傍聴者配布用

資料３－１－４

既存化学物質の人健康影響に関する情報（第一種特定化学物質審議関係①）

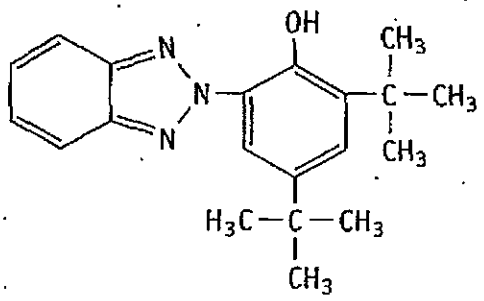
（平成 17 年 11 月 18 日）

2-(2'-Hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾール

[CAS No. 3846-71-7]

Molecular formula: $C_{20}H_{25}N_3O$ Molecular weight: 323.44



ABSTRACT

A single dose oral toxicity test of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole was conducted at doses of 0 and 2000 mg/kg using male and female rats. No deaths were observed in the 0 or 2000 mg/kg groups in either sex, pointing to an LD_{50} value higher than 2000 mg/kg in both sexes.

A repeated dose toxicity test was conducted at doses of 0, 0.5, 2.5, 12.5 and 62.5 mg/kg/day using male and female rats. No deaths of either sex were observed in any of the treatment groups.

The hematocrit value, hemoglobin concentration, and red blood cell count were lowered in males of the 2.5 mg/kg or higher dose groups. The MCHC was lowered in males of the 12.5 and 62.5 mg/kg groups and the fibrinogen concentration was reduced in males of the 2.5 mg/kg or higher dose groups and in females of the 62.5 mg/kg group.

The serum level of glucose was higher in males of the 2.5 mg/kg or higher dose groups and in females of the 62.5 mg/kg group. The total cholesterol and triglyceride levels were elevated in females of the 62.5 mg/kg group.

The liver weights were increased in males of the 0.5 mg/kg or higher dose groups and in females receiving 12.5 mg/kg or higher. The kidney weights were elevated in males of the 62.5 mg/kg group.

Macroscopically, enlarged livers with white patch zones were observed. Histopathologically, the following were noted: degeneration of the myocardium, with cellular infiltration and hypertrophy, extramedullary hematopoiesis of the spleen, vacuolar degeneration, hypertrophy of hepatocytes, increase in mitosis, proliferation of bile ducts and focal necrosis in the liver, basophilic tubules and dilatation of tubules, hypertrophy of tubular epithelium of collecting tubules in the kidney, and follicular cell hyperplasia in the thyroid gland.

These changes were observed in all the treated groups except females dosed 0.5 mg/kg.

The NOEL for repeated dose toxicity is considered to be less than 0.5 mg/kg/day for males and 2.5 mg/kg/day for females.

Genotoxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole was studied by a reverse mutation test in bacteria. This substance was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 or *Escherichia coli* WP2 *uvrA*, with or without an exogenous metabolic activation system.

Genotoxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole was studied by chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells.

2-(2'-Hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole did not induce structural chromosomal aberrations or polyploidy up to 3.2 mg/mL (10 mmol/L) under the present test conditions.

SUMMARIZED DATA FROM THE STUDIES

1. Single Dose Oral Toxicity [1)]

Purity	:	100 %
Test species/strain	:	Rat/Crj:CD(SD)IGS
Test method	:	OECD Test Guideline 401
Route	:	Oral (gavage)
Dosage	:	0 (vehicle), 2000 mg/kg
Number of animals	:	Males and females; 5
Vehicle	:	Corn oil
GLP	:	Yes

Test results:

No deaths were observed in the 0 or 2000 mg/kg group in either sex. The LD_{50} value is higher than 2000 mg/kg in both sexes.

2. Repeated Dose Oral Toxicity [1)]

Purity	:	100 %
Test species/strain	:	Rat/Crj:CD(SD)IGS
Test method	:	Guideline for the 28-Day Repeated Dose Toxicity Test in Mammalian Species (Chemical Substances Control Law of Japan)
Route	:	Oral (gavage)
Dosage	:	0 (vehicle), 0.5, 2.5, 12.5, 62.5 mg/kg/day
Number of animals	:	Males and females; 5
Vehicle	:	Corn oil
Administration period	:	Males and females, 28 days
Terminal killing	:	Males and females, days 29 or 43
GLP	:	Yes

Test results:

No deaths were observed in any of the treatment groups in either sex. There were also no changes in general appearance or body weights.

Hematological examination demonstrated, the hematocrit value, hemoglobin concentration, and red blood cell count to be lowered in males in the 2.5 mg/kg or higher dose groups. The MCHC was lowered in males in the 12.5 and 62.5 mg/kg groups.

On blood coagulation examination, the fibrinogen concentration was found to be lowered in males of the 2.5 mg/kg or higher dose groups and in females of the 62.5 mg/kg group. With the blood chemical examination, the serum level of glucose was elevated in males of the 2.5 mg/kg or higher dose groups and in females of the 62.5 mg/kg group. The total cholesterol and triglyceride levels were higher in females of the 62.5 mg/kg group than in the controls.

The absolute and relative liver weights were increased in males of the 0.5 mg/kg or higher dose groups and in females of the 12.5 mg/kg or higher dose groups. Absolute and relative kidney weights were elevated in males of the 62.5 mg/kg group.

Macroscopically, enlarged livers with white patches were observed.

Histopathologically, the following changes were observed: degeneration of myocardium, with cellular infiltration and hypertrophy, extramedullary hematopoiesis of the spleen, vacuolar degeneration and hypertrophy of hepatocytes, with increased mitosis, bile duct proliferation and focal necrosis in the liver, basophilic tubules and dilatation of tubules, with hypertrophy of tubular epithelium of collecting tubules in the kidneys, and follicular cell hyperplasia in the thyroid gland.

These changes were observed in all the treated groups except females dosed 0.5 mg/kg.

The NOEL for repeated dose toxicity is considered to be less than 0.5 mg/kg/day for males and 2.5 mg/kg/day for females.

3. Genetic Toxicity

3-1. Bacterial test [2)]

Purity	:	100 %
Test species/strains	:	*Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 *uvrA*
Test method	:	Guidelines for Screening Mutagenicity Testing of Chemicals (Chemical Substances Control Law of Japan) and OECD Test Guideline 471
Procedures	:	Pre-incubation method
Solvent	:	Dimethyl sulfoxide
Positive controls	:	−S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2 *uvrA*), Sodium azide (TA1535) and 9-Aminoacridine (TA1537) +S9 mix; 2-Aminoanthracene (five strains)
Dosage	:	−S9 mix; 0, 313, 625, 1250, 2500, 5000 μg/plate (five strains) +S9 mix; 0, 313, 625, 1250, 2500, 5000 μg/plate (five strains)
S9	:	Rat liver, induced with phenobarbital and 5,6-benzoflavone
Plates/test	:	3 (1 for cytotoxicity test)
Number of replicates	:	2 (plus 1 cytotoxicity test)
GLP	:	Yes

Test results:

This chemical did not induce gene mutations in *S. typhimurium* TA100, TA98, TA1535, TA1537 or *E. coli* WP2 *uvrA* strains with or without S9 mix. Growth inhibition was not observed up to 5000 μg/plate in any strain, with or without S9 mix.

Genetic effects:

Salmonella typhimurium TA100, TA98, TA1535, TA1537

	+	?	−
Without metabolic activation:	[ ]	[ ]	[*]
With metabolic activation:	[ ]	[ ]	[*]

Escherichia coli WP2 *uvrA*

	+	?	−
Without metabolic activation:	[ ]	[ ]	[*]
With metabolic activation:	[ ]	[ ]	[*]

3-2. Non-bacterial *in vitro* test (chromosomal aberration test)[2)]

Purity	:	100 %
Type of cell used	:	Chinese hamster lung (CHL/IU) cells
Test method	:	Guidelines for Screening Mutagenicity Testing of Chemicals (Chemical Substances Control Law of Japan) and OECD Test Guideline 473
Vehicle	:	0.5 % Sodium carboxymethylcellulose
Positive controls	:	−S9 mix; Mitomycin C +S9 mix; Cyclophosphamide
Dosage	:	−S9 mix (continuous treatment) : 0, 0.80, 1.6, 3.2 mg/mL −S9 mix (short-term treatment) : 0, 0.80, 1.6, 3.2 mg/mL +S9 mix (short-term treatment) : 0, 0.80, 1.6, 3.2 mg/mL
S9	:	Rat liver, induced with phenobarbital and 5,6-benzoflavone
Plates/test	:	2
GLP	:	Yes

Test results:

With all the test systems, cells with structural chromosomal aberrations and polyploidy were not significantly increased at any dose.

Genotoxic effects:

	clastogenicity			polyploidy		
	+	?	−	+	?	−
Without metabolic activation:	[ ]	[ ]	[*]	[ ]	[ ]	[*]
With metabolic activation:	[ ]	[ ]	[*]	[ ]	[ ]	[*]

1) The tests were performed by the Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), 582-2 Shiosinden Arahama, Fukude-Cho, Iwata-Gun, Shizuoka, 437-1213, Japan. Tel +81-538-58-1266 Fax +81-538-58-1393

2) The tests were performed by the Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-8523, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾー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 2-(2'-Hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazole in Rats

要約

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールは主にプラスチックの紫外線吸収剤として用いられている．2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールの単回経口投与毒性試験をCD(SD)IGS系(SPF)雌雄ラットを用いて実施した．

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールはコーンオイルに懸濁し，雌雄ともに2000 mg/kgを単回強制経口投与した．また，媒体対照としてコーンオイルのみを投与した群も設定した．観察期間は14日間とし，一般状態の観察，体重推移および病理学検査を実施した．

観察期間を通じて雌雄ともに一般状態に異常所見は観察されず，死亡例も認められなかった．体重測定では，雌雄とも対照群に比較して投与後7および14日の測定値に差が認められなかった．

病理学検査では，雌雄ともに異常所見は認められなかった．

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールのラットにおけるLD_{50}値は雌雄ともに2000 mg/kgより大であった．

方法

1. 被験物質

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾール[シプロ化成㈱(福井)，純度100 %，Lot No. S4-034-1]は白色粉末であり，使用時まで室温の被験物質保管庫に保管した．試験終了後，残余被験物質を提供元で再分析し，被験物質が試験期間中安定であったことを確認した．

投与液は，被験物質をコーンオイル(ナカライテスク㈱，Lot No. V7B5849)に懸濁し200 mg/mLの濃度となるように調製した．投与液は投与直前に調製した．投与液中の被験物質濃度を調製後速やかに測定した結果，適切に調製されていたことが確認された．

2. 投与量の設定および投与方法

本試験に先立って実施した予備試験において2000 mg/kgを投与した結果，雌雄とも一般状態に変化が観察されず死亡例もみられなかった．従って，本試験の用量は雌雄ともにOECDガイドライン「急性経口」で上限用量として指定されている2000 mg/kgを設定し，さらに媒体対照群を設定した．投与容量は体重100 g当たり1.0 mLとし，個体別に測定した体重に基づいて投与液量を算出した．

投与回数は1回とし，投与前約16時間絶食させた動物に胃ゾンデを用いて強制経口投与した．なお，対照群にはコーンオイルのみを投与した．給餌は，被験物質投与後約3時間に行った．

3. 供試動物

5週齢のCrj:CD(SD)IGS系ラット(SPF)雌雄各16匹を日本チャールス・リバー㈱から購入し，試験環境への馴化のため1週間予備飼育を行い，6週齢に達した時点で投与した．

動物は23.4～24.3 ℃，湿度51～67 %，照明時間12時間(午前7時点灯，午後7時消灯)に制御された飼育室で，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．動物には，オリエンタル酵母工業㈱製造の固型飼料MF(Lot No. 010205)および水道水を自由に摂取させた．

4. 群構成

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雌雄ともに各群5匹ずつ振り分けた．投与時の体重は，雄が157～176 g，雌が126～141 gであった．

5. 観察および検査

中毒症状および生死の観察は，投与6時間までは1時間毎に，投与翌日からは1日1～2回，14日間にわたって実施した．体重は投与直前，投与後7および14日に測定した．観察終了時に全ての動物をエーテル麻酔後放血死させ解剖した．

結果

1. 死亡率およびLD_{50}値

雌雄の対照群および2000 mg/kg群に死亡例は認められなかった．従って，LD_{50}値は雌雄ともに2000 mg/kgより大であった．

2. 一般状態

観察期間を通じていずれの動物にも一般状態に異常は認められなかった．

3. 体重

対照群に比較して雌雄の2000 mg/kg群で投与後7お

よび14日の測定時とも差がみられなかった.

4. 病理所見

観察期間終了時の解剖でいずれの動物にも異常所見は認められなかった.

考察

2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールの2000 mg/kgを6週齢のCrj:CD(SD)IGS系ラットの雌雄に単回経口投与し，投与後14日間観察した．雌雄とも一般状態に異常は観察されず，死亡例も認められなかった．観察期間中，体重は雌雄とも順調な推移を示した．また，生存動物の病理解剖においても異常所見は認められなかった.

従って2-(2'-ヒドロキシ-3',5'-ジ-*tert*-ブチルフェニル)ベンゾトリアゾールのLD_{50}値は雌雄ともに2000 mg/kgより大と考えられた.

連絡先

試験責任者：渡　修明

試験担当者：保母真由美

(財)食品農医薬品安全性評価センター

〒437-1213　静岡県磐田郡福田町塩新田582-2

Tel 0538-58-1266　Fax 0538-58-1393

Correspondence

Authors: Nobuaki Watari (Study director)

Mayumi Hobo

Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center)

582-2 Shioshinden, Fukude-cho, Iwata-gun,

Shizuoka, 437-1213, Japan

Tel +81-538-58-1266　Fax +81-538-58-1393