比3で除し，中用量を300 mg/kg，低用量を100 mg/kgに設定した．

5. 投与方法

被験物質の投与経路は経口とした．被験物質はコーン

油に溶解し，胃ゾンデを用いて経口投与した．投与容量は体重100 g当り0.5 mlとした．対照群には溶媒のみ投与した．

6. 投与液の調製，分析

被験物質は，各用量(100，300および1000 mg/kg)ごとに所定量を精秤し，コーン油(ナカライテスク㈱)に溶解した．投与液は調製後，冷蔵庫保存で1週間安定であることが確認されているので，本試験においては毎週1回調製を行い，1日分毎に小分けをし使用時まで冷蔵庫に保管した．投与液の濃度分析をすべての群に関し投与1および4週の調製液について実施した結果，設定濃度の98.0～102％の範囲であり，適切に調製されていた．

7. 投与期間

投与期間は28日間とし，投与終了後0および1000 mg/kg群について2週間の回復試験を実施した．

8. 観察，測定および検査

1) 一般状態の観察

全動物を毎日午前，午後の2回観察し，中毒症状の有無，行動異常，死期の迫った動物および死亡動物の有無等を記録した．

2) 体　重

投与開始から回復試験終了時まで，毎週1回測定した．

3) 摂餌量

毎週1回給餌した残量を測定し，飼料摂取量(g/week)を算出した．

4) 臨床検査

投与終了時および回復期間終了時の計2回実施した．採血するに当り，動物は約16時間絶食させた．動物をエーテルで麻酔後開腹し，腹部大動脈から採血した．

a. 血液学検査

EDTA-3Kを添加した初血を用い，白血球数(WBC：暗視野板法)，赤血球数(RBC：暗視野板法)，ヘモグロビン量(HGB：シアンメトヘモグロビン法)，ヘマトクリット値(HCT：RBC, MCVより算出)，平均赤血球容積 (MCV：暗視野板法)，平均赤血球血色素量(MCH：HGB, RBCより算出)，平均赤血球血色素濃度(MCHC：HGB, HCTより算出)，血小板数(PLT：暗視野板法)および白血球百分率(フローサイトケミストリー法)を血液自動分析装置THMS H・1E(米国マイルス社)を用いて測定した．

網赤血球(RC)率算定用に，血液塗抹標本を作製しメイ・グリュンワルド・ギムザで染色後，鏡検した．

また，クエン酸ソーダ添加血液の血漿について，プロトロンビン時間(Quick 1段法)，活性化部分トロンボプラスチン時間(クロット法)およびフィブリノーゲン量(トロンビン時間法)を血液凝固自動測定装置KC-40(独国Amelung社)を用いて測定した．

b. 血液生化学検査

血清を用いて，総蛋白(ビューレット法)，アルブミン(B.C.G.法)，A/G比(計算値)，血糖(グルコースオキシダーゼ法)，中性脂肪(酵素法)，総コレステロール(酵素法)，尿素窒素(BUN：ウレアーゼアンモニア法)，総ビリルビン(ジアゾ色素法)，カルシウム(アルセナゾIII色素法)，無機リン(モリブデン酸ブルー法)，ナトリウム(電極法)，カリウム(電極法)および塩素(電極法)をEKTACHEM 700N(米国コダック社)で，クレアチニン(Jaffé法)，グルタミン酸オキザロ酢酸トランスアミナーゼ(GOT:IFCC法)，グルタミン酸ピルビン酸トランスアミナーゼ(GPT:IFCC法)，γ-グルタミルトランスペプチダーゼ(γ-GTP:Szasz改法)およびアルカリホスファターゼ(ALP:Bessey-Lowry-Brock改良法)をCentrifiChem ENCORE II (米国ベーカー社)で測定した．

c. 尿検査

血液学検査に先立ち，採尿器を用いて24時間(午前10時から翌日午前10時まで)尿を採取し，尿量，色調および濁度を検査後，尿比重計UR-S(㈱アタゴ)を用いて尿比重を測定した．また，尿を遠心分離後Sternheimer変法により沈渣を染色し，鏡検した．pH，潜血，ケトン体，糖，蛋白，ビリルビンおよびウロビリノーゲンについて，N-マルティスティックスSG試験紙(マイルス・三共㈱)およびCLINITEK 200(米国マイルス社)を用いて測定した．

5) 病理学検査

病理解剖は投与終了時および回復期間終了時に動物をエーテル麻酔し，放血致死させ実施した．肉眼的異常を病理解剖所見記録シートに記録した．また，脳，肝臓，腎臓，脾臓，副腎，精巣および卵巣について重量を測定し，器官重量・体重比を算出した．上記重量測定器官と下垂体，眼球，甲状腺(上皮小体を含む)，心臓，肺，胃，膀胱，骨髄(大腿骨)および肉眼所見で変化が認められた雄の肺を10％中性緩衝ホルマリン液で固定した．

病理組織学検査は固定した器官・組織のうち，心臓，肝臓，脾臓，腎臓，副腎および骨髄(大腿骨)については対照群と高用量群，雄の腎臓についてはすべての群について行った．常法に従って薄切標本を作製し，ヘマトキシリン・エオジン染色し鏡検した．

6) データの記録および統計分析

各試験群の体重，摂餌量，血液学検査値，血液生化学検査値，尿検査値(尿量および尿比重のみ)，器官重量および器官重量・体重比は，下記に示した自動判別方式に従い，最初にBartlettの等分散検定を実施した．等分散の場合は一元配置の分散分析を行い，分散が有意で各群の標本数が同数の場合はDunnettの多重比較検定，各群の標本数が異なる場合はDuncanの多重範囲検定で対照群と各投薬群間の有意差を検定した．Bartlettの等分