資料２－４

既存化学物質の人健康影響に関する情報

（平成21年10月23日開催）

官報公示整理番号	CAS No.	物質名称	試験名／評価文書名	頁
2-483	123-63-7	パラアセトアルデヒド	復帰突然変異試験	1
			染色体異常試験	7
			28日間反復投与毒性試験	14
4-1531	31127-54-5	2,3,4,4-テトラヒドロキシベンゾフェノン	復帰突然変異試験	82
			染色体異常試験	92
			反復投与・生殖発生毒性併合試験	116
5-1037	108-80-5	イソシアヌル酸	染色体異常試験	215
			反復投与・生殖発生毒性併合試験	219
			OECD/HPVプログラム初期評価文書（SIDS Initial Assessment Report）	250
3-442	88-73-3	o-クロロニトロベンゼン	OECD/HPVプログラム初期評価文書（SIDS Initial Assessment Report）	308
2-163	112-24-3	トリエチレンテトラミン	OECD/HPVプログラム初期評価文書（SIDS Initial Assessment Report）	414

要約

パラアセトアルデヒドの遺伝子突然変異誘発性の有無を調べるため、細菌を用いる復帰突然変異試験を実施し、陰性の結果を得た。

検定菌として、*Salmonella typhimurium* TA100、TA1535、TA98、TA1537 および *Escherichia coli* WP2 *uvrA* を用い、プレインキュベーション法により、S9 mix 非存在下および存在下で試験を行った。

用量設定試験を 50.0、150、500、1500 および 5000 μg/plate の 5 用量に設定して行ったところ、S9 mix 非存在下および存在下とも、用いたいずれの検定菌においても生育阻害は認められなかった。変異コロニー数は、用いたいずれの検定菌においても、S9 mix の有無にかかわらず、陰性対照値の 2 倍以上となる増加は認められなかった。

これらの結果に基づき、すべての検定菌で最高用量を 5000 μg/plate とし公比 2 で 5 用量(313～5000 μg/plate)を設定して本試験 I および本試験 II を行った。その結果、用いたすべての検定菌において、S9 mix の有無にかかわらず、陰性対照値の 2 倍以上となる変異コロニー数の増加は認められなかった。

以上の結果から、パラアセトアルデヒドは、用いた試験系において遺伝子突然変異誘発性を有しない(陰性)と判定した。

試験目的

パラアセトアルデヒドの遺伝子突然変異誘発性(変異原性)の有無を検討し、安全性評価の資料とするために、パラアセトアルデヒドについて細菌を用いる復帰突然変異試験をプレインキュベーション法[1)]により実施した。

試験ガイドラインと GLP

この試験は、「新規化学物質等に係る試験の方法について」(平成 15 年 11 月 21 日 薬食発第 1121002 号、平成 15・11・13 製局第 2 号、環保企発第 031121002 号、一部改正 平成 17 年 4 月 1 日)および「OECD 化学物質試験法ガイドライン 471/細菌を用いる復帰突然変異試験」(1997 年 7 月 21 日採択)に準拠し、「化学物質 GLP」(平成 15 年 11 月 21 日、薬食発第 1121003 号、平成 15・11・17 製局第 3 号、環保企発第 031121004 号、最終改正 平成 17 年 4 月 1 日)を遵守して実施した。

用することとした。なお、背景データは、2005 年度に実施した各試験の陰性対照値および陽性対照値とした(Appendix 3)。

7. 結果の表示

結果の表示は、各々の平板における変異コロニー数の実測値とその平均値および標準偏差を示した。また、平均値を用いて用量ー反応曲線を作成した。また、被験物質に由来する沈澱および生育阻害が認められた場合は、その旨表示することとした。

8. 判定

用いた5種の検定菌のうち、1種以上の検定菌のS9 mix非存在下あるいはS9 mix存在下において、被験物質を含有する平板上における変異コロニー数の平均値が、陰性対照値の 2 倍以上に増加し、かつ、その増加に用量依存性あるいは再現性が認められた場合に、本試験系において遺伝子突然変異誘発性を有する(陽性)と判定することとした。なお、結果の判定に統計学的手法は用いなかった。

予見することができなかった試験の信頼性に影響を及ぼす疑いのある事態及び試験計画書に従わなかったこと

試験期間中に、「予見することができなかった試験の信頼性に影響を及ぼす疑いのある事態及び試験計画書に従わなかったこと」はなかった。

結果と考察

1. 用量設定試験

パラアセトアルデヒドについて、50.0、150、500、1500および5000 μg/plateの5段階の用量を設定して用量設定試験を行った(Table 1)。その結果、用いたいずれの検定菌においても生育阻害は認められなかった。被験物質に由来する沈澱は、S9 mix非存在下および存在下ともに、用いたいずれの用量においても認められなかった。

変異コロニー数は、用いたいずれの検定菌においても、S9 mixの有無にかかわらず、陰性対照値の2倍以上となる増加は認められなかった。

以上の結果から、本試験における最高用量を、すべての検定菌で5000 μg/plateとした。

2. 本試験

最高用量を5000 μg/plateとし、公比2で5用量(313～5000 μg/plate)を設定して2回の本試験(本試験Iおよび本試験II)を行った(Tables 2、3およびFigures 1、2)。その結果、2回の本試験ともに、用いたいずれの検定菌においても生育阻害は認められなかった。被験物質に由来する沈澱は、S9 mix非存在下および存在下ともに、用いたいずれの用量においても認められなかった。

変異コロニー数は、2回の本試験ともに、用いたいずれの検定菌においても、S9 mixの有無にかかわらず、陰性対照値の2倍以上となる増加は認められなかった。

すべての試験において、最高用量の被験物質調製液およびS9 mixへの雑菌の混入は認められなかった。また、いずれの検定菌においても陽性対照物質の遺伝子突然変異誘発性が検出され、陽性対照値および陰性対照値は、ともに背景データの変動範囲内(平均値±3×標準偏差)であったことから、本試験系の有効性が確認された。

パラアセトアルデヒドについては、当研究所で実施したチャイニーズ・ハムスター培養細胞を用いる染色体異常試験(試験番号:G-05-087)で、構造異常陽性の結果が得られている。また、関連物質である1,3,5-trimethylbenzeneについては復帰突然変異試験、染色体異常試験共に陰性の結果が報告されている[4]。

以上の結果に基づき、パラアセトアルデヒドは、用いた試験系において遺伝子突然変異誘発性を有しない(陰性)と判定した。

参考文献

1) Matsushima, T., Sugimura, T., Nagao, M., Yahagi, T., Shirai, A., Sawamura, M.: Factors modulating mutagenicity in microbial tests. in "Short-term Test Systems for Detecting Carcinogens" Norpoth, K. H., Garner, R. C. eds., Springer, Berlin-Heidelberg-New York (1980) pp. 273-285

2) Maron, D. M., Ames, B. N.: Revised methods for the Salmonella mutagenicity test. Mutation Research 113: 173-215 (1983)

3) Green, M. H. L.: Mutagen testing using Trp^+ reversion in *Escherichia coli*. In "Handbook of

Table 1 Cytotoxicity of 2,4,6-trimethyl-1,3,5-trioxane in bacteria

With (+) or without (-) S9 mix	Test substance dose (μg/ plate)	Number of revertants (number of colonies / plate, Mean ± S.D.)														
		Base - pair substitution type									Frameshift type					
		TA100			TA1535			WP2 *uvrA*			TA98			TA1537		
S9 mix (-)	0	148	126	124	9	10	6	21	32	38	17	27	15	9	4	8
		(133 ± 13)			(8 ± 2)			(30 ± 9)			(20 ± 6)			(7 ± 3)		
	50.0	156			14			25			17			9		
	150	133			5			19			19			3		
	500	149			7			24			22			5		
	1500	160			14			18			20			7		
	5000	146			12			31			13			3		
S9 mix (+)	0	152	121	127	9	12	10	36	43	43	23	22	20	17	19	11
		(133 ± 16)			(10 ± 2)			(41 ± 4)			(22 ± 2)			(16 ± 4)		
	50.0	157			11			36			34			20		
	150	167			10			26			26			10		
	500	156			11			32			27			20		
	1500	143			10			38			24			13		
	5000	155			18			45			27			16		
Positive control S9 mix (-)	Chemical	AF-2			SA			AF-2			AF-2			9AA		
	Dose (μg / plate)	0.01			0.5			0.01			0.1			80		
	Number of colonies / plate	437	439	417	561	598	561	130	102	127	317	401	400	542	449	309
		(431 ± 12)			(573 ± 21)			(120 ± 15)			(373 ± 48)			(433 ± 117)		
Positive control S9 mix (+)	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P		
	Dose (μg / plate)	5			2			10			5			5		
	Number of colonies / plate	1281	1086	1099	277	289	306	562	573	551	294	284	276	170	170	132
		(1155 ± 109)			(291 ± 15)			(562 ± 11)			(285 ± 9)			(157 ± 22)		

Negative control, Water for injection JP

As the purity of the test substance was 88.5%, dose levels were adjusted for purity.

This test substance contained 11.2% acetaldehyde as impurity.

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

Table 2 Mutagenicity of 2,4,6-trimethyl-1,3,5-trioxane in bacteria (I)

With (+) or without (-) S9 mix	Test substance dose (μg/ plate)	Number of revertants (number of colonies / plate, Mean ± S.D.)															
		Base - pair substitution type									Frameshift type						
		TA100			TA1535			WP2 *uvrA*			TA98			TA1537			
S9 mix (-)	0	114	131	126	9	12	12	28	28	24	35	22	24	7	5	5	
		(124 ± 9)			(11 ± 2)			(27 ± 2)			(27 ± 7)			(6 ± 1)			
	313	118	100	110	14	8	14	29	19	29	23	21	27	14	5	8	
		(109 ± 9)			(12 ± 3)			(26 ± 6)			(24 ± 3)			(9 ± 5)			
	625	94	109	118	12	11	16	27	24	24	33	22	30	10	7	5	
		(107 ± 12)			(13 ± 3)			(25 ± 2)			(28 ± 6)			(7 ± 3)			
	1250	108	105	92	13	13	15	30	26	24	33	30	19	7	11	8	
		(102 ± 9)			(14 ± 1)			(27 ± 3)			(27 ± 7)			(9 ± 2)			
	2500	123	121	129	8	13	13	30	24	24	28	28	29	4	7	4	
		(124 ± 4)			(11 ± 3)			(26 ± 3)			(28 ± 1)			(5 ± 2)			
	5000	127	132	130	14	13	16	23	29	27	18	20	27	10	6	5	
		(130 ± 3)			(14 ± 2)			(26 ± 3)			(22 ± 5)			(7 ± 3)			
S9 mix (+)	0	134	122	107	12	15	8	31	38	28	30	27	30	15	13	9	
		(121 ± 14)			(12 ± 4)			(32 ± 5)			(29 ± 2)			(12 ± 3)			
	313	152	135	139	9	13	14	30	29	26	29	28	33	14	6	10	
		(142 ± 9)			(12 ± 3)			(28 ± 2)			(30 ± 3)			(10 ± 4)			
	625	134	150	146	8	8	9	19	25	29	30	39	26	13	19	14	
		(143 ± 8)			(8 ± 1)			(24 ± 5)			(32 ± 7)			(15 ± 3)			
	1250	138	160	125	7	7	10	35	26	33	34	33	34	13	14	12	
		(141 ± 18)			(8 ± 2)			(31 ± 5)			(34 ± 1)			(13 ± 1)			
	2500	139	123	127	9	11	8	27	49	45	25	31	35	14	11	13	
		(130 ± 8)			(9 ± 2)			(40 ± 12)			(30 ± 5)			(13 ± 2)			
	5000	127	117	152	5	8	15	33	33	34	29	31	33	10	16	14	
		(132 ± 18)			(9 ± 5)			(33 ± 1)			(31 ± 2)			(13 ± 3)			
Positive control S9 mix (-)	Chemical	AF-2			SA			AF-2			AF-2			9AA			
	Dose (μg / plate)	0.01			0.5			0.01			0.1			80			
	Number of colonies / plate	443	449	457	443	445	451	95	91	84	468	453	495	226	314	266	
		(450 ± 7)			(446 ± 4)			(90 ± 6)			(472 ± 21)			(269 ± 44)			
Positive control S9 mix (+)	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P			
	Dose (μg / plate)	5			2			10			5			5			
	Number of colonies / plate	1148	1083	1069	297	300	259	510	573	529	296	321	298	116	137	136	
		(1100 ± 42)			(285 ± 23)			(537 ± 32)			(305 ± 14)			(130 ± 12)			

Negative control, Water for injection JP

As the purity of the test substance was 88.5%, dose levels were adjusted for purity.

This test substance contained 11.2% acetaldehyde as impurity.

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

Table 3 Mutagenicity of 2,4,6-trimethyl-1,3,5-trioxane in bacteria (II)

With (+) or without (-) S9 mix	Test substance dose (μg/ plate)	Number of revertants (number of colonies / plate, Mean ± S.D.)															
		Base - pair substitution type									Frameshift type						
		TA100			TA1535			WP2 *uvrA*			TA98			TA1537			
S9 mix (-)	0	137	134	138	5	15	8	40	37	39	22	16	27	8	7	6	
		(136 ± 2)			(9 ± 5)			(39 ± 2)			(22 ± 6)			(7 ± 1)			
	313	123	150	123	10	12	12	33	22	34	20	25	22	7	7	10	
		(132 ± 16)			(11 ± 1)			(30 ± 7)			(22 ± 3)			(8 ± 2)			
	625	145	134	145	8	8	17	36	42	44	28	17	32	6	6	11	
		(141 ± 6)			(11 ± 5)			(41 ± 4)			(26 ± 8)			(8 ± 3)			
	1250	140	107	118	11	7	5	43	36	36	22	20	27	6	9	6	
		(122 ± 17)			(8 ± 3)			(38 ± 4)			(23 ± 4)			(7 ± 2)			
	2500	134	120	108	16	12	10	38	43	36	23	16	22	2	4	8	
		(121 ± 13)			(13 ± 3)			(39 ± 4)			(20 ± 4)			(5 ± 3)			
	5000	122	121	123	9	11	10	47	49	45	30	27	26	11	9	11	
		(122 ± 1)			(10 ± 1)			(47 ± 2)			(28 ± 2)			(10 ± 1)			
S9 mix (+)	0	137	148	148	16	13	8	47	36	38	28	32	25	15	14	14	
		(144 ± 6)			(12 ± 4)			(40 ± 6)			(28 ± 4)			(14 ± 1)			
	313	165	141	142	5	12	21	36	24	39	34	32	34	8	18	20	
		(149 ± 14)			(13 ± 8)			(33 ± 8)			(33 ± 1)			(15 ± 6)			
	625	130	139	126	8	9	7	37	31	46	18	25	32	12	12	13	
		(132 ± 7)			(8 ± 1)			(38 ± 8)			(25 ± 7)			(12 ± 1)			
	1250	126	163	124	15	14	14	32	30	44	34	22	32	8	16	12	
		(138 ± 22)			(14 ± 1)			(35 ± 8)			(29 ± 6)			(12 ± 4)			
	2500	141	153	128	4	10	10	43	39	42	33	22	33	10	14	17	
		(141 ± 13)			(8 ± 3)			(41 ± 2)			(29 ± 6)			(14 ± 4)			
	5000	152	118	112	11	12	18	49	33	36	28	26	31	16	16	13	
		(127 ± 22)			(14 ± 4)			(39 ± 9)			(28 ± 3)			(15 ± 2)			
Positive control S9 mix (-)	Chemical	AF-2			SA			AF-2			AF-2			9AA			
	Dose (μg / plate)	0.01			0.5			0.01			0.1			80			
	Number of colonies / plate	438	432	470	477	466	498	113	110	116	484	504	582	486	480	446	
		(447 ± 20)			(480 ± 16)			(113 ± 3)			(523 ± 52)			(471 ± 22)			
Positive control S9 mix (+)	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P			
	Dose (μg / plate)	5			2			10			5			5			
	Number of colonies / plate	1132	1099	1114	364	323	290	576	538	574	317	275	281	153	150	184	
		(1115 ± 17)			(326 ± 37)			(563 ± 21)			(291 ± 23)			(162 ± 19)			

Negative control, Water for injection JP

As the purity of the test substance was 88.5%, dose levels were adjusted for purity.

This test substance contained 11.2% acetaldehyde as impurity.

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

要約

パラアセトアルデヒドのチャイニーズ・ハムスター雌肺由来細胞(CHL/IU 細胞)を用いる染色体異常試験を実施し、その染色体異常誘発性を検討した。

用量設定のために実施した細胞増殖抑制試験の結果をもとに、短時間処理における最高処理濃度を S9 mix 非存在下および S9 mix 存在下ともに 10 mmol/L(1.3 mg/mL)とし、公比 2 で計 4 段階の濃度群を設定し、染色体異常試験を実施した。

細胞増殖率および分裂指数の結果をもとに以下の観察対象群を決定し、染色体分析を行った。

S9 mix 非存在下の短時間処理:0.33、0.65、1.3 mg/mL

S9 mix 存在下の短時間処理:0.33、0.65、1.3 mg/mL

その結果、S9 mix 非存在下で短時間処理した高濃度群においてのみ構造異常を有する細胞の統計学的に有意な増加(出現率:5.5%)が認められ、傾向性検定も有意となった。それ以外は、S9 mix 非存在下および存在下で短時間処理したいずれの濃度群においても構造異常を有する細胞および倍数性細胞の統計学的に有意な増加は認められなかった。

短時間処理では明らかな陽性結果が得られなかったことから、短時間処理と同様に最高処理濃度を 10 mmol/L(1.3 mg/mL)とし、公比 2 で計 5 段階の濃度群を設定して 24 時間連続処理による染色体異常試験を行った。

細胞増殖率および分裂指数の結果をもとに以下の観察対象群を決定し、染色体分析を行った。

24 時間連続処理:0.33、0.65、1.3 mg/mL

その結果、24 時間連続処理した高濃度群において染色体の構造異常を有する細胞が統計学的に有意に増加(出現率:56.5%)し、傾向性検定も有意となった。倍数性細胞については、いずれの濃度群においても統計学的に有意な増加は認められなかった。

以上のように、高濃度のパラアセトアルデヒドで処理した場合、染色体の構造異常が誘発されたが、今回の試験に用いたパラアセトアルデヒドは、不純物として、0.03 mg/mL 以上の濃度で染色体の構造異常を誘発することが知られているアセトアルデヒドを 11.2%含んでいる。したがって、今回得られた試験結果は、アセトアルデヒトにより構造異常が誘発された可能性も考えられる。

以上の結果より、本試験に用いたパラアセトアルデヒドは、本試験条件において CHL/IU 細胞に染色体異常を誘発するが、それは不純物であるアセトアルデヒドにより誘発された可能性も考えられた。

試験目的

OECD 既存化学物質安全性点検に係る毒性調査事業の一環として、パラアセトアルデヒドの染色体異常誘発作用を評価するため、CHL/IU 細胞を用いる染色体異常試験を実施した。

をスライドグラス(あらかじめフロスト部分に試験番号、コード番号およびスライド番号を記入)上に滴下し、そのまま風乾した。1 ディッシュあたり 6 枚のスライド標本を作製した。

作製したスライド標本を 3 vol%ギムザ液(pH 6.8 の 1/15 mol/L リン酸緩衝液で希釈調製)で染色後、水道水ですすいで風乾した。

8. 染色体分析

染色体分析に先立ち、1 枚のディッシュから得られた 1 枚の標本を用いて、濃度の高い方から分裂指数(500 細胞/標本)を分析した。0.5%未満の分裂指数を示した場合は染色体分析不能と判断し、また、標本あたりの分析可能な分裂中期細胞が少ない場合にはその数を考慮して、分析可能な最高濃度群を決定することとした。

ディッシュ 1 枚から得られたスライド標本 4 枚を、4 人の観察者がそれぞれ処理条件の分からない状態で分析した。染色体がよく広がり、かつ散逸していない分裂中期像を探し、1 群あたり 200 個(100 細胞/ディッシュ、25 細胞/観察者)の分裂中期細胞(染色体数:23～27 本)について構造異常の種類と数を、1 群あたり 800 個(400 細胞/ディッシュ、100 細胞/観察者)の分裂中期細胞について倍数性細胞(染色体数が 38 本以上)の数を調べた。その結果に基づいて構造異常を持つ細胞と倍数性細胞の出現率を求めた。

ギャップおよび切断を除く染色体異常の分類は、日本環境変異原学会・哺乳動物試験分科会 [1]による分類法に基づいて行った。染色分体幅より狭い非染色性部位をギャップ、それ以上幅の広いものを切断と定義し、ギャップについては構造異常誘発性の判定には含めないこととした。

染色体の構造異常(ギャップを除く)を有する細胞および倍数性細胞の出現数について、陰性対照群と被験物質処理群間および陽性対照群間で、フィッシャーの直接確率法 [2](p<0.01、片側)により有意差検定を実施した。また、有意差の認められた処理条件についてはその用量依存性についてコクラン・アーミテッジの傾向性検定 [3](p<0.01、片側)を実施することとした。これらの検定結果を参考とし、生物学的な観点からの判断を加味して染色体異常誘発性の評価を総合的に行った。

予見することができなかった試験の信頼性に影響を及ぼす疑いのある事態及び試験計画書に従わなかったこと

本試験期間中に「予見することができなかった試験の信頼性に影響を及ぼす疑いのある事態及び試験計画書に従わなかったこと」はなかった。

試験成績と考察

用量設定のために実施した細胞増殖抑制試験の結果をもとに、公比 2 で以下の濃度群を設定し、短時間処理による染色体異常試験を実施した。

S9 mix 非存在下の短時間処理:0.16、0.33、0.65、1.3 mg/mL

・S9 mix 存在下の短時間処理:0.16、0.33、0.65、1.3 mg/mL

なお、沈殿の有無を肉眼で観察した結果、いずれの処理群においても培養液中に沈殿は認められなかった。

染色体分析に先立ち実施した分裂指数の分析結果をもとに、観察対象群を以下のように決定し、染色体分析を行った。

S9 mix 非存在下の短時間処理:0.33、0.65、1.3 mg/mL

S9 mix 存在下の短時間処理:0.33、0.65、1.3 mg/mL

染色体分析の結果、S9 mix 非存在下で短時間処理した場合、高濃度群(1.3 mg/mL)においてのみ構造異常を有する細胞の統計学的に有意な増加(出現率:5.5%)が認められ、傾向性検定の結果も有意となった。それ以外は、構造異常を有する細胞および倍数性細胞の統計学的有意差は認められなかった(Table 1)。

S9 mix 存在下で短時間処理した場合には、いずれの濃度群においても構造異常を有する細胞および倍数性細胞の統計学的有意差は認められなかった(Table 2)。

以上のように、S9 mix 非存在下および存在下で短時間処理した場合、明らかな陽性結果が得られなかったことから、細胞増殖抑制試験結果をもとに以下の濃度群(公比 2)を設け、24 時間連続処理による染色体異常試験を実施した。

24 時間連続処理:0.081、0.16、0.33、0.65、1.3 mg/mL

染色体分析に先立ち実施した分裂指数の分析結果をもとに、観察対象群を以下のように決定し、染色体分析を行った。

24 時間連続処理:0.33、0.65、1.3 mg/mL

染色体分析の結果、24 時間連続処理した高濃度群(1.3 mg/mL)で構造異常を有する細胞の統計学的に有意な増加(出現率:56.5%)が認められ、傾向性検定も有意となった。それ以外は、構造異常を有する細胞および倍数性細胞の統計学的に有意な増加は認められなかった(Table 3)。

・陽性結果が得られた S9 mix 非存在下の短時間処理および 24 時間連続処理に関して D20 値[4]を求めたところ、それぞれ 5.3 mg/mL および 0.67 mg/mL となった。

なお、当該試験で使用したパラアセトアルデヒドについては、不純物としてアセトアルデヒドが 11.2%含まれている。アセトアルデヒドは S9 mix 非存在下で短時間処理した場合および連続処理した場合、0.03 mg/mL 以上の濃度で 16%以上の細胞に染色体の構造異常を誘発することが報告[5]されている。今回陽性結果の得られた 1.3 mg/mL は、純度換算しないと 1.47 mg/mL であり、その時のアセトアルデヒドの濃度は 0.16 mg/mL と推定され、被験物質であるパラアセトアルデヒドではなく、不純物であるアセトアルデヒドが染色体の構造異常を誘発した可能性も十分に考えられる。

パラアセトアルデヒドについては、当研究所で実施した細菌を用いる復帰突然変異試験(試験番号:M-05-132)で陰性の結果が得られている。また、ベンゼン環にメチル基の結合した 1,3,5-trimethyl-benzene に関しては復帰突然変異試験、染色体異常試験ともに陰性の結果が報告[6]されている。

陽性対照物質として用いた MMC は、S9 mix 非存在下の短時間処理および 24 時間連続処理におい

て染色体の構造異常を誘発し(Tables 1、3)、CPは短時間処理のS9 mix存在下において染色体の構造異常を誘発した(Table 2)。これらの陽性対照物質の結果より、本実験系の成立が確認された。

以上の結果より、本試験に用いたパラアセトアルデヒドは本試験条件においてCHL/IU細胞に染色体異常を誘発するが、それは不純物であるアセトアルデヒドにより誘発された可能性も考えられた。

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Table 1 Chromosome analysis of Chinese hamster cells (CHL/IU) treated with 2,4,6-trimethyl-1,3,5-trioxane (PAA) for 6 h without S9 mix

Group	Concen-[2] tration (mg/mL)	S 9 mix	Time of exposure (h)	Concurrent[3] cell growth (%)	Mitotic[4] index (%)	Number of cells analyzed	Number of structural aberrations							Others[6]	Number of cells with aberrations		Number[7] of polyploid cells (%)	Trend test[8]	
							gap	ctb	cte	csb	cse	mul[5]	total		+gap (%)	-gap (%)		-gap	POL
Negative[1]	0	−	6 - (18)	100	NA	100	1	0	0	0	1	0	2	1	2 (2.0)	1 (1.0)	0 (0.0)		
						100	1	0	0	0	0	0	1	0	1 (1.0)	0 (0.0)	0 (0.0)		
						200	2	0	0	0	1	0	3	1	3 (1.5)	1 (0.5)	0 (0.0)		
PAA	0.16	−	6 - (18)	98	NA	not observed													
PAA	0.33	−	6 - (18)	95	NA	100	1	1	1	0	0	0	3	0	3 (3.0)	2 (2.0)	1 (0.3)		
						100	3	0	0	4	0	0	7	0	4 (4.0)	1 (1.0)	0 (0.0)		
						200	4	1	1	4	0	0	10	0	7 (3.5)	3 (1.5)	1 (0.1)		
PAA	0.65	−	6 - (18)	89	NA	100	2	1	0	0	0	0	3	0	3 (3.0)	1 (1.0)	1 (0.3)		
						100	0	1	1	0	0	0	2	1	2 (2.0)	2 (2.0)	1 (0.3)		
						200	2	2	1	0	0	0	5	1	5 (2.5)	3 (1.5)	2 (0.3)	+	NA
PAA	1.3	−	6 - (18)	83	8.2, 7.4	100	0	2	1	0	0	0	3	0	3 (3.0)	3 (3.0)	0 (0.0)		
						100	3	5	1	2	1	0	12	0	10 (10.0)	8 (8.0)	4 (1.0)		
						200	3	7	2	2	1	0	15	0	13 (6.5)	11 *(5.5)	4 (0.5)		
MMC	0.1 μg/mL	−	6 - (18)	NA	NA	100	1	17	45	0	0	10	73	0	40 (40.0)	39 (39.0)	0 (0.0)		
						100	5	18	64	0	0	0	87	2	50 (50.0)	47 (47.0)	0 (0.0)		
						200	6	35	109	0	0	10	160	2	90 (45.0)	86 *(43.0)	0 (0.0)		

Abbreviations: gap, chromatid gap and chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; POL, polyploid; MMC, mitomycin C; NA, not analyzed.

1) Water for injection JP was used as a solvent and added at the level of 10 vol% per dish. 2) The concentration of PAA was adjusted for the purity (88.5%). 3) Cell confluency, representing cytotoxicity, was measured with a Monocellater™. 4) Metaphase frequency was calculated by counting 500 cells in each dish. 5) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 6) Others, such as attenuation and premature chromosome condensation, were excluded from the number of structural aberrations. 7) Eight hundred cells were analyzed in each group. 8) Cochran-Armitage's trend test was done at $p<0.01$ (one-side).

*, Significantly different from the negative control at $p<0.01$ (one-side) by Fisher's exact probability test.

Table 2 Chromosome analysis of Chinese hamster cells (CHL/IU) treated with 2,4,6-trimethyl-1,3,5-trioxane (PAA) for 6 h with S9 mix

Group	Concen-[2] tration (mg/mL)	S 9 mix	Time of exposure (h)	Concurrent[3] cell growth (%)	Mitotic[4] index (%)	Number of cells analyzed	Number of structural aberrations gap	ctb	cte	csb	cse	mul[5]	total	Others[6]	Number of cells with aberrations +gap (%)	-gap (%)	Number[7] of polyploid cells (%)	Trend test[8] -gap	POL
Negative[1]	0	+	6 - (18)	100	NA	100	0	1	1	0	0	0	2	0	2 (2.0)	2 (2.0)	1 (0.3)		
						100	0	1	2	0	0	0	3	0	3 (3.0)	3 (3.0)	1 (0.3)		
						200	0	2	3	0	0	0	5	0	5 (2.5)	5 (2.5)	2 (0.3)		
PAA	0.16	+	6 - (18)	99	NA	not observed													
PAA	0.33	+	6 - (18)	97	NA	100	0	1	0	0	0	0	1	1	1 (1.0)	1 (1.0)	1 (0.3)		
						100	0	4	1	0	0	0	5	0	5 (5.0)	5 (5.0)	0 (0.0)		
						200	0	5	1	0	0	0	6	1	6 (3.0)	6 (3.0)	1 (0.1)		
PAA	0.65	+	6 - (18)	94	NA	100	1	2	0	0	0	0	3	2	3 (3.0)	2 (2.0)	0 (0.0)		
						100	0	1	1	0	1	0	3	0	3 (3.0)	3 (3.0)	0 (0.0)		
						200	1	3	1	0	1	0	6	2	6 (3.0)	5 (2.5)	0 (0.0)	NA	NA
PAA	1.3	+	6 - (18)	90	9.2, 7.0	100	2	3	2	0	0	0	7	1	7 (7.0)	5 (5.0)	1 (0.3)		
						100	0	2	2	1	0	0	5	0	4 (4.0)	4 (4.0)	2 (0.5)		
						200	2	5	4	1	0	0	12	1	11 (5.5)	9 (4.5)	3 (0.4)		
CP	10 μg/mL	+	6 - (18)	NA	NA	100	2	25	52	0	1	0	80	1	48 (48.0)	46 (46.0)	0 (0.0)		
						100	5	21	50	0	1	0	77	0	48 (48.0)	45 (45.0)	1 (0.3)		
						200	7	46	102	0	2	0	157	1	96 (48.0)	91 *(45.5)	1 (0.1)		

Abbreviations: gap, chromatid gap and chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; POL, polyploid; CP, cyclophosphamide; NA, not analyzed.

1) Water for injection JP was used as a solvent and added at the level of 10 vol% per dish. 2) The concentration of PAA was adjusted for the purity (88.5%). 3) Cell confluency, representing cytotoxicity, was measured with a Monocellater™. 4) Metaphase frequency was calculated by counting 500 cells in each dish. 5) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 6) Others, such as attenuation and premature chromosome condensation, were excluded from the number structural aberrations. 7) Eight hundred cells were analyzed in each group. 8) Cochran-Armitage's trend test was done at $p<0.01$ (one-side).

*, Significantly different from the negative control at $p<0.01$ (one-side) by Fisher's exact probability test.

Table 3 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with 2,4,6-trimethyl-1,3,5-trioxane (PAA) for 24 h without S9 mix

Group	Concen-[2] tration (mg/mL)	Time of exposure (h)	Concurrent[3] cell growth (%)	Mitotic[4] index (%)	Number of cells analyzed	Number of structural aberrations gap	ctb	cte	csb	cse	mul[5]	total	Others[6]	Number of cells with aberrations +gap (%)	-gap (%)	Number[7] of polyploid cells (%)	Trend test[8] -gap	POL
Negative[1]	0	24	100	NA	100	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	4 (1.0)		
					100	0	2	0	0	0	0	2	0	2 (2.0)	2 (2.0)	1 (0.3)		
					200	0	2	0	0	0	0	2	0	2 (1.0)	2 (1.0)	5 (0.6)		
PAA	0.081	24	100	NA	not observed													
PAA	0.16	24	95	NA	not observed													
PAA	0.33	24	86	NA	100	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	2 (0.5)		
					100	0	0	1	0	0	0	1	2	1 (1.0)	1 (1.0)	1 (0.3)		
					200	0	0	1	0	0	0	1	2	1 (0.5)	1 (0.5)	3 (0.4)		
PAA	0.65	24	76	NA	100	1	1	1	1	0	0	4	0	4 (4.0)	3 (3.0)	2 (0.5)		
					100	2	4	2	0	0	0	8	0	7 (7.0)	6 (6.0)	0 (0.0)		
					200	3	5	3	1	0	0	12	0	11 (5.5)	9 (4.5)	2 (0.3)	+	NA
PAA	1.3	24	57	5.2, 1.8	100	3	23	59	1	1	0	87	2	51 (51.0)	51 (51.0)	3 (0.8)		
					100	4	45	69	1	0	0	119	2	64 (64.0)	62 (62.0)	0 (0.0)		
					200	7	68	128	2	1	0	206	4	115 (57.5)	113 *(56.5)	3 (0.4)		
MMC	0.05 μg/mL	24	NA	NA	100	9	29	60	0	0	0	98	0	59 (59.0)	53 (53.0)	1 (0.3)		
					100	2	21	60	0	0	0	83	0	58 (58.0)	57 (57.0)	0 (0.0)		
					200	11	50	120	0	0	0	181	0	117 (58.5)	110 *(55.0)	1 (0.1)		

Abbreviations: gap, chromatid gap and chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; POL, polyploid; MMC, mitomycin C; NA, not analyzed.

1) Water for injection JP was used as a solvent and added at the level of 10 vol% per dish. 2) The concentration of PAA was adjusted for the purity (88.5%). 3) Cell confluency, representing cytotoxicity, was measured with a Monocellater™. 4) Metaphase frequency was calculated by counting 500 cells in each dish. 5) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 6) Others, such as attenuation and premature chromosome condensation, were excluded from the number of structural aberrations. 7) Eight hundred cells were analyzed in each group. 8) Cochran-Armitage's trend test was done at $p<0.01$ (one-side).

*, Significantly different from the negative control at $p<0.01$ (one-side) by Fisher's exact probability test.

4. 要約

Sprague-Dawley 系 SPF ラット〔Crl:CD(SD)〕を用いて、パラアセトアルデヒドの反復投与による毒性並びにその可逆性を検討した。投与量は 0（コーン油：対照群）、100、300 及び 1000 mg/kg/day とし、28 日間反復強制経口投与した。1 群の動物数は対照群及び 1000 mg/kg 投与群で雌雄各 12 匹、100 及び 300 mg/kg 投与群で雌雄各 6 匹とした。このうち、対照群及び 1000 mg/kg 投与群の雌雄各 6 例については、28 日間投与後 2 週間休薬させた。

投与及び回復期間を通じて死亡動物はみられず、一般状態、詳細な一般状態の観察、握力、体重、摂餌量、尿検査、血液学及び血液化学検査では、被験物質投与の影響は認められなかった。

機能検査では、1000 mg/kg 投与群の雌雄で着地開脚幅の高値が認められた。この変化は休薬により消失し、回復性が認められた。

自発運動量では、1000 mg/kg 投与群の雌雄で測定開始後 20 分以降及び測定開始後 60 分間の合計の値に低値が認められた。この変化は休薬により消失し、回復性が認められた。

病理学検査では、肝臓において 1000 mg/kg 投与群の雌で相対重量の高値がみられ、組織学的にも 300 mg/kg 投与群の雄及び 1000 mg/kg 投与群の雌雄で小葉中心性の肝細胞肥大、300 mg/kg 以上の雄で門脈域における肝細胞の空胞化の減少が認められた。また、胃において 1000 mg/kg 投与群の雌雄で境界縁の肥厚が認められた。これらの変化は休薬により消失あるいは軽減がみられ、回復傾向が認められた。

以上の結果から、パラアセトアルデヒドの本試験条件下における無影響量は、雄で 100 mg/kg/day、雌で 300 mg/kg/day と推定された。なお、投与期間中に認められた変化については、いずれも休薬により消失あるいは軽減し、回復あるいは回復傾向が認められた。

7. 試験結果

7.1 一般状態

成績を Table 1-1~1-3 及び Appendix 1~10 に示した。

いずれの動物においても、投与及び回復期間を通じて異常は認められなかった。

7.2 詳細な一般状態、機能検査、握力及び自発運動量

7.2.1 詳細な一般状態

成績を Table 2-1~2-18 及び Appendix 11~70 に示した。

1) 投与期間

いずれの検査項目においても異常はなく、各被験物質投与群の雌雄とも対照群との間に有意差は認められなかった。

2) 回復期間

回復第 1 週の検査において、オープンフィールド内観察で 1000 mg/kg 投与群の雌の立ち上がり回数に有意な低値が認められたが、投与期間には認められていないことから、偶発性の変化と判断した。

7.2.2 機能検査

成績を Table 2-19、2-20 及び Appendix 71~76 に示した。

1) 投与第 4 週

1000 mg/kg 投与群の雌雄で着地開脚幅に有意な高値が認められた。

2) 回復第 2 週

いずれの検査項目においても異常はなく、1000 mg/kg 投与群の雌雄とも対照群の間に有意差はみられなかった。

7.2.3 握力

成績を Table 2-21、2-22 及び Appendix 77~82 に示した。

1) 投与第 4 週

各被験物質投与群の雌雄とも握力は、対照群とほぼ同様な値を示し、有意差はみられなかった。

2) 回復第 2 週

1000 mg/kg 投与群の雌の後肢で有意な低値が認められたが、機能検査など他の検査項目に異常はなく、投与第 4 週には同様な変化は認められていないことから、偶発性と判断した。

7.2.4 自発運動量

成績を Fig. 1~4、Table 2-23、2-24 及び Appendix 83~88 に示した。

1) 投与第 4 週

1000 mg/kg 投与群の雌雄において、測定開始後 20 分以降及び測定開始後 60 分間の合計の値で有意な低値が認められた。

2) 回復第 2 週

1000 mg/kg 投与群の雌雄とも対照群とほぼ同様に推移し、有意差は認められなかった。

7.3 体重

成績を Fig.5、Table 3-1、3-2 及び Appendix 89~94 に示した。

1) 投与期間

各被験物質投与群の雌雄とも対照群とほぼ同様に推移し、有意差は認められなかった。

2) 回復期間

1000 mg/kg 投与群の雌雄とも対照群とほぼ同様に推移し、有意差は認められなかった。

7.4 摂餌量

成績を Fig.6、Table 4-1、4-2 及び Appendix 95~100 に示した。

1) 投与期間

各被験物質投与群の雌雄とも対照群とほぼ同様に推移し、有意差は認められなかった。

2) 回復期間

1000 mg/kg 投与群の雌雄とも対照群とほぼ同様に推移し、有意差は認められなかった。

7.5 尿検査（摂水量含む）

成績を Table 5-1~5-8 及び Appendix 101~118 に示した。

1) 投与第 4 週

1000 mg/kg 投与群の雄で摂水量に有意な高値が認められたが、ごく軽度な変化であり、また、尿量及び浸透圧などの関連項目に変化がみられないことから、偶発性の変化と判断した。

2) 回復第 2 週

対照群及び 1000 mg/kg 投与群のいずれの動物でも定性的項目及び尿沈渣に異常はなく、尿量、摂水量及び尿浸透圧においても 1000 mg/kg 投与群と対照群との間に有意差はみられなかった。

7.6 血液学検査

成績を Table 6-1~6-4 及び Appendix 119~130 に示した。

1) 投与期間終了時

いずれの検査項目についても、各被験物質投与群の雌雄とも対照群との間に有意差は認められなかった。

2) 回復期間終了時

1000 mg/kg 投与群の雌で、赤血球数の有意な高値が認められたが、投与期間終了時には認められていないことから、偶発性の変化と判断した。

7.7 血液化学検査

成績を Table 7-1~7-4 Appendix 131~142 に示した。

1) 投与期間終了時

1000 mg/kg 投与群の雄で、ALP 活性の有意な低値が認められたが、毒性を示唆する高値ではなく、また、ごく軽度な変化であることから、偶発性の変化と判断した。

2) 回復期間終了時

いずれの検査項目についても、1000 mg/kg 投与群の雌雄とも対照群との間に有意差は認められなかった。

7.8 器官重量

成績を Table 8-1~8-8 及び Appendix 143~166 に示した。

1) 投与期間終了時

肝臓 : 相対重量の有意な高値が 1000 mg/kg 投与群の雌に認められた。

以下に示す所見についてはその出現状況から、偶発性の変化と判断した。

脾臓 : 相対重量の有意な高値が 300 mg/kg 投与群の雄に認められた。

2) 回復期間終了時

以下に示す所見についてはその出現状況から、偶発性の変化と判断した。

脾臓 : 絶対及び相対重量の有意な低値が 1000 mg/kg 投与群の雌に認められた。

7.9 剖検所見

成績を Table 9-1、9-2 及び Appendix 167~238 に示した。

1) 投与期間終了時

以下に示す所見についてはその出現状況などから、いずれも偶発性の変化と判断した。

腎臓 : 陥凹巣が 300 mg/kg 投与群の雌 1 例、のう胞が 1000 mg/kg 投与群の雄 1 例に認められた。

肺　：　暗赤色巣が300及び1000 mg/kg投与群の雄各1例に認められた。

子宮　：　のう胞が100 mg/kg投与群の1例に認められた。

2)　回復期間終了時

いずれの動物においても剖検所見に異常は認められなかった。

7.10　病理組織学検査

成績をTable 10-1~10-4及びAppendix 167~238に示した。

1)　投与期間終了時

被験物質投与によると考えられる変化が肝臓及び胃に認められた。

肝臓　：　軽微あるいは軽度な小葉中心性の肝細胞肥大が300 mg/kg投与群の雄1例、1000 mg/kg投与群の雄5例と雌3例にみられた。また、軽微あるいは軽度な門脈域における肝細胞の空胞化が対照群の雄全例と雌5例、100 mg/kg投与群の雄4例と雌3例、300 mg/kg投与群の雄2例と雌4例、1000 mg/kg投与群の雄1例と雌4例に認められ、300 mg/kg以上の投与群の雄では発現例数が減少した。

胃　：　軽微な境界縁の肥厚が1000 mg/kgの雄1例と雌2例に認められた。

以下に示す所見については、その出現状況あるいは病理組織学的性状からいずれも偶発性の変化と判断した。

心臓　：　軽微な心筋炎が対照群の雄1例に認められた。

盲腸　：　軽微な粘膜の細胞浸潤が対照群の雄1例に認められた。

腎臓　：　軽微な好酸性小体が対照群の雄1例と1000 mg/kg投与群の雄2例に認められた。軽微な再生尿細管が対照群の雄1例、1000 mg/kg投与群の雄2例と雌1例に認められ、また、剖検において陥凹巣がみられた300 mg/kg投与群の雌1例でも軽度な再生尿細管が認められた。さらに、剖検においてのう胞がみられた1000 mg/kg投与群の雄1例では軽微な尿細管のう胞が認められた。

肝臓　：　軽微あるいは軽度な微小肉芽腫が対照群の雄1例と雌4例、100 mg/kg投与群の雄1例と雌4例、300 mg/kg投与群の雌4例、1000 mg/kg投与群の雄2例と雌4例に認められた。

肺　：　軽微な泡沫細胞の集簇が1000 mg/kg投与群の雄1例に認められた。また、剖検において暗赤色巣がみられた

300及び1000 mg/kg投与群の雄各1例では軽度な限局性の出血が認められた。

下垂体 : 軽微なのう胞が対照群の雄1例に認められた。

前立腺 : 軽微あるいは軽度な間質の細胞浸潤が対照群の4例、1000 mg/kg投与群の2例に認められた。

脾臓 : 軽微な髄外造血が対照群の雄4例と雌1例、1000 mg/kg投与群の雄3例に認められた。

胃 : 軽微なびらんが1000 mg/kg投与群の雄1例に認められた。

甲状腺 : 軽微な異所性胸腺が対照群及び1000 mg/kgの雄各1例に、軽微な鰓後体のう胞が対照群及び1000 mg/kg投与群の雌各2例に認められた。

子宮 : 剖検においてのう胞がみられた100 mg/kg投与群の1例に軽微なのう胞が認められた。

2) 回復期間終了時

以下に示す所見については、その出現状況あるいは病理組織学的性状からいずれも偶発性の変化と判断した。

肝臓 : 軽微な門脈域における肝細胞の空胞化が対照群の雄2例と雌3例、1000 mg/kg投与群の雄1例と雌2例に、軽微な微小肉芽腫が対照群の雌雄各2例、1000 mg/kg投与群の雄2例と雌5例に認められた。

8. 考察

Sprague-Dawley系SPFラット〔Crl:CD(SD)〕にパラアセトアルデヒドを0（コーン油：対照群）、100、300及び1000 mg/kg/dayの用量で28日間反復強制経口投与し、その毒性を検討するとともに、対照群及び1000 mg/kg投与群はその後2週間休薬させ、変化の可逆性について検討した。

投与及び回復期間を通じて死亡動物はみられず、一般状態、詳細な一般状態、握力、体重、摂餌量、尿検査、血液学及び血液化学検査では、被験物質投与の影響は認められなかった。

機能検査では、1000 mg/kg投与群の雌雄で着地開脚幅の高値がみられ、被験物質投与の影響が疑われた。この変化は休薬により消失し、回復性が認められた。

自発運動量では、1000 mg/kg投与群の雌雄で測定開始後20分以降及び測定開始後60分間の合計の値に低値がみられ、被験物質投与の影響が疑われた。これらの変化は休薬により消失し、回復性が認められた。

病理学検査では、肝臓において1000 mg/kg投与群の雌で相対重量の高値がみられ、組織学的にも300 mg/kg投与群の雄と1000 mg/kg投与群の雌雄で小葉中心性の肝細胞肥大、300 mg/kg以上の雄で門脈域における肝細胞の空胞化の減少がみられ、被験物質投与の影響が認められた。胃において1000 mg/kg投与群の雌雄で境界縁の肥厚がみられ、被験物質投与の影響が疑われた。これらの変化は休薬により消失あるいは軽減し、回復傾向が認められた。

以上の結果から、本試験条件下におけるパラアセトアルデヒドの無影響量は、雄では300 mg/kg以上の投与群に病理組織学検査で肝臓の変化がみられたことから、100 mg/kg/dayと推定された。また、雌では1000 mg/kg投与群に機能検査で着地開脚幅、自発運動量、器官重量で肝臓、病理組織学検査で肝臓及び胃の変化がみられたことから、300 mg/kg/dayと推定された。なお、投与期間に認められた変化については、いずれの変化も休薬により消失あるいは軽減し、回復性あるいは回復傾向が認められた。

Table 1-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Clinical signs (Administration period)

Sex	Dose mg/kg	Findings	Day of administration													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Male	100	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	300	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	0	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Female	100	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	300	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12

Table 1-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Clinical signs (Administration period)

Sex	Dose mg/kg	Findings	Day of administration													
			15	16	17	18	19	20	21	22	23	24	25	26	27	28
Male	0	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	100	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	300	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Female	0	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	100	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	300	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12
		No abnormality	12	12	12	12	12	12	12	12	12	12	12	12	12	12

Table 1-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Clinical signs (Recovery period)

Sex	Dose mg/kg	Findings	Day of recovery													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Male	0	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Female	0	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1000	No. of animals	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		No abnormality	6	6	6	6	6	6	6	6	6	6	6	6	6	6

Table 2-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 1)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 2)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 3)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 4)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-5 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 1 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Posture					
Normal		6	6	6	6
Convulsion					
None		6	6	6	6
Abnormal behavior					
None		6	6	6	6

No significant difference between treated group and control group.

Table 2-6 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : home cage observations (Week 2 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Posture					
Normal		6	6	6	6
Convulsion					
None		6	6	6	6
Abnormal behavior					
None		6	6	6	6

No significant difference between treated group and control group.

Table 2-7 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 1)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Ease of removal from cage									
Easy		11	6	6	10	12	5	6	11
Some resistance/avoidance		1	0	0	1	0	1	0	1
Difficult		0	0	0	1	0	0	0	0
Fur condition									
Normal		12	6	6	12	12	6	6	12
Skin									
Normal		12	6	6	12	12	6	6	12
Secretions-Eye, Nose									
Absent		12	6	6	12	12	6	6	12
Exophthalmos									
Absent		12	6	6	12	12	6	6	12
Palpebral closure									
Normal		12	6	6	12	12	6	6	12
Mucosal membranes									
Normal		12	6	6	12	12	6	6	12
Lacrimation									
Normal		12	6	6	12	12	6	6	12
Piloerection									
Absent		12	6	6	12	12	6	6	12
Pupil size									
Normal		12	6	6	12	12	6	6	12
Salivation									
None		12	6	6	12	12	6	6	12
Abnormal respiration									
Absent		12	6	6	12	12	6	6	12
Reactivity to handling									
Easy		10	6	6	10	12	5	6	11
Slightly awkward		1	0	0	2	0	1	0	1
Difficult		1	0	0	0	0	0	0	0

No significant difference in any treated groups from control group.

Table 2-8 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 2)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Ease of removal from cage									
Easy		12	6	6	12	12	6	6	12
Fur condition									
Normal		12	6	6	12	12	6	6	12
Skin									
Normal		12	6	6	12	12	6	6	12
Secretions-Eye, Nose									
Absent		12	6	6	12	12	6	6	12
Exophthalmos									
Absent		12	6	6	12	12	6	6	12
Palpebral closure									
Normal		12	6	6	12	12	6	6	12
Mucosal membranes									
Normal		12	6	6	12	12	6	6	12
Lacrimation									
Normal		12	6	6	12	12	6	6	12
Piloerection									
Absent		12	6	6	12	12	6	6	12
Pupil size									
Normal		12	6	6	12	12	6	6	12
Salivation									
None		12	6	6	12	12	6	6	12
Abnormal respiration									
Absent		12	6	6	12	12	6	6	12
Reactivity to handling									
Easy		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-9 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 3)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Ease of removal from cage									
Easy		12	6	6	12	11	6	6	12
Some resistance/avoidance		0	0	0	0	1	0	0	0
Fur condition									
Normal		12	6	6	12	12	6	6	12
Skin									
Normal		12	6	6	12	12	6	6	12
Secretions-Eye, Nose									
Absent		12	6	6	12	12	6	6	12
Exophthalmos									
Absent		12	6	6	12	12	6	6	12
Palpebral closure									
Normal		12	6	6	12	12	6	6	12
Mucosal membranes									
Normal		12	6	6	12	12	6	6	12
Lacrimation									
Normal		12	6	6	12	12	6	6	12
Piloerection									
Absent		12	6	6	12	12	6	6	12
Pupil size									
Normal		12	6	6	12	12	6	6	12
Salivation									
None		12	6	6	12	12	6	6	12
Abnormal respiration									
Absent		12	6	6	12	12	6	6	12
Reactivity to handling									
Easy		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-10 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 4)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Ease of removal from cage									
Easy		12	6	6	12	12	6	6	12
Fur condition									
Normal		12	6	6	12	12	6	6	12
Skin									
Normal		12	6	6	12	12	6	6	12
Secretions-Eye, Nose									
Absent		12	6	6	12	12	6	6	12
Exophthalmos									
Absent		12	6	6	12	12	6	6	12
Palpebral closure									
Normal		12	6	6	12	12	6	6	12
Mucosal membranes									
Normal		12	6	6	12	12	6	6	12
Lacrimation									
Normal		12	6	6	12	12	6	6	12
Piloerection									
Absent		12	6	6	12	12	6	6	12
Pupil size									
Normal		12	6	6	12	12	6	6	12
Salivation									
None		12	6	6	12	12	6	6	12
Abnormal respiration									
Absent		12	6	6	12	12	6	6	12
Reactivity to handling									
Easy		12	6	6	12	12	6	6	12

No significant difference in any treated groups from control group.

Table 2-11 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 1 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Ease of removal from cage					
Easy		6	6	6	6
Fur condition					
Normal		6	6	6	6
Skin					
Normal		6	6	6	6
Secretions-Eye, Nose					
Absent		6	6	6	6
Exophthalmos					
Absent		6	6	6	6
Palpebral closure					
Normal		6	6	6	6
Mucosal membranes					
Normal		6	6	6	6
Lacrimation					
Normal		6	6	6	6
Piloerection					
Absent		6	6	6	6
Pupil size					
Normal		6	6	6	6
Salivation					
None		6	6	6	6
Abnormal respiration					
Absent		6	6	6	6
Reactivity to handling					
Easy		6	6	5	6
Slightly awkward		0	0	1	0

No significant difference between treated group and control group.

Table 2-12 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : in-the-hand observations (Week 2 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Ease of removal from cage					
Easy		6	6	6	6
Fur condition					
Normal		6	6	6	6
Skin					
Normal		6	6	6	6
Secretions-Eye, Nose					
Absent		6	6	6	6
Exophthalmos					
Absent		6	6	6	6
Palpebral closure					
Normal		6	6	6	6
Mucosal membranes					
Normal		6	6	6	6
Lacrimation					
Normal		6	6	6	6
Piloerection					
Absent		6	6	6	6
Pupil size					
Normal		6	6	6	6
Salivation					
None		6	6	6	6
Abnormal respiration					
Absent		6	6	6	6
Reactivity to handling					
Easy		6	6	6	6

No significant difference between treated group and control group.

Table 2-13 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 1)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Arousal									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12
Stereotypy									
None		12	6	6	12	12	6	6	12
Gait									
Normal		12	6	6	12	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Grooming									
None		12	6	6	12	12	6	6	12
Rearing count (Mean±S.D.)		5± 1	4± 2	5± 2	5± 3	8± 3	9± 4	6± 1	6± 2
Defecation count (Mean±S.D.)		0± 1	1± 1	0± 1	1± 1	0± 0	0± 0	0± 0	0± 0
Urination									
None		11	4	6	9	12	6	6	11
Small amount		1	1	0	3	0	0	0	1
Moderate amount		0	1	0	0	0	0	0	0

No significant difference in any treated groups from control group.

Table 2-14 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 2)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Arousal									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior									
None		12	6	6	12	12	6	6	12
Stereotypy									
None		12	6	6	12	12	6	6	12
Gait									
No/minimal location		0	0	0	0	0	0	0	1
Normal		12	6	6	12	12	6	6	11
Posture									
Normal		12	6	6	12	12	6	6	12
Grooming									
None		12	6	6	12	12	6	6	12
Rearing count (Mean±S.D.)		4± 2	3± 1	4± 3	5± 3	8± 2	8± 4	6± 2	7± 4
Defecation count (Mean±S.D.)		0± 1	0± 1	1± 1	1± 1	0± 0	0± 0	0± 0	0± 0
Urination									
None		10	6	5	9	12	6	6	11
Small amount		2	0	1	3	0	0	0	1

No significant difference in any treated groups from control group.

Table 2-15 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 3)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Arousal									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior							a)		
None		12	6	6	12	12	5	6	12
Minor		0	0	0	0	0	1	0	0
Stereotypy									
None		12	6	6	12	12	6	6	12
Gait									
No/minimal location		1	1	0	1	0	0	0	0
Normal		11	5	6	11	12	6	6	12
Posture									
Normal		12	6	6	12	12	6	6	12
Grooming									
None		12	6	6	12	12	6	6	12
Rearing count (Mean±S.D.)		4± 2	2± 1	6± 3	5± 3	9± 1	9± 4	7± 3	7± 3
Defecation count (Mean±S.D.)		0± 1	0± 0	0± 0	0± 1	0± 0	0± 0	0± 0	0± 0
Urination									
None		9	4	4	10	11	6	6	12
Small amount		2	2	1	0	1	0	0	0
Moderate amount		1	0	1	2	0	0	0	0

a): Running
No significant difference in any treated groups from control group.

Table 2-16 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 4)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Arousal									
Normal		12	6	6	12	12	6	6	12
Convulsion									
None		12	6	6	12	12	6	6	12
Abnormal behavior						a)			
None		12	6	6	12	11	6	6	12
Minor		0	0	0	0	1	0	0	0
Stereotypy									
None		12	6	6	12	12	6	6	12
Gait									
No/minimal location		0	1	0	0	0	0	0	1
Normal		12	5	6	12	12	6	6	11
Posture									
Normal		12	6	6	12	12	6	6	12
Grooming									
None		12	6	6	12	12	6	6	12
Rearing count (Mean±S.D.)		5± 3	3± 2	5± 2	5± 2	9± 2	8± 3	8± 2	7± 4
Defecation count (Mean±S.D.)		0± 0	0± 1	0± 0	0± 1	0± 0	0± 0	0± 0	0± 0
Urination									
None		9	4	6	10	12	6	6	12
Small amount		2	2	0	2	0	0	0	0
Moderate amount		1	0	0	0	0	0	0	0

a): Running
No significant difference in any treated groups from control group.

Table 2-17 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 1 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Arousal					
Normal		6	6	6	6
Convulsion					
None		6	6	6	6
Abnormal behavior					
None		6	6	6	6
Stereotypy					
None		6	6	6	6
Gait					
No/minimal location		1	0	0	0
Normal		5	6	6	6
Posture					
Normal		6	6	6	6
Grooming					
None		6	6	6	6
Rearing count (Mean±S.D.)		5± 3	5± 1	8± 2	6± 2*T
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		6	5	5	6
Small amount		0	1	1	0

* : $p<0.05$ (Significant difference from control group)
T : Student's t-test

Table 2-18 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Detailed clinical signs : open field observation (Week 2 of recovery)

Parameter	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
	No. of animals	6	6	6	6
Arousal					
Normal		6	6	6	6
Convulsion					
None		6	6	6	6
Abnormal behavior					
None		6	6	6	6
Stereotypy					
None		6	6	6	6
Gait					
No/minimal location		1	0	0	0
Normal		5	6	6	6
Posture					
Normal		6	6	6	6
Grooming					
None		6	6	6	6
Rearing count (Mean±S.D.)		5± 3	4± 2	10± 2	9± 2
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		5	6	6	6
Small amount		1	0	0	0

No significant difference between treated group and control group.

Table 2-19 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Manipulative test (Week 4)

	Sex	Male				Female			
	Dose (mg/kg)	0	100	300	1000	0	100	300	1000
Parameter	No. of animals	12	6	6	12	12	6	6	12
Auditory response									
Weak		0	0	0	2	0	0	2	1
Normal		12	6	6	10	12	6	4	11
Approach response									
Normal		12	6	6	12	12	6	6	12
Touch response									
Normal		12	6	6	12	12	6	6	12
Tail pinch response									
Normal		11	6	6	12	10	6	6	12
Exaggerate		1	0	0	0	2	0	0	0
Pupillary reflex									
Pass, both		12	6	6	12	12	6	6	12
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		78±11	68±13	72±18	91±10*D	59±19	47±15	63±19	79± 5*DT

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test
DT : Dunnett-type rank test

Table 2-20 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Manipulative test (Week 2 of recovery)

	Sex	Male		Female	
	Dose (mg/kg)	0	1000	0	1000
Parameter	No. of animals	6	6	6	6
Auditory response Normal		6	6	6	6
Approach response Normal		6	6	6	6
Touch response Normal		6	6	6	6
Tail pinch response Normal		6	6	6	6
Pupillary reflex Pass, both		6	6	6	6
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		89±23	81±16	60±16	64±14

No significant difference between treated group and control group.

Table 2-21 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Grip strength (Week 4)

Sex	Dose mg/kg		Fore limb g	Hind limb g
Male	0	No.	12	12
		Mean	1062	494
		S.D.	169	77
	100	No.	6	6
		Mean	951	435
		S.D.	158	94
	300	No.	6	6
		Mean	992	494
		S.D.	62	79
	1000	No.	12	12
		Mean	978	456
		S.D.	108	85
Female	0	No.	12	12
		Mean	871	453
		S.D.	100	87
	100	No.	6	6
		Mean	830	453
		S.D.	123	46
	300	No.	6	6
		Mean	779	356
		S.D.	148	100
	1000	No.	12	12
		Mean	796	371
		S.D.	176	127

No significant difference in any treated groups from control group.

Table 2-22 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Grip strength (Week 2 of recovery)

Sex	Dose mg/kg		Fore limb g	Hind limb g
Male	0	No.	6	6
		Mean	1262	546
		S.D.	111	96
	1000	No.	6	6
		Mean	1092	571
		S.D.	235	86
Female	0	No.	6	6
		Mean	1074	560
		S.D.	126	112
	1000	No.	6	6
		Mean	903	408*
		S.D.	143	28AT

* : $p<0.05$ (Significant difference from control group)
AT : Aspin-Welch t-test

Table 2-23 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Motor activity (Week 4)

Sex	Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
Male	0	No.	12	12	12	12	12	12	12
		Mean	407	382	319	339	289	256	1992
		S.D.	31	62	61	75	124	147	334
	100	No.	6	6	6	6	6	6	6
		Mean	427	390	327	341	317	250	2051
		S.D.	44	54	68	45	64	142	273
	300	No.	6	6	6	6	6	6	6
		Mean	422	385	387	312	275	175	1956
		S.D.	48	73	67	42	124	168	309
	1000	No.	12	12	12	12	12	12	12
		Mean	388	327	163**	126**	101**	49**	1154**
		S.D.	45	71	147D	141DT	118D	89D	408D
Female	0	No.	12	12	12	12	12	12	12
		Mean	418	355	320	234	249	247	1823
		S.D.	38	52	84	103	140	154	387
	100	No.	6	6	6	6	6	6	6
		Mean	413	318	273	276	137	114	1531
		S.D.	37	87	57	97	90	103	249
	300	No.	6	6	6	6	6	6	6
		Mean	401	340	229	238	154	121	1483
		S.D.	25	104	148	180	134	124	512
	1000	No.	12	12	12	12	12	12	12
		Mean	383	263	105**	72**	24**	20**	867**
		S.D.	59	125	98D	121D	42DT	40DT	265D

** : $p<0.01$ (Significant difference from control group)
D : Dunnett's test
DT : Dunnett-type rank test

Table 2-24 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Motor activity (Week 2 of recovery)

Sex	Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
Male	0	No.	6	6	6	6	6	6	6
		Mean	397	349	315	227	218	221	1726
		S.D.	31	58	63	74	73	111	265
	1000	No.	6	6	6	6	6	6	6
		Mean	408	372	280	276	274	208	1819
		S.D.	27	46	63	51	139	125	256
Female	0	No.	6	6	6	6	6	6	6
		Mean	392	277	197	241	242	225	1573
		S.D.	19	74	150	147	206	122	528
	1000	No.	6	6	6	6	6	6	6
		Mean	378	292	247	286	258	209	1670
		S.D.	39	58	140	79	125	132	288

No significant difference between treated group and control group.

Table 3-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Body weight (Administration period)

Sex	Dose mg/kg		Day of administration 1	4	7	10	14	17	21	24	28	Gain 1-28
	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	210	236	264	289	322	343	365	380	398	188
		S.D.	7	9	10	12	16	18	23	25	28	26
Male	100	No.	6	6	6	6	6	6	6	6	6	6
		Mean	210	235	264	291	322	345	367	380	401	191
		S.D.	7	8	9	8	6	8	5	10	10	7
	300	No.	6	6	6	6	6	6	6	6	6	6
		Mean	209	235	261	285	317	339	364	377	398	189
		S.D.	9	14	19	23	31	34	39	39	46	38
	1000	No.	12	12	12	12	12	12	12	12	12	12
		Mean	209	232	260	286	314	337	358	369	387	178
		S.D.	9	10	12	15	17	20	24	28	29	23
	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	158	168	181	190	206	218	229	236	248	90
		S.D.	7	11	13	16	19	19	22	25	27	22
Female	100	No.	6	6	6	6	6	6	6	6	6	6
		Mean	157	168	177	187	201	209	221	228	241	84
		S.D.	7	8	9	14	20	20	22	23	31	28
	300	No.	6	6	6	6	6	6	6	6	6	6
		Mean	159	172	182	192	204	215	223	227	238	79
		S.D.	6	9	11	10	13	15	17	18	18	16
	1000	No.	12	12	12	12	12	12	12	12	12	12
		Mean	157	168	180	191	203	210	222	229	239	82
		S.D.	4	5	7	8	11	11	12	12	13	12

Unit : g
No significant difference in any treated groups from control group.

Table 3-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Body weight (Recovery period)

Sex	Dose mg/kg		Day of recovery 1	3	7	10	14	Gain 1-14
Male	0	No.	6	6	6	6	6	6
		Mean	404	416	436	448	463	60
		S.D.	35	39	42	44	50	16
	1000	No.	6	6	6	6	6	6
		Mean	394	405	424	434	452	58
		S.D.	38	37	41	41	46	11
Female	0	No.	6	6	6	6	6	6
		Mean	253	261	269	271	273	21
		S.D.	27	30	33	33	38	11
	1000	No.	6	6	6	6	6	6
		Mean	231	237	249	254	259	28
		S.D.	16	15	18	18	14	7

Unit : g
No significant difference between treated group and control group.

Table 4-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Food consumption (Administration period)

Sex	Dose mg/kg		Day of administration 1	4	7	10	14	17	21	24	28
	0	No.	12	12	12	12	12	12	12	12	12
		Mean	24	23	26	25	27	26	26	24	25
		S.D.	2	2	2	2	2	2	2	2	2
Male	100	No.	6	6	6	6	6	6	6	6	6
		Mean	24	23	25	25	26	25	25	24	25
		S.D.	1	1	2	2	1	1	1	1	1
	300	No.	6	6	6	6	6	6	6	6	6
		Mean	23	23	25	24	26	26	26	24	25
		S.D.	2	3	4	4	5	5	4	4	5
	1000	No.	12	12	12	12	12	12	12	12	12
		Mean	24	22	25	24	26	26	25	24	24
		S.D.	2	2	2	2	2	2	2	3	2
	0	No.	12	12	12	12	12	12	12	12	12
		Mean	19	17	17	17	18	18	18	17	19
		S.D.	2	2	1	1	2	1	2	2	2
Female	100	No.	6	6	6	6	6	6	6	6	6
		Mean	18	16	17	16	17	17	18	16	18
		S.D.	3	2	1	3	3	2	2	3	2
	300	No.	6	6	6	6	6	6	6	6	6
		Mean	20	17	18	17	18	17	17	16	18
		S.D.	2	2	2	1	2	2	2	2	2
	1000	No.	12	12	12	12	12	12	12	12	12
		Mean	18	16	17	17	18	17	18	17	18
		S.D.	1	2	1	2	1	2	2	1	2

Unit : g/rat/day
No significant difference in any treated groups from control group.

Table 4-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Food consumption (Recovery period)

Sex	Dose mg/kg		Day of recovery 3	7	10	14
Male	0	No.	6	6	6	6
		Mean	30	31	31	30
		S.D.	3	3	3	2
	1000	No.	6	6	6	6
		Mean	27	29	29	29
		S.D.	2	2	2	3
Female	0	No.	6	6	6	6
		Mean	22	22	21	20
		S.D.	2	3	2	3
	1000	No.	6	6	6	6
		Mean	21	21	20	20
		S.D.	2	2	2	1

Unit : g/rat/day
No significant difference between treated group and control group.

Table 5-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 4)

Sex	Dose mg/kg	No.	pH									1) Protein						2) Ketone body						3) Glucose					
			5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++
Male	0	12	0	0	0	1	2	1	3	3	2	0	3	8	1	0	0	1	4	7	0	0	0	12	0	0	0	0	0
	100	6	0	0	0	0	0	1	1	3	1	0	3	2	1	0	0	3	1	2	0	0	0	6	0	0	0	0	0
	300	6	0	0	0	4	0	0	1	1	0	0	0	6	0	0	0	0	2	3	1	0	0	6	0	0	0	0	0
	1000	12	0	0	0	0	2	0	4	6	0	0	2	9	1	0	0	2	3	7	0	0	0	12	0	0	0	0	0
Female	0	12	0	0	0	3	3	3	3	0	0	5	3	4	0	0	0	4	4	4	0	0	0	12	0	0	0	0	0
	100	6	0	0	0	2	1	1	1	1	0	0	2	4	0	0	0	0	3	3	0	0	0	6	0	0	0	0	0
	300	6	0	0	2	3	0	0	1	0	0	0	2	4	0	0	0	0	1	5	0	0	0	6	0	0	0	0	0
	1000	12	0	0	1	6	1	3	1	0	0	0	2	8	2	0	0	0	4	8	0	0	0	12	0	0	0	0	0

1) - : <10 mg/dL +- : 10 - 25 mg/dL + : 26 - 85 mg/dL ++ : 86 - 250 mg/dL +++ : 251 - 600 mg/dL ++++ : >600 mg/dL

2) - : <5 mg/dL +- : 5 - 7.5 mg/dL + : 7.6 - 30 mg/dL ++ : 31 - 70 mg/dL +++ : 71 - 125 mg/dL ++++ : >125 mg/dL

3) - : <30 mg/dL +- : 30 - 60 mg/dL + : 61 - 125 mg/dL ++ : 126 - 250 mg/dL +++ : 251 - 750 mg/dL ++++ : >750 mg/dL

Table 5-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 4)

Sex	Dose mg/kg	No.	4) Occult blood					5) Bilirubin					6) Urobilinogen					7) Color		
			-	+-	+	++	+++	-	+	++	+++	++++	+-	+	++	+++	++++	LY	Y	DY
	0	12	12	0	0	0	0	12	0	0	0	0	10	2	0	0	0	0	12	0
Male	100	6	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0	0	6	0
	300	6	6	0	0	0	0	6	0	0	0	0	4	2	0	0	0	0	6	0
	1000	12	12	0	0	0	0	12	0	0	0	0	8	4	0	0	0	0	12	0
	0	12	12	0	0	0	0	12	0	0	0	0	10	2	0	0	0	0	12	0
Female	100	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	0	6	0
	300	6	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0	0	6	0
	1000	12	12	0	0	0	0	12	0	0	0	0	5	7	0	0	0	0	12	0

4) - : <0.03 mg/dL +- : 0.03 - 0.05 mg/dL + : 0.06 - 0.15 mg/dL ++ : 0.16 - 0.75 mg/dL +++ : >0.75 mg/dL
5) - : <0.5 mg/dL + : 0.5 - 1.5 mg/dL ++ : 1.6 - 5.0 mg/dL +++ : 5.1 - 10.0 mg/dL ++++ : >10.0 mg/dL
6) +- : <2.0 mg/dL + : 2.0 - 3.5 mg/dL ++ : 3.6 - 7.0 mg/dL +++ : 7.1 - 12.0 mg/dL ++++ : >12.0 mg/dL
7) LY : Light yellow Y : Yellow DY : Dark yellow

Table 5-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 4)

Sex	Dose mg/kg	No.	URINE SEDIMENT																																	
			RBC					WBC					SEC					SREC					Cast			CRYSTALLIZATION PS						CRYSTALLIZATION CO				
			-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	-	+-	+	++	+++	-	+-	+	++	+++
Male	0	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	12	0	0	0	0	12	0	0	12	0	0	0	0	12	0	0	0	0
	100	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	6	0	0	0	0	6	0	0	0	0
	300	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	6	0	0	0	0	5	1	0	0	0
	1000	12	12	0	0	0	0	12	0	0	0	0	0	11	1	0	0	11	1	0	0	0	12	0	0	12	0	0	0	0	11	1	0	0	0
Female	0	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	12	0	0	0	0	12	0	0	12	0	0	0	0	12	0	0	0	0
	100	6	6	0	0	0	0	5	1	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	6	0	0	0	0	5	1	0	0	0
	300	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	6	0	0	0	0	6	0	0	0	0
	1000	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	12	0	0	0	0	12	0	0	11	1	0	0	0	10	2	0	0	0

SEC : Squamous Epithelial Cell
SREC : Small Round Epithelial Cell
PS : Phosphate Salts
CO : Calcium Oxalate

\- : Negative
+- : Slight
\+ : Mild
++ : Moderate
+++ : Severe

Table 5-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Water intake and urinalysis (Week 4)

Sex	Dose mg/kg	No.		Water intake mL/24h	Urine volume mL/24h	Osmolality mOsm/kg
	0	12	Mean	30	6.5	2136
			S.D.	5	3.0	382
Male	100	6	Mean	37	9.1	1734
			S.D.	5	3.0	354
	300	6	Mean	31	7.6	2068
			S.D.	5	1.4	285
	1000	12	Mean	38*	8.3	2082
			S.D.	9D	3.4	490
	0	12	Mean	28	5.4	2183
			S.D.	7	4.3	648
Female	100	6	Mean	30	6.0	2027
			S.D.	11	2.8	493
	300	6	Mean	29	5.3	2241
			S.D.	9	4.2	686
	1000	12	Mean	32	4.8	2325
			S.D.	7	2.1	445

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test

Table 5-5 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 2 of recovery)

Sex	Dose mg/kg	No.	pH									1) Protein						2) Ketone body						3) Glucose					
			5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++
Male	0	6	0	0	0	0	0	0	1	4	1	0	4	2	0	0	0	1	2	3	0	0	0	6	0	0	0	0	0
	1000	6	0	0	0	0	0	0	0	3	3	1	3	2	0	0	0	3	0	3	0	0	0	6	0	0	0	0	0
Female	0	6	0	0	0	0	2	2	0	1	1	3	0	3	0	0	0	3	0	3	0	0	0	6	0	0	0	0	0
	1000	6	0	0	0	2	0	1	0	3	0	2	3	1	0	0	0	1	2	3	0	0	0	6	0	0	0	0	0

1) - : <10 mg/dL +- : 10 - 25 mg/dL + : 26 - 85 mg/dL ++ : 86 - 250 mg/dL +++ : 251 - 600 mg/dL ++++ : >600 mg/dL
2) - : <5 mg/dL +- : 5 - 7.5 mg/dL + : 7.6 - 30 mg/dL ++ : 31 - 70 mg/dL +++ : 71 - 125 mg/dL ++++ : >125 mg/dL
3) - : <30 mg/dL +- : 30 - 60 mg/dL + : 61 - 125 mg/dL ++ : 126 - 250 mg/dL +++ : 251 - 750 mg/dL ++++ : >750 mg/dL

Table 5-6 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 2 of recovery)

Sex	Dose mg/kg	No.	4) Occult blood					5) Bilirubin					6) Urobilinogen					7) Color		
			-	+-	+	++	+++	-	+	++	+++	++++	+-	+	++	+++	++++	LY	Y	DY
Male	0	6	5	1	0	0	0	6	0	0	0	0	6	0	0	0	0	0	6	0
	1000	6	5	1	0	0	0	6	0	0	0	0	6	0	0	0	0	0	6	0
Female	0	6	6	0	0	0	0	5	1	0	0	0	5	1	0	0	0	0	6	0
	1000	6	5	1	0	0	0	6	0	0	0	0	6	0	0	0	0	0	6	0

4) - : <0.03 mg/dL +- : 0.03 - 0.05 mg/dL + : 0.06 - 0.15 mg/dL ++ : 0.16 - 0.75 mg/dL +++ : >0.75 mg/dL
5) - : <0.5 mg/dL + : 0.5 - 1.5 mg/dL ++ : 1.6 - 5.0 mg/dL +++ : 5.1 - 10.0 mg/dL ++++ : >10.0 mg/dL
6) +- : <2.0 mg/dL + : 2.0 - 3.5 mg/dL ++ : 3.6 - 7.0 mg/dL +++ : 7.1 - 12.0 mg/dL ++++ : >12.0 mg/dL
7) LY : Light yellow Y : Yellow DY : Dark yellow

Table 5-7 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Urinalysis (Week 2 of recovery)

Sex	Dose mg/kg	No.	URINE SEDIMENT																													
			RBC					WBC					SEC					SREC					Cast			CRYSTALLIZATION PS					CRYSTALLIZATION CO				
			-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	-	+-	+	++	+++	-	+-	+	++	+++
Male	0	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	4	2	0	0	0	6	0	0	0	0
	1000	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	5	1	0	0	0	6	0	0	0	0
Female	0	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	5	1	0	0	0	6	0	0	0	0
	1000	6	6	0	0	0	0	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	6	0	0	5	1	0	0	0	6	0	0	0	0

SEC : Squamous Epithelial Cell
SREC : Small Round Epithelial Cell
PS : Phosphate Salts
CO : Calcium Oxalate

\- : Negative
+- : Slight
\+ : Mild
++ : Moderate
+++ : Severe

Table 5-8 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Water intake and urinalysis (Week 2 of recovery)

Sex	Dose mg/kg	No.		Water intake mL/24h	Urine volume mL/24h	Osmolality mOsm/kg
Male	0	6	Mean	35	15.2	2000
			S.D.	5	4.4	230
	1000	6	Mean	38	12.9	1716
			S.D.	6	4.6	291
Female	0	6	Mean	32	9.0	2122
			S.D.	8	3.9	542
	1000	6	Mean	29	7.8	2064
			S.D.	4	3.5	557

No significant difference between treated group and control group.

Table 6-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Hematology (Day 28)

Sex	Dose mg/kg	No.		RBC $X10^4/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	Reticul. %	PLT $X10^4/\mu L$	PT s	APTT s	FIB mg/dL
	0	6	Mean	790	16.1	42.6	54.0	20.4	37.8	2.1	134.1	13.9	21.9	358
			S.D.	37	0.6	1.0	1.9	0.7	0.5	0.5	31.2	1.4	4.1	30
Male	100	6	Mean	799	16.4	43.0	53.8	20.5	38.1	2.2	120.8	13.7	20.5	370
			S.D.	40	0.6	1.8	1.1	0.5	0.5	0.4	11.0	0.5	3.0	26
	300	6	Mean	807	16.5	43.8	54.2	20.5	37.7	2.3	115.3	15.6	21.7	349
			S.D.	22	0.4	1.3	1.9	0.6	0.4	0.4	12.1	2.3	2.8	25
	1000	6	Mean	802	16.6	44.1	55.1	20.7	37.6	2.2	111.9	15.7	24.7	359
			S.D.	35	0.5	1.1	1.3	0.4	0.2	0.3	9.3	2.7	2.4	22
	0	6	Mean	769	15.8	40.9	53.2	20.6	38.8	2.0	130.5	12.5	16.0	272
			S.D.	43	0.6	1.2	1.5	0.5	0.4	0.5	9.6	0.7	1.8	17
Female	100	6	Mean	801	16.4	42.7	53.4	20.5	38.3	1.9	137.5	12.4	17.8	268
			S.D.	41	0.8	2.2	1.9	0.6	0.3	0.5	18.2	0.7	2.4	26
	300	6	Mean	805	16.6	43.3	53.9	20.6	38.3	1.5	127.0	12.3	18.0	267
			S.D.	25	0.7	1.6	0.8	0.3	0.3	0.3	11.8	0.6	2.0	29
	1000	6	Mean	812	16.2	42.2	51.9	19.9	38.4	1.7	136.1	12.5	18.1	277
			S.D.	25	0.7	1.5	1.6	0.7	0.3	0.3	12.3	0.4	2.0	20

No significant difference in any treated groups from control group.

Table 6-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Hematology (Day 28)

Sex	Dose mg/kg	No.		WBC	Differential leukocyte counts (%)					
				X10²/μL	LYM	NE	EOSINO	BASO	MONO	LUC
	0	6	Mean	95.2	76.6	19.2	1.3	0.4	1.9	0.7
			S.D.	19.3	5.8	4.9	0.5	0.1	0.5	0.3
Male	100	6	Mean	76.7	76.8	19.2	0.9	0.4	2.1	0.7
			S.D.	15.8	8.4	7.6	0.4	0.1	1.0	0.2
	300	6	Mean	114.1	77.8	18.3	1.0	0.5	1.9	0.6
			S.D.	30.3	4.1	3.6	0.3	0.1	0.5	0.1
	1000	6	Mean	82.6	75.2	20.3	1.0	0.5	2.1	0.9
			S.D.	25.8	10.1	10.0	0.3	0.2	0.5	0.1
	0	6	Mean	69.6	76.2	19.1	1.1	0.3	2.5	0.7
			S.D.	20.5	8.1	6.7	0.5	0.1	1.6	0.4
Female	100	6	Mean	71.5	77.2	18.8	1.3	0.4	1.6	0.8
			S.D.	11.9	9.5	9.0	0.5	0.2	0.4	0.2
	300	6	Mean	77.2	81.0	14.7	1.3	0.5	1.8	0.8
			S.D.	26.4	6.2	6.0	0.3	0.1	0.7	0.2
	1000	6	Mean	85.7	77.5	18.6	0.9	0.4	1.6	1.0
			S.D.	22.0	5.4	5.6	0.4	0.1	0.5	0.2

LUC : Large unstained cells
No significant difference in any treated groups from control group.

Table 6-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Hematology (Week 2 of recovery)

Sex	Dose mg/kg	No.		RBC $\times 10^4/\mu L$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	Reticul. %	PLT $\times 10^4/\mu L$	PT s	APTT s	FIB mg/dL
Male	0	6	Mean	836	16.5	42.6	51.0	19.8	38.8	2.0	117.3	13.9	20.2	373
			S.D.	40	0.6	1.4	2.2	0.8	0.3	0.4	8.0	1.2	2.0	29
	1000	6	Mean	862	17.1	43.7	50.8	19.8	39.0	1.8	118.1	14.4	21.8	381
			S.D.	15	0.4	1.2	0.7	0.2	0.3	0.3	9.9	1.4	3.6	31
Female	0	6	Mean	807	16.1	41.2	51.1	20.0	39.2	1.8	130.1	11.8	15.6	276
			S.D.	36	0.6	1.7	2.7	0.8	0.6	0.3	7.6	0.5	2.4	20
	1000	6	Mean	850*	16.6	42.3	49.8	19.6	39.2	1.4	137.0	12.2	17.3	281
			S.D.	23T	0.3	0.6	1.3	0.5	0.3	0.2	9.1	0.7	3.1	22

* : $p<0.05$ (Significant difference from control group)
T : Student's t-test

Table 6-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Hematology (Week 2 of recovery)

Sex	Dose mg/kg	No.		WBC	Differential leukocyte counts (%)					
				X10²/μL	LYM	NE	EOSINO	BASO	MONO	LUC
Male	0	6	Mean	105.8	73.5	21.8	1.5	0.4	2.3	0.6
			S.D.	31.4	5.9	6.0	0.2	0.1	0.6	0.2
	1000	6	Mean	98.1	78.7	16.6	1.1	0.5	2.4	0.7
			S.D.	36.1	4.8	4.5	0.4	0.2	0.5	0.3
Female	0	6	Mean	68.3	79.9	15.8	1.0	0.3	2.1	0.9
			S.D.	12.4	6.6	6.0	0.3	0.1	1.2	0.2
	1000	6	Mean	81.2	75.7	19.3	1.4	0.4	2.1	1.2
			S.D.	22.4	5.1	6.0	0.5	0.1	0.7	0.5

LUC : Large unstained cells
No significant difference between treated group and control group.

Table 7-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Blood chemistry (Day 28)

Sex	Dose mg/kg	No.		AST IU/L	ALT IU/L	LDH IU/L	γ-GTP IU/L	ALP IU/L	T-CHO mg/dL	TG mg/dL	PL mg/dL	T-BIL mg/dL	GLU mg/dL
Male	0	6	Mean	59	27	53	1	774	51	31	91	0.1	135
			S.D.	6	2	9	0	60	11	9	13	0.1	17
	100	6	Mean	56	27	51	1	631	59	38	103	0.1	141
			S.D.	3	5	8	0	114	9	19	10	0.1	4
	300	6	Mean	59	27	51	1	628	49	36	92	0.0	139
			S.D.	5	2	7	0	156	9	18	13	0.1	10
	1000	6	Mean	58	27	65	1	604*	59	31	100	0.1	140
			S.D.	5	2	19	1	97D	10	10	9	0.1	15
Female	0	6	Mean	65	27	68	2	452	51	9	93	0.1	112
			S.D.	9	10	17	1	93	10	2	14	0.0	12
	100	6	Mean	58	22	60	1	428	51	8	98	0.1	122
			S.D.	5	2	13	0	107	17	4	28	0.1	13
	300	6	Mean	58	24	53	1	366	56	10	101	0.1	119
			S.D.	6	3	10	0	55	21	4	27	0.1	16
	1000	6	Mean	55	25	59	1	405	63	12	112	0.1	124
			S.D.	6	2	9	1	74	13	6	24	0.1	14

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test

Table 7-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Blood chemistry (Day 28)

Sex	Dose mg/kg	No.		BUN mg/dL	CRNN mg/dL	Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	ALB g/dL	A/G
	0	6	Mean	11	0.23	142	4.9	107	9.8	8.0	5.9	2.9	0.93
			S.D.	2	0.03	1	0.3	1	0.3	0.8	0.2	0.1	0.02
Male	100	6	Mean	11	0.21	142	5.2	108	10.0	7.9	6.0	2.8	0.91
			S.D.	1	0.01	2	0.3	1	0.3	0.6	0.3	0.1	0.04
	300	6	Mean	12	0.23	142	5.0	107	9.9	8.0	5.7	2.8	0.93
			S.D.	1	0.03	1	0.3	2	0.3	0.4	0.2	0.1	0.05
	1000	6	Mean	11	0.22	142	5.0	107	10.0	7.9	6.1	2.9	0.93
			S.D.	1	0.01	1	0.3	1	0.2	0.5	0.2	0.1	0.07
	0	6	Mean	15	0.27	142	4.5	109	9.9	7.4	6.1	3.0	0.98
			S.D.	2	0.03	1	0.1	1	0.3	0.6	0.2	0.1	0.04
Female	100	6	Mean	14	0.25	142	4.7	110	9.9	7.3	5.9	3.0	1.01
			S.D.	2	0.03	1	0.4	1	0.3	0.4	0.2	0.1	0.03
	300	6	Mean	16	0.29	141	4.5	109	10.0	7.9	6.0	3.0	1.00
			S.D.	1	0.02	1	0.2	2	0.3	0.6	0.2	0.1	0.06
	1000	6	Mean	15	0.26	142	4.5	108	10.1	7.9	6.3	3.1	0.97
			S.D.	2	0.03	2	0.3	1	0.2	0.3	0.3	0.1	0.07

No significant difference in any treated groups from control group.

Table 7-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Blood chemistry (Week 2 of recovery)

Sex	Dose mg/kg	No.		AST IU/L	ALT IU/L	LDH IU/L	γ-GTP IU/L	ALP IU/L	T-CHO mg/dL	TG mg/dL	PL mg/dL	T-BIL mg/dL	GLU mg/dL
Male	0	6	Mean	60	28	59	1	576	56	50	99	0.1	149
			S.D.	5	5	16	0	156	8	16	9	0.0	20
	1000	6	Mean	60	32	59	1	486	62	51	107	0.1	151
			S.D.	7	6	10	1	42	12	11	16	0.0	21
Female	0	6	Mean	60	24	45	1	274	68	17	122	0.1	117
			S.D.	8	3	9	0	47	13	9	22	0.0	20
	1000	6	Mean	62	24	49	1	383	66	17	119	0.1	115
			S.D.	7	1	15	1	128	11	4	15	0.0	11

No significant difference between treated group and control group.

Table 7-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Blood chemistry (Week 2 of recovery)

Sex	Dose mg/kg	No.		BUN mg/dL	CRNN mg/dL	Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	ALB g/dL	A/G
Male	0	6	Mean	15	0.24	144	4.6	107	9.9	7.3	6.1	2.8	0.84
			S.D.	1	0.03	2	0.3	1	0.3	0.5	0.1	0.1	0.05
	1000	6	Mean	14	0.24	143	4.5	107	9.7	7.4	6.0	2.8	0.88
			S.D.	2	0.02	3	0.3	3	0.3	0.5	0.3	0.1	0.03
Female	0	6	Mean	16	0.30	143	4.5	109	10.1	7.3	6.4	3.1	0.94
			S.D.	1	0.04	1	0.2	1	0.2	0.5	0.2	0.1	0.08
	1000	6	Mean	15	0.28	144	4.6	110	10.0	7.2	6.3	3.0	0.92
			S.D.	2	0.02	1	0.3	1	0.3	0.3	0.4	0.2	0.04

No significant difference between treated group and control group.

Table 8-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Day 28)

Male

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)	Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
	0	No.	6	6	6	6	6	6	6	6
		Mean	374	2.05	431	1.21	11.68	0.62	2.88	59
		S.D.	24	0.05	130	0.06	1.34	0.05	0.25	8
Absolute	100	No.	6	6	6	6	6	6	6	6
		Mean	376	2.01	478	1.25	12.40	0.66	2.86	55
		S.D.	11	0.09	76	0.07	1.09	0.09	0.14	6
	300	No.	6	6	6	6	6	6	6	6
		Mean	370	2.00	559	1.25	11.71	0.71	2.83	61
		S.D.	40	0.09	92	0.18	2.70	0.13	0.45	13
	1000	No.	6	6	6	6	6	6	6	6
		Mean	360	1.99	447	1.18	11.77	0.60	2.83	62
		S.D.	26	0.07	71	0.08	0.89	0.12	0.26	11
	0	No.		6	6	6	6	6	6	6
		Mean		0.55	115	0.33	3.12	0.16	0.77	16
		S.D.		0.03	35	0.04	0.19	0.01	0.05	2
Relative	100	No.		6	6	6	6	6	6	6
		Mean		0.54	127	0.33	3.30	0.18	0.76	15
		S.D.		0.03	19	0.02	0.30	0.02	0.04	2
	300	No.		6	6	6	6	6	6	6
		Mean		0.54	152	0.34	3.13	0.19*	0.76	16
		S.D.		0.04	26	0.03	0.38	0.02D	0.06	3
	1000	No.		6	6	6	6	6	6	6
		Mean		0.55	125	0.33	3.27	0.17	0.79	17
		S.D.		0.03	21	0.03	0.13	0.03	0.04	2

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test

Table 8-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Day 28)

Male

	Dose mg/kg		Testis (R+L) g(g/100g BW)	Epididymis (R+L) mg(mg/100g BW)
	0	No.	6	6
		Mean	3.21	862
		S.D.	0.18	49
Absolute	100	No.	6	6
		Mean	3.18	863
		S.D.	0.27	94
	300	No.	6	6
		Mean	3.05	844
		S.D.	0.52	66
	1000	No.	6	6
		Mean	3.01	836
		S.D.	0.32	96
	0	No.	6	6
		Mean	0.86	232
		S.D.	0.05	26
Relative	100	No.	6	6
		Mean	0.85	230
		S.D.	0.07	23
	300	No.	6	6
		Mean	0.82	229
		S.D.	0.08	16
	1000	No.	6	6
		Mean	0.84	233
		S.D.	0.09	30

No significant difference in any treated groups from control group.

Table 8-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Day 28)

Female

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)	Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
	0	No.	6	6	6	6	6	6	6	6
		Mean	231	1.92	484	0.83	6.90	0.50	1.78	69
		S.D.	24	0.06	190	0.10	1.01	0.09	0.16	11
Absolute	100	No.	6	6	6	6	6	6	6	6
		Mean	224	1.91	444	0.82	6.68	0.53	1.74	67
		S.D.	25	0.09	181	0.10	1.10	0.14	0.19	9
	300	No.	6	6	6	6	6	6	6	6
		Mean	219	1.88	437	0.77	6.63	0.48	1.58	68
		S.D.	15	0.06	103	0.09	0.95	0.11	0.14	7
	1000	No.	6	6	6	6	6	6	6	6
		Mean	228	1.89	470	0.84	7.62	0.55	1.76	72
		S.D.	8	0.07	145	0.06	0.28	0.09	0.12	5
	0	No.		6	6	6	6	6	6	6
		Mean		0.84	206	0.36	2.97	0.22	0.77	30
		S.D.		0.08	62	0.01	0.16	0.03	0.03	5
Relative	100	No.		6	6	6	6	6	6	6
		Mean		0.86	194	0.37	2.98	0.24	0.78	30
		S.D.		0.07	53	0.02	0.25	0.04	0.05	4
	300	No.		6	6	6	6	6	6	6
		Mean		0.86	200	0.35	3.02	0.22	0.72	31
		S.D.		0.04	46	0.02	0.24	0.04	0.04	1
	1000	No.		6	6	6	6	6	6	6
		Mean		0.83	206	0.37	3.35*	0.24	0.77	32
		S.D.		0.02	59	0.02	0.13D	0.04	0.05	3

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test

Table 8-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Day 28)

Female

	Dose mg/kg		Ovary (R+L) mg(mg/100g BW)	Uterus mg(mg/100g BW)
Absolute	0	No.	6	6
		Mean	91.7	432
		S.D.	14.6	112
	100	No.	6	6
		Mean	89.3	487
		S.D.	15.6	195
	300	No.	6	6
		Mean	74.9	419
		S.D.	5.9	156
	1000	No.	6	6
		Mean	92.7	438
		S.D.	14.3	95
Relative	0	No.	6	6
		Mean	39.8	186
		S.D.	6.4	40
	100	No.	6	6
		Mean	39.9	213
		S.D.	4.3	62
	300	No.	6	6
		Mean	34.4	189
		S.D.	3.2	60
	1000	No.	6	6
		Mean	40.7	192
		S.D.	6.1	41

No significant difference in any treated groups from control group.

Table 8-5 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Week 2 of recovery)

Male

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)	Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
Absolute	0	No.	6	6	6	6	6	6	6	6
		Mean	435	2.06	432	1.32	12.77	0.75	3.05	59
		S.D.	46	0.08	126	0.17	2.11	0.16	0.25	9
	1000	No.	6	6	6	6	6	6	6	6
		Mean	421	2.12	495	1.29	12.29	0.72	2.93	65
		S.D.	43	0.07	110	0.17	2.26	0.12	0.33	13
Relative	0	No.		6	6	6	6	6	6	6
		Mean		0.48	99	0.30	2.92	0.17	0.71	14
		S.D.		0.04	27	0.02	0.19	0.03	0.06	2
	1000	No.		6	6	6	6	6	6	6
		Mean		0.51	118	0.31	2.90	0.17	0.70	15
		S.D.		0.04	25	0.06	0.23	0.02	0.05	2

No significant difference between treated group and control group.

Table 8-6 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Week 2 of recovery)

Male

	Dose mg/kg		Testis (R+L) g(g/100g BW)	Epididymis (R+L) mg(mg/100g BW)
Absolute	0	No.	6	6
		Mean	3.20	1054
		S.D.	0.27	93
	1000	No.	6	6
		Mean	3.23	1063
		S.D.	0.25	58
Relative	0	No.	6	6
		Mean	0.74	246
		S.D.	0.11	46
	1000	No.	6	6
		Mean	0.77	254
		S.D.	0.08	23

No significant difference between treated group and control group.

Table 8-7 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Week 2 of recovery)

Female

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)	Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
Absolute	0	No.	6	6	6	6	6	6	6	6
		Mean	258	1.96	374	0.84	6.96	0.56	1.79	67
		S.D.	33	0.05	61	0.07	1.00	0.08	0.15	15
	1000	No.	6	6	6	6	6	6	6	6
		Mean	242	1.94	353	0.84	6.67	0.45*	1.83	69
		S.D.	16	0.07	82	0.07	0.57	0.06T	0.14	14
Relative	0	No.		6	6	6	6	6	6	6
		Mean		0.77	147	0.33	2.70	0.22	0.70	26
		S.D.		0.09	26	0.02	0.07	0.02	0.05	5
	1000	No.		6	6	6	6	6	6	6
		Mean		0.81	146	0.35	2.76	0.19*	0.76	29
		S.D.		0.07	30	0.02	0.09	0.02T	0.05	5

* : $p<0.05$ (Significant difference from control group)
T : Student's t-test

Table 8-8 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Absolute and relative organ weight (Week 2 of recovery)

Female

	Dose mg/kg		Ovary (R+L) mg(mg/100g BW)	Uterus mg(mg/100g BW)
Absolute	0	No.	6	6
		Mean	85.3	476
		S.D.	8.8	155
	1000	No.	6	6
		Mean	76.6	418
		S.D.	15.4	106
Relative	0	No.	6	6
		Mean	33.4	186
		S.D.	4.1	59
	1000	No.	6	6
		Mean	31.6	173
		S.D.	5.1	45

No significant difference between treated group and control group.

Table 9-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Gross pathological findings (Day 28)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg):	0	100	300	1000	0	100	300	1000
	Number:	6	6	6	6	6	6	6	6
Kidney									
Focus,depressed		0	0	0	0	0	0	1	0
Cyst		0	0	0	1	0	0	0	0
Lung(bronchus)									
Focus,dark red		0	0	1	1	0	0	0	0
Uterus									
Cyst		-	-	-	-	0	1	0	0

- : Not applicable

Table 9-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Gross pathological findings (Week 2 of recovery)

Organs	Sex:	M	M	F	F
	Dose(mg/kg):	0	1000	0	1000
Findings	Number:	6	6	6	6
All tissues					
Not remarkable		6	6	6	6

Table 10-1 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Histopathological findings (Day 28)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg):	0	100	300	1000	0	100	300	1000
	Number:	6	6	6	6	6	6	6	6
Adrenal									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Bone+Bone marrow,femoral									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Bone+Bone marrow,sternal									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Cerebellum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Cerebrum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Epididymis									
Number examined		6	0	0	6	-	-	-	-
Not remarkable		6	0	0	6	-	-	-	-
Eye									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Heart									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		5	0	0	6	6	0	0	6
Cardiomyopathy		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Intestine,duodenum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Intestine,jejunum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Intestine,ileum(Peyer's patch)									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Intestine,cecum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		5	0	0	6	6	0	0	6
Cell infiltration,mucosal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Intestine,colon									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Intestine,rectum									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Kidney									
Number examined		6	0	0	6	6	0	1	6
Not remarkable		5	0	0	3	6	0	0	5

- : Not applicable

Table 10-2 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Histopathological findings (Day 28)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg):	0	100	300	1000	0	100	300	1000
	Number:	6	6	6	6	6	6	6	6
Kidney (continued)									
Cyst		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Regeneration,tubular		1	0	0	2	0	0	1	1
minimal		1	0	0	2	0	0	0	1
mild		0	0	0	0	0	0	1	0
Eosinophilic body,tubular cell		1	0	0	2	0	0	0	0
minimal		1	0	0	2	0	0	0	0
Liver									
Number examined		6	6	6	6	6	6	6	6
Not remarkable		0	1	3	0	0	1	1	1
Vacuolation,hepatocyte,periportal		6	4	2	1	5	3	4	4
minimal		5	4	2	1	3	3	3	3
mild		1	0	0	0	2	0	1	1
Microgranuloma		1	1	0	2	4	4	4	4
minimal		1	1	0	2	3	4	4	4
mild		0	0	0	0	1	0	0	0
Hypertrophy,hepatocytic,central		0	0	1	5	0	0	0	3
minimal		0	0	1	2	0	0	0	3
mild		0	0	0	3	0	0	0	0
Lung(bronchus)									
Number examined		6	0	1	6	6	0	0	6
Not remarkable		6	0	0	4	6	0	0	6
Hemorrhage,focal		0	0	1	1	0	0	0	0
mild		0	0	1	1	0	0	0	0
Accumulation,foamy cell		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Lymph node,mesenteric									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Lymph node,submandibular									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Ovary									
Number examined		-	-	-	-	6	0	0	6
Not remarkable		-	-	-	-	6	0	0	6
Parathyroid									
Number examined		5	0	0	6	6	0	0	5
Not remarkable		5	0	0	6	6	0	0	5
No sample		1	0	0	0	0	0	0	1
Pituitary									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		5	0	0	6	6	0	0	6
Cyst		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Prostate									
Number examined		6	0	0	6	-	-	-	-
Not remarkable		2	0	0	4	-	-	-	-
Cell infiltration,interstitial		4	0	0	2	-	-	-	-
minimal		3	0	0	2	-	-	-	-
mild		1	0	0	0	-	-	-	-
Sciatic nerve									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6

- : Not applicable

Table 10-3 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Histopathological findings (Day 28)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg):	0	100	300	1000	0	100	300	1000
	Number:	6	6	6	6	6	6	6	6
Skeletal muscle,femoral									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Spinal cord,thoracic									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Spleen									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		2	0	0	3	5	0	0	6
Hematopoiesis,extramedullary		4	0	0	3	1	0	0	0
minimal		4	0	0	3	1	0	0	0
Stomach									
Number examined		6	6	6	6	6	6	6	6
Not remarkable		6	6	6	4	6	6	6	4
Erosion		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Thickening,limiting ridge		0	0	0	1	0	0	0	2
minimal		0	0	0	1	0	0	0	2
Testis									
Number examined		6	0	0	6	-	-	-	-
Not remarkable		6	0	0	6	-	-	-	-
Thymus									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Thyroid									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		5	0	0	5	4	0	0	4
Ectopic thymus		1	0	0	1	0	0	0	0
minimal		1	0	0	1	0	0	0	0
Cyst,ultimobranchial		0	0	0	0	2	0	0	2
minimal		0	0	0	0	2	0	0	2
Trachea									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Urinary bladder									
Number examined		6	0	0	6	6	0	0	6
Not remarkable		6	0	0	6	6	0	0	6
Uterus									
Number examined		-	-	-	-	6	1	0	6
Not remarkable		-	-	-	-	6	0	0	6
Cyst		-	-	-	-	0	1	0	0
minimal		-	-	-	-	0	1	0	0

- : Not applicable

Table 10-4 A 28-day oral toxicity study of paracetaldehyde in rats with a recovery period of 2 weeks

Histopathological findings (Week 2 of recovery)

Organs / Findings	Sex:	M	M	F	F
	Dose(mg/kg):	0	1000	0	1000
	Number:	6	6	6	6
Liver					
Number examined		6	6	6	6
Not remarkable		2	3	3	1
Vacuolation,hepatocyte,periportal		2	1	3	2
minimal		2	1	3	2
Microgranuloma		2	2	2	5
minimal		2	2	2	5
Stomach					
Number examined		6	6	6	6
Not remarkable		6	6	6	6

要　約

2,3,4,4'-テトラヒドロキシベンゾフェノンの遺伝子突然変異誘発性の有無を検討するため，復帰突然変異試験を指標菌株として *Salmonella typhimurium* TA100，TA1535，TA98，TA1537 および *Escherichia coli* WP2*uvrA* を用い，S9 mix 非存在（直接法）および存在（代謝活性化法）下でプレインキュベーション法により行った。

用量は，用量設定試験（予備試験）の結果，菌の生育阻害が認められる用量を最高用量とし，直接法においては TA100 および TA1535 で 31.3～1000μg/プレート，TA98 および TA1537 で 1.56～50μg/プレート，WP2*uvrA* では 62.5～2000μg/プレートの範囲（公比 2），また，代謝活性化法ではいずれの菌株とも 156～5000μg/プレートの範囲（公比 2）で設定した。

試験は 2 回実施した。その結果，全ての菌株において代謝活性化の有無にかかわらず，復帰変異コロニー数の増加は認められなかった。菌の生育阻害については，直接法では TA100 の 1000μg/プレート，TA1535 の 500μg/プレート以上，TA98 および TA1537 の 25μg/プレート以上，および WP2*uvrA* の 2000μg/プレートの用量で，また，代謝活性化法では TA100，TA1535 および WP2*uvrA* の 2500μg/プレート以上，TA98 および TA1537 の 5000μg/プレートの用量で認められた。

以上の成績から，本実験条件下では，2,3,4,4'-テトラヒドロキシベンゾフェノンの細菌に対する遺伝子突然変異誘発性は陰性と判定した。

(2) 被験物質用量の増加とともに復帰変異コロニー数が増加する（用量依存性）。

(3) 2回にわたる本試験の結果から復帰変異コロニー数の増加に再現性が認められる。但し，明確な用量依存性が認められない場合においても，陽性値を示す試験結果に再現性が認められれば陽性と判定する。

結　果

試験を2回実施した結果（表2-1-1，2-1-2，2-2，3-1-1，3-1-2，3-2および図1-1，1-2，1-3，1-4，1-5，2-1，2-2，2-3，2-4，2-5），直接法および代謝活性化法のいずれの場合も，供試したすべての菌株において復帰変異コロニー数は，陰性対照値の2倍を超えることはなかった。菌の生育阻害については，直接法ではTA100の1000μg/プレート，TA1535の500μg/プレート以上，TA98およびTA1537の25μg/プレート以上，およびWP2*uvrA*の2000μg/プレートの用量で，また，代謝活性化法ではTA100，TA1535およびWP2*uvrA*の2500μg/プレート以上，TA98およびTA1537の5000μg/プレートの用量で認められた。

陰性対照群では背景データ（添付資料）の範囲内の復帰変異コロニー数が認められた。陽性対照群においては明らかな復帰変異コロニー数の増加が認められ，その程度は，それぞれ背景データ（添付資料）の範囲内の陽性値を示すものであった。また，試験に用いた菌液，溶媒，被験物質の供試液およびS9 mixなどには，雑菌の混入は認められなかった。その他，実験中被験物質の析出等，特記すべき変化は認められなかった。

結　論

2,3,4,4'-テトラヒドロキシベンゾフェノンについて遺伝子突然変異誘発性の有無を調べるため，細菌を用いる復帰突然変異試験を実施した。その結果，代謝活性化の有無にかかわらず，全ての指標菌株で復帰変異コロニー数の増加は認められなかった。

試験の有効性については，2回にわたる本試験ともに有効であることが確認された。

したがって，本実験条件下では2,3,4,4'-テトラヒドロキシベンゾフェノンの遺伝子突然変異誘発性は陰性と判定した。

表 1-1　　S9 mix 非存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの用量設定試験結果〔直接法〕

用　量 〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照〔ジメチルスルホキシド〕	100	15	20	21	12
20	106	11	21	35	13
50	132	14	18	34 *	19 *
100	115	16	19	14 *	21 *
200	124	19	22	27 *	17 *
500	125	16	22	26 *	17 *
1000	136 *	4 *	23	30 *	17 *
2000	0 *	0 *	10 *	1 *	1 *
5000	0 *	0 *	0 *	0 *	0 *
陽性対照	AF−2	SA	AF−2	AF−2	9−AA
μg/プレート	0.01	0.5	0.04	0.1	80
復帰変異コロニー数／プレート	1172	439	419	285	351

＊　：菌の生育阻害が認められた。
AF-2：2-(2-フリル)-3-(5-ニトロ-2-フリル)アクリルアミド
SA　：アジ化ナトリウム
9-AA：9-アミノアクリジン

表 1-2　S9 mix 存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの用量設定試験結果〔代謝活性化法〕

用量〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照〔ジメチルスルホキシド〕	110	14	28	26	18
20	138	22	20	28	22
50	117	22	13	37	15
100	129	22	19	21	19
200	129	25	20	23	11
500	137	24	23	14	15
1000	128	13	11	15	9
2000	117	8	18	18	12
5000	0 *	0 *	2 *	0 *	0 *
陽性対照	2-AA	2-AA	2-AA	2-AA	2-AA
μg/プレート	1	2	10	1	2
復帰変異コロニー数／プレート	205	108	443	280	55

＊　：菌の生育阻害が認められた。
2-AA: 2-アミノアントラセン

表 2-1-1　S9 mix 非存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの復帰突然変異試験結果〔本試験1回目－直接法〕

用量〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照	99	10	17	--	--
〔ジメチル	96	11	21	--	--
スルホキシド〕	107	10	17	--	--
	(101 ± 6)	(10 ± 1)	(18 ± 2)	--	--
	115	8	--	--	--
31.3	127	10	--	--	--
	90	10	--	--	--
	(111 ± 19)	(9 ± 1)	--	--	--
	108	12	29	--	--
62.5	100	11	24	--	--
	103	13	27	--	--
	(104 ± 4)	(12 ± 1)	(27 ± 3)	--	--
	115	5	19	--	--
125	124	14	18	--	--
	118	7	25	--	--
	(119 ± 5)	(9 ± 5)	(21 ± 4)	--	--
	127	7	30	--	--
250	105	6	21	--	--
	104	6	28	--	--
	(112 ± 13)	(6 ± 1)	(26 ± 5)	--	--
	125	4 *	24	--	--
500	109	6 *	23	--	--
	131	7 *	20	--	--
	(122 ± 11)	(6 ± 2)	(22 ± 2)	--	--
	13 *	0 *	25	--	--
1000	23 *	0 *	36	--	--
	14 *	0 *	39	--	--
	(17 ± 6)	(0 ± 0)	(33 ± 7)	--	--
	--	--	15 *	--	--
2000	--	--	19 *	--	--
	--	--	24 *	--	--
	--	--	(19 ± 5)	--	--
陽性対照	AF－2	SA	AF－2	AF－2	9－AA
μg/プレート	0.01	0.5	0.04	0.1	80
復帰変異	998	327	585	--	--
コロニー数	948	324	572	--	--
／プレート	984	328	642	--	--
	(977 ± 26)	(326 ± 2)	(600 ± 37)	--	--

( ): 平均値±標準偏差
* : 菌の生育阻害が認められた。
AF-2: 2-(2-フリル)-3-(5-ニトロ-2-フリル)アクリルアミド
SA : アジ化ナトリウム
9-AA: 9-アミノアクリジン

表 2-1-2　S9 mix 非存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの復帰突然変異試験結果〔本試験1回目－直接法〕

用量 〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照	--	--	--	21	9
〔ジメチル	--	--	--	21	8
スルホキシド〕	--	--	--	18	10
	--	--	--	(20 ± 2)	(9 ± 1)
	--	--	--	21	13
1.56	--	--	--	17	12
	--	--	--	13	9
	--	--	--	(17 ± 4)	(11 ± 2)
	--	--	--	24	14
3.13	--	--	--	16	16
	--	--	--	20	13
	--	--	--	(20 ± 4)	(14 ± 2)
	--	--	--	19	7
6.25	--	--	--	32	15
	--	--	--	27	18
	--	--	--	(26 ± 7)	(13 ± 6)
	--	--	--	24	16
12.5	--	--	--	34	13
	--	--	--	22	18
	--	--	--	(27 ± 6)	(16 ± 3)
	--	--	--	30 *	13 *
25	--	--	--	36 *	12 *
	--	--	--	23 *	17 *
	--	--	--	(30 ± 7)	(14 ± 3)
	--	--	--	30 *	0 *
50	--	--	--	18 *	0 *
	--	--	--	28 *	0 *
	--	--	--	(25 ± 6)	(0 ± 0)
陽性対照	AF－2	SA	AF－2	AF－2	9－AA
μg/プレート	0.01	0.5	0.04	0.1	80
復帰変異	--	--	--	329	228
コロニー数	--	--	--	242	215
／プレート	--	--	--	272	239
	--	--	--	(281 ± 44)	(227 ± 12)

( ) : 平均値±標準偏差
* : 菌の生育阻害が認められた。
AF-2: 2-(2-フリル)-3-(5-ニトロ-2-フリル)アクリルアミド
SA : アジ化ナトリウム
9-AA: 9-アミノアクリジン

表 2-2　　S9 mix 存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの
復帰突然変異試験結果〔本試験1回目－代謝活性化法〕

用量	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
〔μg/プレート〕	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照	108	16	25	28	9
〔ジメチル	95	9	25	24	14
スルホキシド〕	90	10	16	28	17
	(98 ± 9)	(12 ± 4)	(22 ± 5)	(27 ± 2)	(13 ± 4)
	139	9	36	33	14
156	127	10	30	13	10
	112	1	28	29	9
	(126 ± 14)	(7 ± 5)	(31 ± 4)	(25 ± 11)	(11 ± 3)
	104	5	24	16	12
313	110	12	25	18	7
	99	8	14	17	15
	(104 ± 6)	(8 ± 4)	(21 ± 6)	(17 ± 1)	(11 ± 4)
	113	16	32	25	6
625	107	10	22	14	13
	118	6	31	19	11
	(113 ± 6)	(11 ± 5)	(28 ± 6)	(19 ± 6)	(10 ± 4)
	124	6	25	22	13
1250	101	5	25	17	7
	102	5	26	13	7
	(109 ± 13)	(5 ± 1)	(25 ± 1)	(17 ± 5)	(9 ± 3)
	76 *	9 *	14 *	9	6
2500	93 *	3 *	21 *	18	10
	95 *	3 *	16 *	14	9
	(88 ± 10)	(5 ± 3)	(17 ± 4)	(14 ± 5)	(8 ± 2)
	0 *	0 *	8 *	0 *	0 *
5000	0 *	0 *	9 *	0 *	0 *
	0 *	0 *	7 *	0 *	0 *
	(0 ± 0)	(0 ± 0)	(8 ± 1)	(0 ± 0)	(0 ± 0)
陽性対照	2-AA	2-AA	2-AA	2-AA	2-AA
μg/プレート	1	2	10	1	2
復帰変異	422	173	448	270	65
コロニー数	444	164	408	266	77
／プレート	501	170	410	283	98
	(456 ± 41)	(169 ± 5)	(422 ± 23)	(273 ± 9)	(80 ± 17)

( ) : 平均値±標準偏差
* : 菌の生育阻害が認められた。
2-AA: 2-アミノアントラセン

表 3-1-1　S9 mix 非存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの復帰突然変異試験結果〔本試験2回目－直接法〕

用量〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照〔ジメチルスルホキシド〕	114	15	13	--	--
	95	15	13	--	--
	114	8	17	--	--
	(108 ± 11)	(13 ± 4)	(14 ± 2)	--	--
31.3	110	13	--	--	--
	108	16	--	--	--
	120	12	--	--	--
	(113 ± 6)	(14 ± 2)	--	--	--
62.5	122	9	20	--	--
	128	8	16	--	--
	59	12	20	--	--
	(103 ± 38)	(10 ± 2)	(19 ± 2)	--	--
125	37	10	19	--	--
	90	13	13	--	--
	102	8	20	--	--
	(76 ± 35)	(10 ± 3)	(17 ± 4)	--	--
250	101	5	13	--	--
	112	10	20	--	--
	110	14	24	--	--
	(108 ± 6)	(10 ± 5)	(19 ± 6)	--	--
500	119	3 *	13	--	--
	124	7 *	28	--	--
	117	4 *	15	--	--
	(120 ± 4)	(5 ± 2)	(19 ± 8)	--	--
1000	133 *	0 *	16	--	--
	111 *	0 *	17	--	--
	87 *	0 *	13	--	--
	(110 ± 23)	(0 ± 0)	(15 ± 2)	--	--
2000	--	--	9 *	--	--
	--	--	7 *	--	--
	--	--	8 *	--	--
	--	--	(8 ± 1)	--	--
陽性対照	AF－2	SA	AF－2	AF－2	9－AA
μg/プレート	0.01	0.5	0.04	0.1	80
復帰変異コロニー数／プレート	1036	324	504	--	--
	1083	300	555	--	--
	1124	318	536	--	--
	(1081 ± 44)	(314 ± 12)	(532 ± 26)	--	--

( ) : 平均値±標準偏差
* : 菌の生育阻害が認められた。
AF-2: 2-(2-フリル)-3-(5-ニトロ-2-フリル)アクリルアミド
SA : アジ化ナトリウム
9-AA: 9-アミノアクリジン

表 3-1-2 S9 mix 非存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの復帰突然変異試験結果〔本試験2回目－直接法〕

用量 〔μg/プレート〕	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照 〔ジメチルスルホキシド〕	--	--	--	37	8
	--	--	--	32	16
	--	--	--	31	11
	--	--	--	(33 ± 3)	(12 ± 4)
1.56	--	--	--	27	6
	--	--	--	24	9
	--	--	--	22	14
	--	--	--	(24 ± 3)	(10 ± 4)
3.13	--	--	--	35	13
	--	--	--	21	24
	--	--	--	29	13
	--	--	--	(28 ± 7)	(17 ± 6)
6.25	--	--	--	25	19
	--	--	--	28	26
	--	--	--	29	16
	--	--	--	(27 ± 2)	(20 ± 5)
12.5	--	--	--	33	21
	--	--	--	41	24
	--	--	--	33	14
	--	--	--	(36 ± 5)	(20 ± 5)
25	--	--	--	27 *	15 *
	--	--	--	22 *	16 *
	--	--	--	22 *	22 *
	--	--	--	(24 ± 3)	(18 ± 4)
50	--	--	--	24 *	12 *
	--	--	--	12 *	18 *
	--	--	--	30 *	19 *
	--	--	--	(22 ± 9)	(16 ± 4)
陽性対照	AF－2	SA	AF－2	AF－2	9－AA
μg/プレート	0.01	0.5	0.04	0.1	80
復帰変異コロニー数／プレート	--	--	--	363	367
	--	--	--	380	406
	--	--	--	404	486
	--	--	--	(382 ± 21)	(420 ± 61)

( ): 平均値±標準偏差
* : 菌の生育阻害が認められた。
AF-2: 2-(2-フリル)-3-(5-ニトロ-2-フリル)アクリルアミド
SA : アジ化ナトリウム
9-AA: 9-アミノアクリジン

表 3-2　　S9 mix 存在下における2, 3, 4, 4'-テトラヒドロキシベンゾフェノンの復帰突然変異試験結果〔本試験2回目－代謝活性化法〕

用　量	復帰変異コロニー数／プレート				
	塩基対置換型			フレームシフト型	
〔μg/プレート〕	TA100	TA1535	WP2*uvrA*	TA98	TA1537
陰性対照	121	6	22	27	16
〔ジメチル	117	14	16	25	17
スルホキシド〕	110	14	24	35	17
	(116 ± 6)	(11 ± 5)	(21 ± 4)	(29 ± 5)	(17 ± 1)
	120	7	28	30	18
156	118	11	20	33	19
	107	11	21	23	22
	(115 ± 7)	(10 ± 2)	(23 ± 4)	(29 ± 5)	(20 ± 2)
	105	10	20	21	15
313	98	10	17	26	21
	118	6	12	26	17
	(107 ± 10)	(9 ± 2)	(16 ± 4)	(24 ± 3)	(18 ± 3)
	126	14	16	30	20
625	107	7	22	21	13
	107	13	16	28	16
	(113 ± 11)	(11 ± 4)	(18 ± 3)	(26 ± 5)	(16 ± 4)
	110	9	23	24	13
1250	115	9	15	29	12
	97	13	17	19	10
	(107 ± 9)	(10 ± 2)	(18 ± 4)	(24 ± 5)	(12 ± 2)
	89 *	7 *	11 *	26 *	9 *
2500	72 *	6 *	9 *	14 *	9 *
	66 *	4 *	13 *	22 *	9 *
	(76 ± 12)	(6 ± 2)	(11 ± 2)	(21 ± 6)	(9 ± 0)
	2 *	0 *	8 *	0 *	1 *
5000	22 *	0 *	10 *	0 *	0 *
	15 *	2 *	7 *	1 *	0 *
	(13 ± 10)	(1 ± 1)	(8 ± 2)	(0 ± 1)	(0 ± 1)
陽性対照	2-AA	2-AA	2-AA	2-AA	2-AA
μg/プレート	1	2	10	1	2
復帰変異	358	170	402	258	90
コロニー数	415	159	406	253	85
／プレート	404	172	503	260	91
	(392 ± 30)	(167 ± 7)	(437 ± 57)	(257 ± 4)	(89 ± 3)

( ): 平均値±標準偏差
* : 菌の生育阻害が認められた。
2-AA: 2-アミノアントラセン

要　約

2、3、4、4'-テトラヒドロキシベンゾフェノンの染色体異常誘発能の有無を検討するため、チャイニーズ・ハムスター培養細胞（CHL/IU）を用いて染色体異常試験を実施した。

初めに、最高用量を毒性試験ガイドラインに定められた 10mM に相当する 2500 μg/mL として、細胞増殖抑制試験を実施した。その結果、短時間処理法の代謝活性化では 625 μg/mL 以上の用量で、短時間処理法の非代謝活性化では 313 μg/mL 以上の用量で、連続処理法の 24 時間処理では 313 μg/mL 以上の用量で、連続処理法の 48 時間処理では 39.1 μg/mL 以上の用量で、50%以上の細胞増殖抑制が認められ、50%細胞増殖抑制濃度（概略値）は短時間処理の代謝活性化では 464.273 μg/mL、非代謝活性化では 184.036 μg/mL、連続処理法の 24 時間処理では 205.955 μg/mL、48 時間処理では 33.500 μg/mL と算出された。これより、短時間処理法の代謝活性化では 625 μg/mL を、短時間処理法の非代謝活性化では 313 μg/mL を、連続処理法の 24 時間処理では 313 μg/mL を、連続処理法の 48 時間処理では 39.1 μg/mL を最高用量として、以下公比 2 で希釈した各 5 試験用量を設定し染色体異常誘発能を検討した。

染色体異常試験の結果、短時間処理法では、代謝活性化及び非代謝活性化ともに染色体数的異常（倍数体）の増加は認められなかったが、染色体構造異常の出現率が増加し、代謝活性化では疑陽性を、非代謝活性化では陽性を示した。また、連続処理法においても、24 時間処理及び 48 時間処理ともに染色体数的異常（倍数体）の増加は認められなかったが、染色体構造異常の出現率が増加し、疑陽性を示した。一方、陽性対照群では、染色体構造異常の顕著な誘発が認められた。また、陰性対照群における染色体数的異常（倍数体）の出現率は各々陰性の判定基準内にあり、さらに試験施設の背景値と同様であった。従って試験は適切に実施されたものと考えられた。

染色体異常試験において、染色体構造異常の出現率（TA）が短時間処理法の代謝活性化では疑陽性、非代謝活性化では陽性の結果が得られたが、いずれも用量依存的な増加が認められなかったため確認試験を実施した。その結果、確認試験の代謝活性化においては、320 及び 400 μg/mL で TA 値が疑陽性を示したが、用量依存性は認められなかった。染色体異常試験の代謝活性化においても 78.1～313 μg/mL で TA 値は疑陽性を示し、明確な用量依存性は得られていなかったことから、再現性があることが確認された。一方、確認試験の非代謝活性化においては、TA 値は 9.88 μg/mL では陰性、14.8 μg/mL で疑陽性、22.2 μg/mL で陽性、33.3 μg/mL で陽性、50 μg/mL で疑陽性を示した。22.2～50 μg/mL では用量依存性は認められなかったも

のの、9.88～22.2 μg/mL ではTA値の用量依存的な増加が認められた。これらの結果から総合的に判断すると、本被験物質の染色体構造異常誘発性は陽性であり、染色体構造異常の誘発能を有するものと判定された。一方、染色体数的異常（倍数体）の出現頻度の増加はいずれの処理法においても認められなかった。確認試験における陽性対照群では、染色体構造異常の顕著な誘発が認められた。また、陰性対照群における染色体数的異常（倍数体）の出現率は各々陰性の判定基準内にあり、さらに試験施設の背景値と同様であった。従って試験は適切に実施されたものと考えられた。

以上の結果から、2、3、4、4'-テトラヒドロキシベンゾフェノンは、本試験条件下において染色体数的異常（倍数体）の誘発能は有さないものの、弱い染色体の構造異常の誘発能を有するものと判定した。

試験結果

1. 細胞増殖抑制試験

1) 短時間処理法

短時間処理法における代謝活性化の結果を Fig. 1-1 及び Table 1-1 に、非代謝活性化の結果を Fig. 1-2 及び Table 1-2 に示した。

(1) 50%細胞増殖抑制濃度

代謝活性化では 625 μg/mL 以上の濃度で 50%以上の細胞増殖抑制が認められ、50%細胞増殖抑制濃度は 464.273 μg/mL であった。また非代謝活性化では 313 μg/mL 以上の濃度で 50%以上の細胞増殖抑制が認められ、50%細胞増殖抑制濃度は 184.036 μg/mL であった。

(2) 被験物質処理終了時の培養細胞の観察

被験物質処理群の細胞の状態を倒立位相差顕微鏡下で観察し、陰性対照群と比較すると、代謝活性化では 156 μg/mL 以上の濃度で細胞の不連続性が認められ、1250 μg/mL 以上の濃度では被験物質と思われる析出物のため細胞状態の観察が不可能であった。一方、非代謝活性化では 39.1 μg/mL 以上の濃度で細胞の不連続性が認められた。肉眼による培養液の色調の観察では、代謝活性化では 156 μg/mL 以上の濃度で、非代謝活性化では 78.1 μg/mL 以上の濃度で変化が認められた。肉眼による被験物質の析出の観察では、代謝活性化及び非代謝活性化ともに 2500 μg/mL で析出が認められた。

2) 連続処理法

連続処理法における 24 時間処理の結果を Fig. 1-3 及び Table 1-3 に、48 時間処理の結果を Fig. 1-4 及び Table 1-4 に示した。

(1) 50%細胞増殖抑制濃度

細胞増殖抑制は、24 時間処理では 313 μg/mL 以上の濃度で 50%以上の細胞増殖抑制が認められ、50%細胞増殖抑制濃度は 205.955 μg/mL であった。48 時間処理では 39.1 μg/mL 以上の濃度で 50%以上の細胞増殖抑制が認められ、50%細胞増殖抑制濃度は 33.500 μg/mL であった。

(2) 被験物質処理終了時の培養細胞の観察

被験物質処理群の細胞の状態を倒立位相差顕微鏡下で観察し、陰性対照群と比較すると、24 時間処理では 39.1 μg/mL 以上の濃度で細胞の不連続性が認められた。一方、48 時間処理では 19.5 μg/mL 以上の濃度で細胞の不連続性が認められた。肉眼による培養液

の色調の観察では、24時間処理及び48時間処理ともに78.1 μg/mL以上の濃度で変化が認められた。肉眼による被験物質の析出の観察では、代謝活性化及び非代謝活性化ともに2500 μg/mLで析出が認められた。

2. 染色体異常試験

短時間処理法の結果をFig. 2-1、2-2、Table 2-1、2-2、3-1、3-2に、連続処理法の結果をFig. 2-3、2-4、Table 2-3、2-4、3-3、3-4に示した。

1) 被験物質処理終了時の培養細胞の観察

被験物質処理群の細胞の状態は、細胞増殖抑制試験の場合とほぼ同様であった。すなわち、被験物質処理群の細胞の状態を倒立位相差顕微鏡下で観察し、陰性対照群と比較すると、短時間処理法の代謝活性化においては156 μg/mLでは微少に、313 μg/mLでは半数に、625 μg/mLでは多数に細胞の不連続性が認められた。短時間処理法の非代謝活性化においては39.1 μg/mL及び78.1 μg/mLでは微少に、156 μg/mLでは半数に、313 μg/mLでは多数に細胞の不連続性が認められた。連続処理法の24時間処理においては、39.1 μg/mL及び78.1 μg/mLでは微少に、156 μg/mLでは半数に、313 μg/mLでは多数に細胞の不連続性が認められた。連続処理法の48時間処理においては、19.5 μg/mLでは微少に、39.1 μg/mLでは半数に細胞の不連続性が認められた。肉眼による培養液の色調の観察では、短時間処理法の代謝活性化では156 μg/mL以上の濃度で、非代謝活性化では78.1 μg/mL以上の濃度で、連続処理法の24時間処理では78.1 μg/mL以上の濃度で淡黄色から淡褐色の色調変化が認められた。肉眼による被験物質の析出の観察では、短時間処理法及び連続処理法ともに析出は認められなかった。

2) 構造異常

構造異常の出現率（TA）は、短時間処理法の代謝活性化では625 μg/mLではTOX、313 μg/mLで7.5%、156 μg/mLで9.0%、78.1 μg/mLで9.5%及び39.1 μg/mLで3.0%と疑陽性の判定基準である5%以上10%未満から、陰性の判定基準である5%未満を示した。また、非代謝活性化においても、313 μg/mLでTOX、156 μg/mLで2.5%、78.1 μg/mLで4.0%、39.1 μg/mLで9.0%及び19.5 μg/mLで11.5%と、陽性の判定基準である10%以上から、陰性の判定基準である5%未満を示した。さらに、連続処理法の24時間処理では313 μg/mL、156 μg/mL及び78.1 μg/mLでTOX、39.1 μg/mLで5.5%及び19.5 μg/mLで5.0%と疑陽性の判定基準である5%以上10%未満を示した。また、48時間処理では、39.1 μg/mLで7.0%、19.5 μg/mLで2.5%、9.77 μg/mLで2.0%、4.88 μg/mLで1.0%及び2.44 μg/mL

で 1.5%と疑陽性の判定基準である 5%以上 10%未満から、陰性の判定基準である 5%未満を示した。なお、連続処理法の 24 時間処理の 156 μg/mL 及び 78.1 μg/mL では、観察細胞数が規定数に満たなかったため TOX と判定したが、TA はそれぞれ 27.3 及び 14.0%を示し、それ以下の濃度を含めて用量依存的な出現率の増加が認められた。

各処理法ともに陰性及び陽性対照群における染色体構造異常の出現率は各々陰性及び陽性の判定基準内にあり、また試験施設の背景値（Attached Data 5）と同様であったことから試験は適切に実施されたと考えられた。

3) 数的異常

染色体数的異常（倍数体）の出現率は、短時間処理法の代謝活性化では 625 μg/mL で TOX、313 μg/mL で 1.5%、156 μg/mL で 3.5%、78.1 μg/mL で 4.0%及び 39.1 μg/mL で 1.5%と陰性の判定基準である 5%未満であった。また、非代謝活性化においては、313 μg/mL で TOX、156 μg/mL で 1.0%、78.1 μg/mL で 0.5%、39.1 μg/mL で 3.5%及び 19.5 μg/mL で 0%と陰性の判定基準である 5%未満であった。さらに、連続処理法の 24 時間処理では 313 μg/mL、156 μg/mL 及び 78.1 μg/mL で TOX、39.1 μg/mL で 0%及び 19.5 μg/mL で 1.0%と陰性の判定基準である 5%未満であった。また、48 時間処理では、39.1 μg/mL で 0.5%、19.5 μg/mL で 0.5%、9.77 μg/mL で 1.0%、4.88 μg/mL で 0.5%及び 2.44 μg/mL で 0%と陰性の判定基準である 5%未満であった。

各処理法ともに陰性対照群における染色体数的異常（倍数体）の出現率は各々陰性の判定基準内にあり、また試験施設の背景値（Attached Data 5）と同様であったことから試験は適切に実施されたと考えられた。

3. 確認試験

結果を Fig. 2-5、2-6、Table 2-5、2-6、3-5、3-6 に示した。

1) 被験物質処理終了時の培養細胞の観察

被験物質処理群の細胞の状態を倒立位相差顕微鏡下で観察し、陰性対照群と比較すると、代謝活性化においては 205 μg/mL 及び 256 μg/mL では微少に、320 μg/mL、400 μg/mL 及び 500 μg/mL では半数に細胞の不連続性が認められた。非代謝活性化においては 14.8 μg/mL 及び 22.2 μg/mL では微少に、33.3 μg/mL 及び 50 μg/mL では半数に細胞の不連続性が認められた。肉眼による培養液の色調の観察では、代謝活性化においては全用量で、非代謝活性化においては 33.3 μg/mL 以上の用量で色調変化が認められた。肉眼による被験物質の析出の観察では、析出は認められなかった。

2) 構造異常

構造異常の出現率(TA)は、代謝活性化では500 μg/mLではTOX、400 μg/mLで5.0%、320 μg/mLで5.5%、256 μg/mLで2.5%及び205 μg/mLで3.5%と疑陽性の判定基準である5%以上10%未満から、陰性の判定基準である5%未満を示した。また、非代謝活性化においては、50 μg/mLで6.0%、33.3 μg/mLで10.5%、22.2 μg/mLで12.5%、14.8 μg/mLで7.5%、9.88 μg/mLで1.0%及び6.58 μg/mLで1.5%と、陽性の判定基準である10%以上から、陰性の判定基準である5%未満を示した。

各処理法ともに陰性及び陽性対照群における染色体構造異常の出現率は各々陰性及び陽性の判定基準内にあり、また試験施設の背景値（Attached Data 5）と同様であったことから試験は適切に実施されたと考えられた。

3) 数的異常

数的異常(倍数体)の出現率は、代謝活性化では500 μg/mLでTOX、400 μg/mLで1.0%、320 μg/mLで2.0%、256 μg/mLで2.0%及び205 μg/mLで0.5%と陰性の判定基準である5%未満であった。また、非代謝活性化においては、50 μg/mLで1.0%、33.3 μg/mLで0.5%、22.2 μg/mLで0.5%、14.8 μg/mLで0%、9.88 μg/mLで0%及び6.58 μg/mLで0%と陰性の判定基準である5%未満であった。

各処理法ともに陰性対照群における染色体数的異常（倍数体）の出現率は各々陰性の判定基準内にあり、また試験施設の背景値（Attached Data 5）と同様であったことから試験は適切に実施されたと考えられた。

考　察

被験物質は、染色体異常試験の短時間処理法・非代謝活性化において、19.5 μg/mL で構造異常を有する細胞の出現率（TA）が陽性を示したが、連続処理法の 24 時間処理及び 48 時間処理の同一用量においては、TA はそれぞれ疑陽性及び陰性を示し、被験物質に対する暴露時間の経過とともに構造異常の出現率が増加する傾向は認められなかった。また、短時間処理法及び連続処理法ともに、19.5 μg/mL 以外の用量において明確な用量依存性は認められないものの TA の増加が認められ、疑陽性を示した。これらの結果から総合的に判断すると、本被験物質の染色体構造異常誘発性は疑陽性であり、弱い構造異常の誘発能を有するものと判定された。一方、染色体数的異常（倍数体）の出現頻度の増加はいずれの処理法においても認められなかった。

なお、陰性対照群における染色体の構造異常を有する細胞及び染色体数的異常（倍数体）の出現頻度は、いずれの処理法においても 5%未満であった。また、陽性対照物質の CP あるいは MMC を処理した細胞では、染色体構造異常の顕著な誘発が認められた。更に、2 枚のシャーレ間における染色体異常細胞の出現頻度に著しい差はなく、培養条件などの試験環境の異常も認められなかった。これらのことから、試験は適切に実施されたものと考えられた。

染色体異常試験において、短時間処理法の代謝活性化では疑陽性の、非代謝活性化では陽性の結果が得られたが、いずれの場合も構造異常を有する細胞の出現率（TA）に用量依存的な増加が認められなかったため確認試験を実施した。

その結果、確認試験の代謝活性化においては、2 用量で疑陽性を示したが TA 値の用量依存的な増加は認められず、染色体異常試験の代謝活性化と同様な結果が得られ再現性が確認された。一方、確認試験の非代謝活性化においては、染色体異常試験で陽性を示した近傍の用量で試験を実施したが、低用量では TA 値の用量依存的な増加が認められたが、高用量では用量依存的な増加は認められず、D20 値は求められなかった。以上の結果から判断すると、本被験物質の染色体構造異常誘発性は陽性であり、染色体構造異常の誘発能を有するものと判定された。一方、染色体数的異常（倍数体）の出現頻度の増加はいずれの処理法においても認められなかった。確認試験における陽性対照群では、染色体構造異常の顕著な誘発が認められた。また、陰性対照群における染色体の構造異常を有する細胞及び染色体数的異常（倍数体）の出現率は各々陰性の判定基準内にあり、さらに試験施設の背景値と同様であった。従って試験は適切に実施されたものと考えられた。

以上の結果から、2、3、4、4'-テトラヒドロキシベンゾフェノンは、本試験条件下において

染色体数的異常（倍数体）の誘発能は有さないものの、弱い染色体構造異常の誘発能を有するものと判定した。

Table 1-1 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [short-term treatment:+S9 mix]

Cell-growth inhibition test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
+	6-18	0(NC)		100 [a]	100	—	—	—
				100		—	—	—
		Test article	19.5	83	83	—	—	—
				83		—	—	—
			39.1	83	83	—	—	—
				83		—	—	—
			78.1	100	92	—	—	—
				83		—	—	—
			156	66	75	+	Light-yellow	—
				83		+	Light-yellow	—
			313	66	66	+	Orange	—
				66		+	Orange	—
			625	33	33	++	Light-brown	—
				33		++	Light-brown	—
			1250	33	33	g)	Brown	—
				33		g)	Brown	—
			2500	133	125 [h]	g)	Brown	+
				116		g)	Brown	+
Concentration of 50% cell-growth inhibition: 464.273 μg/mL								

NC : Negative Control(dimethylsulfoxide)

a) The plate in the negative control group was regarded as a 100% growth.

b) The mean showed as a growth ratio against the negative control value.

c) Observation of plate at the end of treatment

d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among approximately half of the surviving cells.

e) — : No changes of color

f) — : Absence of precipitates/crystals
+ : Presence of precipitates

g) Condition of cells could not be observed due to severe precipitate of the test article.

h) These values are unreliable since adherence of precipitation at the bottom of the plastic plate inhibited accurate measurement of the cell density.

Table 1-2 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [short-term treatment:-S9 mix]

Cell-growth inhibition test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	6-18	0(NC)		100 [a]	100	—	—	—
				83		—	—	—
		Test article	19.5	66	72	—	—	—
				66		—	—	—
			39.1	66	72	+	—	—
				66		+	—	—
			78.1	66	72	++	Light-yellow	—
				66		++	Light-yellow	—
			156	50	55	++	Orange	—
				50		++	Orange	—
			313	33	27	+++	Orange	—
				16		+++	Orange	—
			625	16	17	+++	Light-brown	—
				16		+++	Light-brown	—
			1250	16	17	+++	Brown	—
				16		+++	Brown	—
			2500	50	55 [g]	+++	Brown	+
				50		+++	Brown	+
Concentration of 50% cell-growth inhibition: 184.036 μg/mL								

NC : Negative Control(dimethylsulfoxide)

a) The plate in the negative control group was regarded as a 100% growth.

b) The mean showed as a growth ratio against the negative control value.

c) Observation of plate at the end of treatment

d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among apploximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.

e) — : No changes of color

f) — : Absence of precipitates/crystals
+ : Presence of precipitates

g) These values are unreliable since adherence of precipitation at the bottom of the plastic plate inhibited accurate measurement of the cell density.

Table 1-3 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [continuous treatment: 24hr]

Cell-growth inhibition test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	24	0(NC)		100 [a]	100	—	—	—
				100		—	—	—
		Test article	19.5	71	71	—	—	—
				71		—	—	—
			39.1	71	64	+	—	—
				57		+	—	—
			78.1	57	57	++	Light-yellow	—
				57		++	Light-yellow	—
			156	57	57	++	Orange	—
				57		++	Orange	—
			313	42	35	+++	Orange	—
				28		+++	Orange	—
			625	42	42	+++	Light-brown	—
				42		+++	Light-brown	—
			1250	42	50	+++	Brown	—
				57		+++	Brown	—
			2500	150	150[g]	+++	Brown	+
				150		+++	Brown	+
Concentration of 50% cell-growth inhibition: 205.955 μg/mL								

NC : Negative Control(dimethylsulfoxide)

a) The plate in the negative control group was regarded as a 100% growth.

b) The mean showed as a growth ratio against the negative control value.

c) Observation of plate at the end of treatment

d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among apploximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.

e) — : No changes of color

f) — : Absence of precipitates/crystals
+ : Presence of precipitates

g) These values are unreliable since adherence of precipitation at the bottom of the plastic plate inhibited accurate measurement of the cell density.

Table 1-4 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [continuous treatment: 48hr]

Cell-growth inhibition test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	48	0(NC)		100 [a]	100	—	—	—
				92		—	—	—
		Test article	19.5	53	55	+	—	—
				53		+	—	—
			39.1	46	48	+	—	—
				46		+	—	—
			78.1	38	40	+	Light-yellow	—
				38		+	Light-yellow	—
			156	30	31	++	Orange	—
				30		++	Orange	—
			313	23	24	+++	Orange	—
				23		+++	Orange	—
			625	23	24	+++	Light-brown	—
				23		+++	Light-brown	—
			1250	30	31	+++	Brown	—
				30		+++	Brown	—
			2500	99	95 [g]	+++	Brown	+
				84		+++	Brown	+
Concentration of 50% cell-growth inhibition: 33.500 μg/mL								

NC : Negative Control(dimethylsulfoxide)

a) The plate in the negative control group was regarded as a 100% growth.

b) The mean showed as a growth ratio against the negative control value.

c) Observation of plate at the end of treatment

d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among apploximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.

e) — : No changes of color

f) — : Absence of precipitates/crystals
+ : Presence of precipitates

g) These values are unreliable since adherence of precipitation at the bottom of the plastic plate inhibited accurate measurement of the cell density.

Table 2-1 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [short-term treatment:+S9 mix]

Chromosome aberration test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
+	6-18	0(NC)		100 [a]	100	—	—	—
				99		—	—	—
		Test article	39.1	83	83	—	—	—
				83		—	—	—
			78.1	83	83	—	—	—
				83		—	—	—
			156	83	83	+	Light-yellow	—
				83		+	Light-yellow	—
			313	66	66	++	Orange	—
				66		++	Orange	—
			625	33	33	+++	Light-brown	—
				33		+++	Light-brown	—
		PC		83	83	—	—	—
				83		—	—	—

NC : Negative Control(dimethylsulfoxide)

PC : Positive Control(cyclophosphamide, 14μg/mL)

a) The plate in the negative control group was regarded as a 100% growth.

b) The mean showed as a growth ratio against the negative control value.

c) Observation of plate at the end of treatment

d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among apploximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.

e) — : No changes of color

f) — : Absence of precipitates/crystals

Table 2-2 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [short-term treatment:-S9 mix]

Chromosome aberration test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	6-18	0(NC)		100 [a]	100	—	—	—
				100		—	—	—
		Test article	19.5	80	80	—	—	—
				80		—	—	—
			39.1	80	70	+	—	—
				60		+	—	—
			78.1	60	60	+	Light-yellow	—
				60		+	Light-yellow	—
			156	60	60	++	Orange	—
				60		++	Orange	—
			313	20	30	+++	Orange	—
				40		+++	Orange	—
		PC		80	80	—	—	—
				80		—	—	—

NC : Negative Control(dimethylsulfoxide)
PC : Positive Control(mitomycin C, 0.05μg/mL)

a) The plate in the negative control group was regarded as a 100% growth.
b) The mean showed as a growth ratio against the negative control value.
c) Observation of plate at the end of treatment
d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among apploximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.
e) — : No changes of color
f) — : Absence of precipitates/crystals

Table 2-3 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [continuous treatment: 24hr]

Chromosome aberration test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
−	24	0(NC)		100 [a]	100	−	−	−
				100		−	−	−
		Test article	19.5	79	79	−	−	−
				79		−	−	−
			39.1	59	59	+	−	−
				59		+	−	−
			78.1	59	59	+	Light-brown	−
				59		+	Light-brown	−
			156	59	59	++	Light-brown	−
				59		++	Light-brown	−
			313	39	39	+++	Light-brown	−
				39		+++	Light-brown	−
		PC		100	100	−	−	−
				100		−	−	−

NC : Negative Control(dimethylsulfoxide)
PC : Positive Control(mitomycin C, 0.05μg/mL)
a) The plate in the negative control group was regarded as a 100% growth.
b) The mean showed as a growth ratio against the negative control value.
c) Observation of plate at the end of treatment
d) − : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among approximately half of the surviving cells.
+++ : There was discontinuity among most of the surviving cells.
e) − : No changes of color
f) − : Absence of precipitates/crystals

Table 2-4 Cell-growth ratio in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone [continuous treatment: 48hr]

Chromosome aberration test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	48	0(NC)		100 [a]	100	—	—	—
				100		—	—	—
		Test article	2.44	100	100	—	—	—
				100		—	—	—
			4.88	89	89	—	—	—
				89		—	—	—
			9.77	89	89	—	—	—
				89		—	—	—
			19.5	70	65	+	—	—
				59		+	—	—
			39.1	50	55	++	—	—
				59		++	—	—
		PC		89	89	—	—	—
				89		—	—	—

NC : Negative Control(dimethylsulfoxide)
PC : Positive Control(mitomycin C, 0.05μg/mL)

a) The plate in the negative control group was regarded as a 100% growth.
b) The mean showed as a growth ratio against the negative control value.
c) Observation of plate at the end of treatment
d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among approximately half of the surviving cells.
e) — : No changes of color
f) — : Absence of precipitates/crystals

Table 2-5 Cell-growth ratio in the confirmation test in cultured Chinese hamster cells treated with 2,3,4,4'-tetrahydroxybenzophenone
[short-term treatment:+S9 mix]

Confirmation test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
+	6-18	0(NC)		100 [a]	100	−	−	−
				125		−	−	−
		Test article	205	74	66	+	Light-brown	−
				74		+	Light-brown	−
			256	74	66	+	Light-brown	−
				74		+	Light-brown	−
			320	99	77	++	Light-brown	−
				74		++	Light-brown	−
			400	74	66	++	Brown	−
				74		++	Brown	−
			500	74	66	++	Brown	−
				74		++	Brown	−
		PC		99	88	−	−	−
				99		−	−	−

NC : Negative Control(dimethylsulfoxide)
PC : Positive Control(cyclophosphamide, 14μg/mL)
a) The plate in the negative control group was regarded as a 100% growth.
b) The mean showed as a growth ratio against the negative control value.
c) Observation of plate at the end of treatment
d) − : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among approximately half of the surviving cells.
e) − : No changes of color
f) − : Absence of precipitates/crystals

Table 2-6 Cell-growth ratio in the confirmation test in cultured Chinese hamster cells treated with 2,3,4,4'-tetrahydroxybenzophenone
[short-term treatment:-S9 mix]

Confirmation test							
Study type		Treatment and Concentration (μg/mL)		Cell-growth ratio		Observation [c]		
S9 mix	time (hr)			Plate 1 and 2	Mean [b] (%)	Condition of cells [d]	Color of medium [e]	Precipitates /Crystals [f]
—	6-18	0(NC)		100 [a]	100	—	—	—
				100		—	—	—
		Test article	6.58	83	83	—	—	—
				83		—	—	—
			9.88	83	83	—	—	—
				83		—	—	—
			14.8	83	75	+	—	—
				66		+	—	—
			22.2	66	66	+	—	—
				66		+	—	—
			33.3	50	58	++	Light-brown	—
				66		++	Light-brown	—
			50	66	58	++	Light-brown	—
				50		++	Light-brown	—
		PC		83	83	—	—	—
				83		—	—	—

NC : Negative Control(dimethylsulfoxide)
PC : Positive Control(mitomycin C, 0.075μg/mL)
a) The plate in the negative control group was regarded as a 100% growth.
b) The mean showed as a growth ratio against the negative control value.
c) Observation of plate at the end of treatment
d) — : Most of the cells were attached to the surface of plates and their shape was normal.
+ : There was discontinuity among a small number of surviving cells.
++ : There was discontinuity among approximately half of the surviving cells.
e) — : No changes of color
f) — : Absence of precipitates/crystals

Table 3-1 Chromosome aberration in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone

[short-term treatment:+S9 mix]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
+	6-18	NC	200	1.5	−	0	0	0	0	1	0	0.5	0.5	−	76-1
			(100)	(1)		(0)	(0)	(0)	(0)	(1)	(0)	(1)	(1)		02-1
			(100)	(2)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		
		625	0	0.0	TOX	0	0	0	0	0	0	0.0	0.0	TOX	55-1
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		55-2
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		92-1
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		92-2
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		
		313	200	1.5	−	3	10	4	0	0	1	7.5	9.0	±	41-1
			(100)	(1)		(1)	(6)	(1)	(0)	(0)	(0)	(7)	(8)		98-1
			(100)	(2)		(2)	(4)	(3)	(0)	(0)	(1)	(8)	(10)		
		156	200	3.5	−	4	10	7	0	0	1	9.0	11.0	±	28-1
			(100)	(2)		(2)	(6)	(1)	(0)	(0)	(1)	(8)	(10)		67-1
			(100)	(5)		(2)	(4)	(6)	(0)	(0)	(0)	(10)	(12)		
		78.1	200	4.0	−	1	4	14	0	1	0	9.5	10.0	±	58-1
			(100)	(4)		(1)	(1)	(6)	(0)	(0)	(0)	(7)	(8)		40-1
			(100)	(4)		(0)	(3)	(8)	(0)	(1)	(0)	(12)	(12)		
		39.1	200	1.5	−	0	0	6	0	0	0	3.0	3.0	−	61-1
			(100)	(2)		(0)	(0)	(2)	(0)	(0)	(0)	(2)	(2)		25-1
			(100)	(1)		(0)	(0)	(4)	(0)	(0)	(0)	(4)	(4)		
		PC	200	1.0	−	5	18	106	0	1	1	62.5	63.5	+	99-1
			(100)	(1)		(1)	(11)	(54)	(0)	(0)	(1)	(66)	(67)		59-1
			(100)	(1)		(4)	(7)	(52)	(0)	(1)	(0)	(59)	(60)		

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation
TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.
TOX: cell toxicity was observed.
NC: Negative control (dimethyl sulfoxide)
PC: Positive control (cyclophosphamide, 14 μg/mL)

Table 3-2 Chromosome aberration in CHL/IU cells treated with 2.3.4.4'-tetrahydroxybenzophenone

[short-term treatment:-S9 mix]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
			200	1.0		0	0	0	0	0	0	0.0	0.0		
		NC	(100)	(2)	−	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	−	42-1
			(100)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		48-1
			0	0.0		0	0	0	0	0	0	0.0	0.0		
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		70-1
		313	(0)	(0)	TOX	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	TOX	70-2
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		90-1
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		90-2
			200	1.0		1	1	4	0	0	0	2.5	3.0		
		156	(100)	(0)	−	(0)	(0)	(2)	(0)	(0)	(0)	(2)	(2)	−	75-1
			(100)	(2)		(1)	(1)	(2)	(0)	(0)	(0)	(3)	(4)		22-1
			200	0.5		4	2	6	0	0	0	4.0	6.0		
−	6-18	78.1	(100)	(1)	−	(1)	(2)	(4)	(0)	(0)	(0)	(6)	(7)	−	03-1
			(100)	(0)		(3)	(0)	(2)	(0)	(0)	(0)	(2)	(5)		88-1
			200	3.5		0	8	8	0	1	1	9.0	9.0		
		39.1	(100)	(5)	−	(0)	(5)	(6)	(0)	(0)	(1)	(12)	(12)	±	47-1
			(100)	(2)		(0)	(3)	(2)	(0)	(1)	(0)	(6)	(6)		79-1
			200	0.0		0	2	20	0	1	0	11.5	11.5		
		19.5	(100)	(0)	−	(0)	(1)	(9)	(0)	(1)	(0)	(11)	(11)	+	06-1
			(100)	(0)		(0)	(1)	(11)	(0)	(0)	(0)	(12)	(12)		18-1
			200	0.0		3	14	16	0	1	0	15.0	16.5		
		PC	(100)	(0)	−	(1)	(5)	(6)	(0)	(1)	(0)	(12)	(13)	+	51-1
			(100)	(0)		(2)	(9)	(10)	(0)	(0)	(0)	(18)	(20)		91-1

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation
TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.
TOX: cell toxicity was observed.
NC: Negative control (dimethyl sulfoxide)
PC: Positive control (mitomycin C, 0.05 μg/mL)

Table 3-3 Chromosome aberration in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone

[continuous treatment:24hr]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
			200	0.0		1	0	0	0	0	0	0.0	0.5		
		NC	(100)	(0)	−	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	−	50-1
			(100)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		65-1
			8	0.0		0	0	1	0	0	0	12.5	12.5		
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		11-1
		313	(0)	(0)	TOX	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	TOX	11-2
			(7)	(0)		(0)	(0)	(1)	(0)	(0)	(0)	(1)	(1)		87-1
			(1)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		87-2
			11	0.0		0	0	2	0	1	0	27.3	27.3		
			(3)	(0)		(0)	(0)	(1)	(0)	(0)	(0)	(1)	(1)		60-1
		156	(8)	(0)	TOX	(0)	(0)	(1)	(0)	(1)	(0)	(2)	(2)	TOX	60-2
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		85-1
			(0)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		85-2
			50	0.0		0	4	3	0	0	0	14.0	14.0		
			(13)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		01-1
−	24-0	78.1	(14)	(0)	TOX	(0)	(1)	(1)	(0)	(0)	(0)	(2)	(2)	TOX	01-2
			(13)	(0)		(0)	(1)	(1)	(0)	(0)	(0)	(2)	(2)		07-1
			(10)	(0)		(0)	(2)	(1)	(0)	(0)	(0)	(3)	(3)		07-2
			200	0.0		1	2	9	0	0	0	5.5	6.0		
		39.1	(100)	(0)	−	(1)	(1)	(4)	(0)	(0)	(0)	(5)	(6)	±	33-1
			(100)	(0)		(0)	(1)	(5)	(0)	(0)	(0)	(6)	(6)		14-1
			200	1.0		2	2	8	0	0	0	5.0	6.0		
		19.5	(100)	(1)	−	(1)	(0)	(4)	(0)	(0)	(0)	(4)	(5)	±	80-1
			(100)	(1)		(1)	(2)	(4)	(0)	(0)	(0)	(6)	(7)		27-1
			200	1.0		4	14	43	0	0	0	28.0	30.0		
		PC	(100)	(2)	−	(0)	(8)	(22)	(0)	(0)	(0)	(30)	(30)	+	43-1
			(100)	(0)		(4)	(6)	(21)	(0)	(0)	(0)	(26)	(30)		73-1

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation
TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.
TOX: cell toxicity was observed.
NC: Negative control (dimethyl sulfoxide)
PC: Positive control (mitomycin C, 0.05 μg/mL)

Table 3-4 Chromosome aberration in CHL/IU cells treated with 2,3,4,4'-tetrahydroxybenzophenone

[continuous treatment:48hr]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
			200	1.0		1	0	0	0	0	0	0.0	0.5		
		NC	(100)	(0)	−	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	−	94-1
			(100)	(2)		(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)		26-1
			200	0.5		0	5	8	1	0	0	7.0	7.0		
		39.1	(100)	(1)	−	(0)	(2)	(5)	(1)	(0)	(0)	(8)	(8)	±	81-1
			(100)	(0)		(0)	(3)	(3)	(0)	(0)	(0)	(6)	(6)		05-1
			200	0.5		0	2	4	0	0	0	2.5	2.5		
		19.5	(100)	(1)	−	(0)	(1)	(1)	(0)	(0)	(0)	(2)	(2)	−	04-1
			(100)	(0)		(0)	(1)	(3)	(0)	(0)	(0)	(3)	(3)		68-1
			200	1.0		1	2	2	0	0	0	2.0	2.5		
−	48-0	9.77	(100)	(1)	−	(1)	(0)	(1)	(0)	(0)	(0)	(1)	(2)	−	77-1
			(100)	(1)		(0)	(2)	(1)	(0)	(0)	(0)	(3)	(3)		30-1
			200	0.5		0	0	1	0	1	0	1.0	1.0		
		4.88	(100)	(0)	−	(0)	(0)	(1)	(0)	(0)	(0)	(1)	(1)	−	24-1
			(100)	(1)		(0)	(0)	(0)	(0)	(1)	(0)	(1)	(1)		49-1
			200	0.0		0	2	1	0	0	0	1.5	1.5		
		2.44	(100)	(0)	−	(0)	(2)	(1)	(0)	(0)	(0)	(3)	(3)	−	39-1
			(100)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		46-1
			200	0.0		1	17	98	0	2	1	58.5	58.5		
		PC	(100)	(0)	−	(1)	(6)	(46)	(0)	(1)	(0)	(52)	(52)	+	45-1
			(100)	(0)		(0)	(11)	(52)	(0)	(1)	(1)	(65)	(65)		52-1

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation
TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.
NC: Negative control (dimethyl sulfoxide)
PC: Positive control (mitomycin C, 0.05 μg/mL)

Table 3-5 Chromosome aberration in CHL/IU cells treated with 2.3.4.4'-tetrahydroxybenzophenone

[confirmation test:+S9 mix]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
			200	0.0		1	0	0	0	0	0	0.0	0.5		
		NC	(100)	(0)	−	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	−	023-1
			(100)	(0)		(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)		116-1
			39	0.0		2	0	0	0	0	1	2.6	7.7		
			(9)	(0)		(1)	(0)	(0)	(0)	(0)	(1)	(1)	(2)		074-1
		500	(8)	(0)	TOX	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	TOX	074-2
			(9)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		066-1
			(13)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		066-2
			200	1.0		3	3	6	0	0	1	5.0	6.5		
			(63)	(0)		(0)	(2)	(2)	(0)	(0)	(0)	(4)	(4)		057-1
		400	(37)	(1)	−	(2)	(1)	(2)	(0)	(0)	(0)	(3)	(5)	±	057-2
			(76)	(1)		(1)	(0)	(1)	(0)	(0)	(1)	(2)	(3)		064-1
			(24)	(0)		(0)	(0)	(1)	(0)	(0)	(0)	(1)	(1)		064-2
			200	2.0		4	5	6	0	1	0	5.5	7.5		
			(100)	(2)		(2)	(3)	(2)	(0)	(1)	(0)	(5)	(7)		111-1
+	6-18	320	(93)	(2)	−	(2)	(2)	(4)	(0)	(0)	(0)	(6)	(8)	±	107-1
			(7)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		107-2
			200	2.0		3	4	1	0	0	0	2.5	3.5		
		256	(100)	(2)	−	(2)	(1)	(0)	(0)	(0)	(0)	(1)	(2)	−	063-1
			(100)	(2)		(1)	(3)	(1)	(0)	(0)	(0)	(4)	(5)		008-1
			200	0.5		1	5	2	0	0	0	3.5	4.0		
			(100)	(0)		(1)	(4)	(1)	(0)	(0)	(0)	(5)	(6)		084-1
		205	(75)	(1)	−	(0)	(1)	(1)	(0)	(0)	(0)	(2)	(2)	−	121-1
			(25)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		121-2
			200	0.5		7	34	106	0	0	0	60.5	62.5		
		PC	(100)	(0)	−	(4)	(17)	(59)	(0)	(0)	(0)	(66)	(67)	+	118-1
			(100)	(1)		(3)	(17)	(47)	(0)	(0)	(0)	(55)	(58)		104-1

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation

TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.

TOX: cell toxicity was observed.

NC: Negative control (dimethyl sulfoxide)

PC: Positive control (cyclophosphamide, 14 μg/mL)

Table 3-6 Chromosome aberration in CHL/IU cells treated with 2.3.4.4'-tetrahydroxybenzophenone

[confirmation test:-S9 mix]

S9 mix	Time(h)	Conc. (μg/mL)	Cells observed	Polyploid cells (%)	Judge	Number of aberration g	ctb	cte	csb	cse	other	TA (%)	TAG (%)	Judge	Slide No.
			200	0.0		0	1	1	0	0	0	1.0	1.0		
		NC	(100)	(0)	−	(0)	(1)	(1)	(0)	(0)	(0)	(2)	(2)	−	037-1
			(100)	(0)		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)		095-1
			200	1.0		0	5	5	0	0	3	6.0	6.0		
			(53)	(0)		(0)	(1)	(0)	(0)	(0)	(0)	(1)	(1)		062-1
		50	(47)	(0)	−	(0)	(1)	(2)	(0)	(0)	(1)	(4)	(4)	±	062-2
			(67)	(1)		(0)	(2)	(2)	(0)	(0)	(1)	(4)	(4)		053-1
			(33)	(1)		(0)	(1)	(1)	(0)	(0)	(1)	(3)	(3)		053-2
			200	0.5		1	9	16	0	0	0	10.5	11.0		
			(84)	(1)		(0)	(1)	(5)	(0)	(0)	(0)	(6)	(6)		114-1
		33.3	(16)	(0)	−	(0)	(2)	(3)	(0)	(0)	(0)	(4)	(4)	+	114-2
			(61)	(0)		(1)	(3)	(4)	(0)	(0)	(0)	(6)	(7)		021-1
			(39)	(0)		(0)	(3)	(4)	(0)	(0)	(0)	(5)	(5)		021-2
			200	0.5		2	8	17	0	1	1	12.5	13.5		
−	6-18	22.2	(100)	(1)	−	(1)	(5)	(11)	(0)	(1)	(0)	(15)	(16)	+	119-1
			(100)	(0)		(1)	(3)	(6)	(0)	(0)	(1)	(10)	(11)		071-1
			200	0.0		1	5	14	0	0	0	7.5	8.0		
		14.8	(100)	(0)	−	(0)	(5)	(5)	(0)	(0)	(0)	(6)	(6)	±	035-1
			(100)	(0)		(1)	(0)	(9)	(0)	(0)	(0)	(9)	(10)		122-1
			200	0.0		1	1	1	0	0	0	1.0	1.5		
		9.88	(100)	(0)	−	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	−	093-1
			(100)	(0)		(1)	(1)	(1)	(0)	(0)	(0)	(2)	(3)		108-1
			200	0.0		0	0	3	0	0	0	1.5	1.5		
		6.58	(100)	(0)	−	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	−	102-1
			(100)	(0)		(0)	(0)	(3)	(0)	(0)	(0)	(3)	(3)		044-1
			200	0.5		2	16	35	0	0	0	24.0	25.0		
		PC	(100)	(1)	−	(2)	(10)	(16)	(0)	(0)	(0)	(25)	(27)	+	069-1
			(100)	(0)		(0)	(6)	(19)	(0)	(0)	(0)	(23)	(23)		034-1

g: chromatid or chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, other: including fragmentation
TA: total number of cells with aberration excluding gap, TAG: total number of cells with aberration including gap.
NC: Negative control (dimethyl sulfoxide)
PC: Positive control (mitomycin C, 0.075 μg/mL)

4. 要約

2,3,4,4'-Tetrahydroxybenzophenone の 0（対照群）、100、300、及び 1000 mg/kg を、Sprague-Dawley 系 SPF ラットの雄には交配前 14 日間及び交配期間を通して剖検前日（42 日間投与）まで、雌には交配前 14 日間及び交配期間並びに妊娠期間を通して授乳 4 日まで（41~45 日間投与）投与し、反復投与毒性及び生殖発生毒性を検討した。更に、0 及び 1000 mg/kg 投与群については 42 日間投与した後、14 日間の回復期間を設け、毒性変化の可逆性を検討した。

1) 反復投与毒性

1000 mg/kg 投与群の雌 1 例が授乳 0 日に死亡した。本例の死亡前の一般状態に異常はみられなかったが、剖検では脾臓及び胸腺の小型化がみられ、組織学的に白脾髄の萎縮、胸腺の萎縮がみられた。

詳細な一般状態の観察、機能検査、握力測定、自発運動量の測定には被験物質投与による影響は認められなかった。

一般状態では、1000 mg/kg 投与群の雄で投与 4 週以降に投与後の流涎がみられた。

体重及び摂餌量では、1000 mg/kg 投与群の雌雄で投与初期に摂餌量の低値、投与期間中に体重増加抑制が認められた。300 mg/kg 投与群の雌では投与初期に摂餌量の低値がみられた。

尿検査では、投与期間終了時検査において、尿潜血が各投与群の全例にみられ、また暗黄色の色調を示す例もみられた。これらの変化については、被験物質の排泄に関連した変化と推察され、毒性変化ではなかった。

血液学検査では、投与期間終了時検査において、1000 mg/kg 投与群の雄で赤血球数、ヘモグロビン量、ヘマトクリット値及び平均赤血球血色素濃度の低値、好中球数及び単球数の高値がみられた。更に、1000 mg/kg 投与群の雌雄で血小板数の高値がみられた。

血液化学検査では、投与期間終了時検査において、300 mg/kg 以上の投与群の雄で無機リンの有意な高値がみられた。

病理学検査では、投与期間終了時検査において、300 mg/kg 以上の投与群の雌で胸腺重量が減少し、肉眼的な小型化、組織学的な萎縮がみられた。盲腸における粘膜上皮細胞の単細胞壊死及び粘膜のび漫性過形成が 100mg/kg 以上の投与群の雌雄で認められた。また、肝臓では、重量の増加が 1000 mg/kg 投与群の雌雄でみられた。また、小葉辺縁性肝細胞の空胞化が対照群、100 及び 300 mg/kg 投与群の雌雄でみられ、300 mg/kg 以上の投与群で用量の増加に伴って減少した。

尿検査、血液検査、血液化学検査及び病理学検査における変化は、いずれも休薬により軽減するか、回復した。

2)　生殖発生毒性

性周期、交尾までに要した日数、交尾率、授精率及び受胎率には被験物質投与の影響は認められなかった。また、母動物では 1000 mg/kg 投与群の 1 例が分娩後（授乳 0 日）に死亡したが、本例の分娩状態に異常はみられなかった。更に、出産率、妊娠期間、黄体数、着床痕数、着床率、死産児率、出生児数、出生率及び性比に被験物質投与の影響は認められず、授乳期間中の授乳状態にも異常は認められなかった。

出生児では、1000 mg/kg 投与群の雌雄で出生時及び生後 4 日の雌雄体重に、300 mg/kg 投与群の雌雄で生後 4 日の雌雄体重にそれぞれ低値がみられた。出生時の外表観察及び生後 4 日剖検所見及び生存率には被験物質投与による変化は認められなかった。

これらの結果から、2,3,4,4'-Tetrahydroxybenzophenone の反復投与毒性に対する無影響量は、盲腸における粘膜上皮細胞の単細胞壊死及び粘膜のび漫性過形成が 100mg/kg 以上の投与群の雌雄で認められたため雌雄ともに 100 mg/kg/day 未満、生殖発生毒性に対しては雌雄親動物に対する無影響量は 1000 mg/kg/day、児動物に対する無影響量は 100 mg/kg/day と判断した。

7. 試験結果

7.1 一般状態（Table 1-1~1-8、Appendix 1~24）

主群では、1000 mg/kg 投与群の雌 1 例（動物番号 4110）が分娩後（授乳 0 日）に死亡した。本例の死亡前の一般状態には異常はみられなかった。1000 mg/kg 投与群の雄では投与後の流涎が投与 4 週以降に計 5 例にみられた。

回復群では、1000 mg/kg の雄で投与後の流涎が投与 4 週以降に計 3 例にみられた。回復期間中には異常はみられなかった。

その他の主群及び回復群の動物には異常はみられなかった。

7.2 詳細な一般状態の観察、機能検査、握力測定及び自発運動量の測定（Fig. 1~6、Table 2-1~2-105、Appendix 25~324）

1) ホームケージ内観察（Table 2-1~2-29、Appendix 25~108）

主群及び回復群のいずれの動物にも異常はみられなかった。

2) 手に持っての観察（Table 2-30~2-58、Appendix 109~192）

主群及び回復群のいずれの動物にも異常はみられなかった。

3) オープンフィールド内観察（Table 2-59~2-87、Appendix 193~276）

主群及び回復群のいずれの動物にも異常はみられなかった。また、立ち上がり回数及び糞数にも対照群と各投与群との間に有意差は認められなかった。

4) 機能検査（Table 2-88~2-93、Appendix 277~292）

1000 mg/kg 投与群の雌で授乳 4 日に着地開脚幅の有意な高値がみられた。他には主群及び回復群のいずれの動物にも異常はみられなかった。また、空中正向反射には対照群と各投与群との間に有意差は認められなかった。

5) 握力測定（Table 2-94~2-99、Appendix 293~308）

1000 mg/kg 投与群の雄で投与 6 週に後肢握力の有意な低値がみられた。他には主群及び回復群のいずれの動物にも対照群と各投与群との間に有意差は認められなかった。

6) 自発運動量の測定（Fig. 1~6、Table 2-100~2-105、Appendix 309~324）

主群では、300 mg/kg 投与群の雌で授乳 4 日の測定開始後 20~40 分の自発運動量に有意な高値がみられたが、用量との関連はなかった。

回復群では、1000 mg/kg 投与群の雌で投与 6 週の測定開始後 20~50 分の自発運動量及び総自発運動量に有意な高値がみられたが、同時期の主群の検査に変化はみられなかった。

7.3 体重（Fig. 7~10、Table 3-1~3-8、Appendix 325~348）

主群では、1000 mg/kg 投与群の雄で投与 8 日以降の体重に低値がみられ、投与期間の体重及び体重増加量に有意差がみられた。同群の雌では投与 4 日以降の体重に低値がみられ、交配前投与期間、妊娠期間及び授乳期間中のほとんどの測定値と交配前投与期間及び妊娠期間の体重増加量に有意差がみられた。300 mg/kg 投与群の雌では交

配前投与期間、妊娠期間及び授乳期間中の体重が対照群を下回って推移し、授乳4日の体重に有意差がみられた。300 mg/kg投与群の雄及び100 mg/kgの雌雄の体重は対照群と同等値を示し、有意差は認められなかった。

回復群では、1000 mg/kg投与群の雌雄で投与期間中の体重に低値がみられた。なお、同群では回復期間中の体重増加量に有意な高値がみられた。

7.4 摂餌量（Fig. 11~14、Table 4-1~4-8、Appendix 349~372）

主群では、1000 mg/kg投与群の雌雄で投与4日に有意な低値がみられた。その後、同群の雌では投与15日に有意な高値もみられたが、妊娠20日及び授乳2日に有意な低値がみられた。300 mg/kg投与群の雌では投与4日及び授乳2日に有意な低値がみられた。300 mg/kg投与群の雄及び100 mg/kgの雌雄の摂餌量には被験物質投与による影響は認められなかった。

回復群では、1000 mg/kg投与群の雌雄で投与4日に有意な低値がみられた。その後、同群の雄では投与42日と回復8及び11日に、雌では回復4日に有意な高値がみられた。

7.5 尿検査（摂水量測定を含む）（Table 5-1~5-8、Appendix 373~390）

定性項目については、投与期間終了週検査で尿潜血が各投与群の全例にみられ、その程度は投与量の増加に伴って増強した。また、色調で暗黄色が100、300及び1000 mg/kg投与群で2、3及び3例みられた。その他の検査項目では、主群及び回復群のいずれの動物にも異常はみられなかった。

定量項目については、1000 mg/kg投与群で摂水量の有意な増加がみられた。その他の検査項目では、対照群と各投与群との間に有意差は認められなかった。

7.6 血液学検査（Table 6-1~6-8、Appendix 391~398）

1) 投与期間終了時検査

1000 mg/kg投与群の雄で赤血球数、ヘモグロビン量、ヘマトクリット値及び平均赤血球血色素濃度の有意な低値、血小板数、好中球数及び単球数の有意な高値がみられた。同群の雌では血小板数の有意な高値がみられた。300 mg/kg投与群の雄で赤血球数の有意な低値がみられたが、100 mg/kg投与群と大差ない値であり、他の赤血球項目に変化がみられないことから生理的変動範囲内の変化と考えられた。また、300 mg/kg投与群の雄ではフィブリノーゲン量の有意な高値が、100 mg/kg投与群の雌では平均赤血球血色素濃度の有意な高値が認められたが、1000 mg/kg投与群に同様な変化が認められないことから、生理的変動範囲内の変化と考えられた。その他の検査項目では、対照群と各被験物質投与群との間に有意差は認められなかった。

2) 回復期間終了時検査

1000 mg/kg投与群の雄で赤血球数及びヘモグロビン量の有意な低値、網赤血球率の有意な高値がみられた。また、好酸球比率及び好酸球数の有意な低値が認められたが、

投与期間終了時にみられない変化であることから、生理的変動範囲内の変化と判断した。

なお、雌動物では対照群と 1000 mg/kg 投与群との間に有意差は認められなかった。

7.7 血液化学検査（Table 7-1~7-8、Appendix 399~406）

1) 投与期間終了時検査

300 mg/kg 以上の投与群の雄で無機リンの有意な高値が認められた。

その他、1000 mg/kg 投与群の雄でグルコースの有意な低値、300 mg/kg 投与群の LDH 及びカリウムと 100 mg/kg 投与群のグルコース及びカルシウムに有意な高値がみられたが、用量との関連がないことから、生理的変動範囲内の変化と判断した。

なお、雌動物では、対照群と各被験物質投与群との間に有意差は認められなかった。

2) 回復期間終了時検査

1000 mg/kg 投与群の雄で AlP の有意な低値、雌でナトリウムの有意な高値、アルブミン及び A/G 比の有意な低値が認められたが、投与期間終了時にみられない変化であることから、生理的変動範囲内の変化と判断した。

7.8 器官重量（Table 8-1~8-8、Appendix 407~436）

1) 投与期間終了時検査

300 mg/kg 以上の投与群の雌で胸腺の絶対及び相対重量に有意な低値、1000 mg/kg 投与群の雌雄で肝臓の相対重量の有意な高値がみられた。

なお、以下の器官に対照群との間に有意差がみられたが、体重増加抑制に伴う生理的変動範囲内の変化と考えられた。

脳	:	相対重量の有意な高値が 1000 mg/kg 投与群の雌雄にみられた。
心臓	:	絶対重量の有意な低値が 1000 mg/kg 投与群の雌にみられた。
脾臓	:	相対重量の有意な高値が 1000 mg/kg 投与群の雄にみられた。
腎臓	:	相対重量の有意な高値が 1000 mg/kg 投与群の雌雄にみられた。
副腎	:	絶対重量の有意な低値が 1000 mg/kg 投与群の雌にみられた。
精巣上体	:	相対重量の有意な高値が 1000 mg/kg 投与群にみられた。

2) 回復期間終了時検査

肝臓	:	相対重量の有意な高値が 1000 mg/kg 投与群の雌にみられた。

7.9 剖検所見（Table 9-1~9-3、Appendix 437~552）

1) 死亡動物

分娩後（授乳0日）に死亡した主群の1000 mg/kg投与群の雌1例（動物番号4110）で、脾臓及び胸腺の小型化がみられた。

2) 投与期間終了時検査

被験物質投与によると考えられる変化が雄の肝臓、雌の外表及び胸腺にみられた。

外表	:	低栄養状態が1000 mg/kg投与群の雌1例にみられた。
肝臓	:	暗調化が1000 mg/kg投与群の雄6例にみられた。
胸腺	:	小型化が300 mg/kg投与群の雌1例及び1000 mg/kg投与群の雌3例にみられた。

他に、以下の器官・組織に所見がみられたが、出現頻度及び病理学的性状から偶発的変化と考えられた。

肝臓	:	癒着が100 mg/kg投与群の雄1例にみられた。
大脳	:	陥凹巣が100 mg/kg投与群の雌1例にみられた。
精巣上体	:	小型化が100 mg/kg投与群で1例、黄色巣が1000 mg/kg投与群で1例にみられた。
胃	:	腺胃の白色巣が対照群の雄1例に、前胃の陥凹巣、前胃の暗赤色巣又は境界縁の肥厚が300 mg/kg投与群の雌各1例に、腺胃の暗赤色巣が300及び1000 mg/kg投与群の雌各1例にみられた。
精巣	:	小型化が100 mg/kg投与群で1例にみられた。
子宮	:	低形成が100 mg/kg投与群で1例にみられた。

3) 回復期間終了時検査

異常はみられなかった。

7.10 病理組織学検査（Table 10-1~10-4、Appendix 437~552）

1) 死亡動物

授乳0日に死亡した主群の1000 mg/kg投与群の雌1例（動物番号4110）では、被験物質投与によると考えられる変化として脾臓で軽微な白脾髄の萎縮、胸腺で中等度の萎縮がみられた。

その他、以下の所見がみられたが、出現状態及び病理組織学的性状から偶発病変と考えられた。

骨及び骨髄（胸骨）	:	軽微な軟骨粘液変性がみられた。
肝臓	:	軽微な小葉辺縁性肝細胞の空胞化がみられた。

2) 投与期間終了時検査

被験物質投与によると考えられる変化が盲腸、肝臓及び胸腺にみられた。

盲腸	:	軽微又は軽度な粘膜上皮細胞の単細胞壊死が100 mg/kg投与群の雄4例と雌2例、300 mg/kg投与群の雌

雄各3例、1000 mg/kg投与群の雄8例と雌7例に、軽微な粘膜のび漫性過形成が100 mg/kg投与群の雌雄各1例、300 mg/kg投与群の雄3例と雌4例、1000 mg/kg投与群の雄7例と雌6例にみられた。

肝臓 : 軽微又は軽度な小葉辺縁性肝細胞の空胞化が対照群の雄11例と雌5例、100 mg/kg投与群の雄10例と雌4例、300 mg/kg投与群の雄7例と雌1例にみられ、300 mg/kg以上の投与群の雌雄で用量の増加に伴って減少した。

胸腺 : 軽度な萎縮が対照群の雌1例でみられたのに対し、軽微な萎縮が100 mg/kg投与群の雌1例、軽微から中等度の萎縮が300 mg/kg投与群の雌4例、軽微から高度の萎縮が1000 mg/kg投与群の雌5例にみられ、300 mg/kg以上の投与群の雌で用量の増加に伴って増強した。

その他、以下の所見がみられたが、出現状態及び病理組織学的性状から偶発病変と考えられた。

骨及び骨髄（胸骨） : 軽微な軟骨粘液変性が対照群の雌雄各5例及び1000 mg/kg投与群の雄5例と雌4例にみられた。

大脳 : 軽微な形成異常が100 mg/kg投与群の雌1例にみられた。

精巣上体 : 高度な精子低形成及び軽微な管腔内の細胞残屑が100 mg/kg投与群で1例みられた。

心臓 : 軽微な心筋症が1000 mg/kg投与群の雌1例にみられた。

盲腸 : 軽微又は軽度な粘膜の細胞浸潤が対照群の雄3例と雌1例、100 mg/kg投与群の雄1例、300 mg/kg投与群の雌雄各1例、1000 mg/kg投与群の雄5例と雌3例に、軽微な漿膜の細胞浸潤が1000 mg/kg投与群の雌1例にみられた。

結腸 : 軽微な漿膜の細胞浸潤が1000 mg/kg投与群の雌1例にみられた。

腎臓 : 軽微な尿細管細胞の空胞化が1000 mg/kg投与群の雌1例に、軽微な再生尿細管が対照群の雄4例と雌1例及び1000 mg/kg投与群の雄2例に、軽微な鉱質沈着が1000 mg/kg投与群の雌2例に、軽微な移行上皮細胞の過形成が1000 mg/kg投与群の雌1例にみられた。

肝臓 : 軽微な壊死巣が300 mg/kg投与群の雌1例に、軽微な

髄外造血が対照群の雌1例と1000 mg/kg投与群の雌1例に、軽微な微小肉芽腫が対照群の雄10例と雌2例、100 mg/kg投与群の雄10例と雌2例、300 mg/kg投与群の雄8例と雌2例、1000 mg/kg投与群の雄8例と雌3例に、軽微な皮膜の線維化が100 mg/kg投与群の雄1例に、軽微な好酸性変異細胞巣が100 mg/kg投与群の雌1例にみられた。

肺（気管支を含む）：軽微な動脈壁の鉱質沈着が対照群の雄1例と雌2例に、軽微または軽度な泡沫細胞の集簇が対照群の雄2例及び1000 mg/kg投与群の雄2例と雌1例に、軽微な炎症巣が対照群の雌1例にみられた。

脾臓：軽微又は軽度な髄外造血が対照群の雄2例と雌12例、100 mg/kg投与群の雌12例、300 mg/kg投与群の雌11例及び1000 mg/kg投与群の雄3例と雌8例にみられた。

胃：軽度な筋層又は漿膜部の炎症が1000 mg/kg投与群の雌1例に、軽微な腺胃の糜爛が対照群の雄2例、300及び1000 mg/kg投与群の雌各1例に、軽微又は軽度な前胃の潰瘍が300 mg/kg投与群の雌2例にみられた。

精巣：軽微な精細管の萎縮が対照群の1例に、高度な精細管の萎縮が100 mg/kg投与群の1例にみられた。

甲状腺：軽微な鰓後体の嚢胞が対照群の雌雄各1例及び1000 mg/kg投与群の雌1例にみられた。

膀胱：軽微な粘膜の細胞浸潤が対照群の雄1例に、軽度なび漫性の粘膜過形成が1000 mg/kg投与群の雌1例にみられた。

子宮：軽度な低形成が100 mg/kg投与群で1例にみられた。

3) 回復期間終了時検査

盲腸：軽微な粘膜の細胞浸潤が対照群の雄1例及び1000 mg/kg投与群の雌雄各2例に、軽微なび漫性の粘膜過形成が1000 mg/kg投与群の雄1例にみられた。

肝臓：軽微な小葉辺縁性肝細胞の空胞化が対照群の雌雄各1例に、軽微な微小肉芽腫が対照群及び1000 mg/kg投与群の雄4例と雌5例にみられた。

脾臓：軽微な髄外造血が対照群及び1000 mg/kg投与群の雌各2例にみられた。

7.11　性周期（Table 11、Appendix 553~556）

性周期異常の動物はみられず、平均性周期日数には対照群と各被験物質投与群との間に有意差は認められなかった。

7.12　交配成績（Table 12、Appendix 557~560）

交配開始後5日までに全組み合わせで交尾が成立した。なお、不妊であった組み合わせは1000 mg/kg投与群の1組のみであった。したがって、交尾までに要した日数、交尾率、授精率及び受胎率には対照群と各被験物質投与群との間に有意差は認められなかった。

7.13　分娩成績及び分娩・授乳状態（Table 13、Appendix 561~564）

分娩状態では、妊娠21.5~23.0日に全例が正常に分娩し、出産率、妊娠期間、黄体数、着床痕数、着床率、死産児率、出生児数及び出生率には有意差は認められなかった。

哺育状態では、いずれの母動物にも巣作り、児集め及び授乳行動に異常はみられなかった。

7.14　出生児の観察（Table 14、Appendix 565~568）

出生時体重において1000 mg/kg投与群の雌雄に有意な低値がみられた。性比には対照群と各投与群との間に有意差は認められず、外表異常はみられなかった。

7.15　出生児の生存率（Table 15、Appendix 569~572）

授乳期間中の死亡児は300 mg/kg投与群で3例及び1000 mg/kg投与群で5例みられたのみであり、生後4日生存率には対照群と各投与群との間に有意差は認められなかった。

7.16　出生児の体重（Table 16、Appendix 573~576）

出生時及び生後4日の雌雄体重において1000 mg/kg投与群の雌雄に、生後4日の雌雄体重において300 mg/kg投与群の雌雄に有意な低値がみられた。他には対照群と各投与群との間に有意差は認められなかった。

7.17　出生児の生後4日剖検所見（Table 17、Appendix 577~580）

低栄養状態が1000 mg/kg投与群の雄8例と雌4例にみられた。他に胸腺の頸部残留が対照群の雌1例、100 mg/kg投与群の雄1例及び1000 mg/kg投与群の雌1例に、横隔膜ヘルニアが1000 mg/kg投与群の雄1例にみられたが、いずれも1例のみの変化で発現状況に用量との関連はなかった。

8. 考察

2,3,4,4'-Tetrahydroxybenzophenone の 0（対照群）、100、300、及び 1000 mg/kg を、Sprague-Dawley 系 SPF ラットの雄には交配前 14 日間及び交配期間を通して剖検前日（42 日間投与）まで、雌には交配前 14 日間及び交配期間並びに妊娠期間を通して授乳 4 日まで（41~45 日間投与）投与し、反復投与毒性及び生殖発生毒性を検討した。更に、0 及び 1000 mg/kg 投与群については 42 日間投与した後、14 日間の回復期間を設け、毒性変化の可逆性を検討した。

1) 反復投与毒性

1000 mg/kg 投与群の雌 1 例が授乳 0 日に死亡した。本例の一般状態に異常はみられなかったが、剖検では脾臓及び胸腺の小型化がみられ、組織学的に白脾髄の萎縮、胸腺の萎縮がみられた。明らかな死因は不明であるが、被験物質投与の影響と妊娠及び分娩のストレスの関与が推察された。

詳細な一般状態の観察、機能検査、握力測定、自発運動量の測定に被験物質投与による影響は認められなかった。

一般状態では、1000 mg/kg 投与群の雄で投与 4 週以降に投与後の流涎がみられた。しかし、詳細な一般状態の観察及び各種機能検査で異常はみられなかったことから、中枢性の変化ではなく、被験物質の刺激に基づく変化と推察された。

体重及び摂餌量では、1000 mg/kg 投与群の雌雄で投与初期に摂餌量の低値、投与期間中に体重増加抑制が認められた。300 mg/kg 投与群の雌では投与初期に摂餌量の低値がみられた。

尿検査では、尿潜血が各投与群の全例にみられ、その程度は投与量の増加に伴って増強した。また暗黄色の色調を示す例もみられた。これらの変化については、尿沈渣中に赤血球がみられなかったこと、病理組織検査で腎臓、尿路系に異常はなく、ヘモジデリン沈着のような溶血を示唆する変化もみられていないことから、赤血球への直接の影響に基づく変化とは考え難かった。また、被験物質をラット正常尿中に添加し潜血反応を確認した検討を実施したところ、潜血反応が確認され、その程度が被験物質添加量に応じて増強した。この結果から尿潜血及び色調の変化は被験物質の排泄に関連した変化と推察され、被験物質の毒性を示す変化ではないと判断した。また、1000 mg/kg 投与群の雄では摂水量の増加がみられたが、尿量及び糞の形態などに影響がない軽度な変化であることから、生理学的変動範囲内の変化と考えられた。

血液学検査では、1000 mg/kg 投与群の雄で赤血球数、ヘモグロビン量、ヘマトクリット値及び平均赤血球血色素濃度の低値がみられ、貧血が示唆された。また、同群では好中球数及び単球数の高値がみられたが、その発現機序は不明であった。さらに、1000 mg/kg 投与群の雌雄で血小板数の高値がみられたが、炎症性変化、組織損傷といった関連する変化がみられないこと、凝固系検査値は反応していないことから、その発現機序は不明であった。

血液化学検査では、投与期間終了時検査において300 mg/kg以上の投与群の雄で無機リンの高値がみられたが、その原因は不明であった。

病理学検査では、投与期間終了時検査において、300 mg/kg 以上の投与群の雌で胸腺重量が減少し、肉眼的な小型化、組織学的な萎縮がみられた。なお、胸腺の萎縮は皮質におけるリンパ球の減少であった。胸腺萎縮は前述の死亡例や低栄養状態を示した例でもみられ、被験物質投与の影響と妊娠及び分娩のストレスが関与した変化と考えられた。また、盲腸で粘膜上皮細胞の単細胞壊死及び粘膜のび漫性過形成が100mg/kg以上の投与群の雌雄で認められた。盲腸での単細胞壊死は極めて軽度な粘膜への障害作用、過形成変化はそれに対する反応性変化である可能性が考えられた。肝臓では、重量の増加が1000 mg/kg投与群の雌雄でみられ、被験物質投与の影響が疑われた。また、小葉辺縁性肝細胞の空胞化が対照群、100及び300 mg/kg投与群の雌雄でみられたが、回復群で減弱したことと、その形態的特徴から脂肪空胞と推察され、媒体であるコーンオイルによる変化と考えられた。また、本変化は300 mg/kg以上の投与群で用量の増加に伴って減少し、雌での変化がやや強いことから、肝細胞の空胞化の減少と体重増加抑制との関連性が考えられた。また、1000 mg/kg投与群の雄でみられた肝色調の変化は、脂肪化した対照群との比較で生じた変化と考えられた。

尿検査、血液検査、血液化学検査及び病理学検査でみられた変化は、いずれも休薬により軽減するか、回復したことからいずれも可逆性の変化と考えられた。

2)　生殖発生毒性

性周期、交尾までに要した日数、交尾率、授精率及び受胎率には被験物質投与の影響は認められなかった。また、母動物では、1000 mg/kg投与群の1例が分娩後（授乳0日）に死亡したが、本例の分娩状態に異常はみられなかった。更に、出産率、妊娠期間、黄体数、着床痕数、着床率、死産児率、出生児数、出生率及び性比に被験物質投与の影響は認められず、授乳期間中の授乳状態にも異常が認められないことから、1000 mg/kg投与群においても雌雄動物の交尾能、授精能及び受胎能、母動物の妊娠維持、分娩及び哺育行動などの生殖機能への影響はないと考えられた。

出生児では、1000 mg/kg 投与群の雌雄で出生時及び生後 4 日の雌雄体重に、300 mg/kg 投与群の雌雄で生後 4 日の雌雄体重にそれぞれ低値がみられた。出生時の外表観察及び生後4日剖検所見及び生存率には被験物質投与による変化は認められなかった。

これらの結果から、2,3,4,4'-Tetrahydroxybenzophenoneの反復投与毒性に対する無影響量は、盲腸における粘膜上皮細胞の単細胞壊死及び粘膜のび漫性過形成が100mg/kg以上の投与群の雌雄で認められたため雌雄ともに100 mg/kg/day未満、生殖発生毒性に対しては雌雄親動物に対する無影響量は1000 mg/kg/day、児動物に対する無影響量は100 mg/kg/dayと判断した。

Table 1-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in male rats (Main group)

Dose mg/kg	Signs	Day of administration					
		1-7	8-14	15-21	22-28	29-35	36-42
0	No. of animals	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0
100	No. of animals	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0
300	No. of animals	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0
1000	No. of animals	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	3	2	4
	Salivation	0	0	0	3	2	4

Table 1-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in female rats during the pre-mating period (Main group)

Dose mg/kg	Signs	Administration														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15a)
0	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
300	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	No. of animals	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a): Day of administration

Table 1-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in dams during the gestation period (Main group)

Dose mg/kg	Signs	Administration																						
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23a)
0	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	0	
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
100	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	10	0	
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
300	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	9	0	
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
1000	No. of dams	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	10	1	0
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

a): Day of gestation

Table 1-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in dams during the lactation period (Main group)

Dose mg/kg	Signs	Administration				
		0	1	2	3	4a)
0	No. of dams	12	12	12	12	12
	No. of dams with abnormal findings	0	0	0	0	0
100	No. of dams	12	12	12	12	12
	No. of dams with abnormal findings	0	0	0	0	0
300	No. of dams	12	12	12	12	12
	No. of dams with abnormal findings	0	0	0	0	0
1000	No. of dams	11	10	10	10	10
	No. of dams with abnormal findings	1	0	0	0	0
	Dead	1	0	0	0	0

a): Day of lactation

Table 1-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in male rats (Recovery group, administration period)

Dose mg/kg	Signs	Day of administration 1-7	8-14	15-21	22-28	29-35	36-42
0	No. of animals	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0
1000	No. of animals	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	3	1	2
	Salivation	0	0	0	3	1	2

R-944

Table 1-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in female rats (Recovery group, administration period)

Dose mg/kg	Signs	Day of administration 1-7	8-14	15-21	22-28	29-35	36-42
0	No. of animals	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0
1000	No. of animals	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0

Table 1-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in male rats (Recovery group, recovery period)

Dose mg/kg	Signs	Day of recovery													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0

R-944

Table 1-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Clinical signs in female rats (Recovery group, recovery period)

Dose mg/kg	Signs	Day of recovery													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No. of animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 1 of administration)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

R-944

Table 2-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 2 of administration)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

Table 2-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

R-944

Table 2-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 4 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

Table 2-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

R-944

Table 2-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Main group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

Table 2-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Week 1 of administration)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

R-944

Table 2-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Week 2 of administration)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	12
Posture					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12

Table 2-9 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Day 1 of gestation)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	11
Posture					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11

R-944

Table 2-10 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Day 7 of gestation)

	Dose (mg/kg)	0	100	300	1000
Parameter	No. of animals	12	12	12	11
Posture					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11

Table 2-11 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Day 14 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Posture					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11

Table 2-12 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Day 20 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Posture					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11

Table 2-13 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Main group, Day 4 of lactation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 10
Posture					
Normal		12	12	12	10
Convulsion					
None		12	12	12	10
Abnormal behavior					
None		12	12	12	10

R-944

Table 2-14 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-15 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-16 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 3 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-17 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 4 of administration)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-18 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 5 of administration)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-19 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 8 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-20 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 1 of recovery)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-21 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: home cage observations (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-22 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-23 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-24 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-25 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 4 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-26 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 5 of administration)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-27 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 6 of administration)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-28 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 1 of recovery)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

Table 2-29 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: home cage observations (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Posture			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5

R-944

Table 2-30 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
Easy		12	12	11	11
Some resistance/avoidance		0	0	1	1
Fur condition					
Normal		12	12	12	12
Skin					
Normal		12	12	12	12
Secretions-Eye, Nose					
Absent		12	12	12	12
Exophthalmos					
Absent		12	12	12	12
Palpebral closure					
Normal		12	12	12	12
Mucosal membranes					
Normal		12	12	12	12
Lacrimation					
Normal		12	12	12	12
Piloerection					
Absent		12	12	12	12
Pupil size					
Normal		12	12	12	12
Salivation					
None		12	12	12	12
Abnormal respiration					
Absent		12	12	12	12
Vocalization					
None		11	9	9	10
Soft		1	2	3	1
Moderate		0	1	0	1
Reactivity to handling					
Easy		10	11	12	10
Slightly awkward		2	1	0	2

Table 2-31 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
Easy		12	12	12	12
Fur condition					
Normal		12	12	12	12
Skin					
Normal		12	12	12	12
Secretions-Eye, Nose					
Absent		12	12	12	12
Exophthalmos					
Absent		12	12	12	12
Palpebral closure					
Normal		12	12	12	12
Mucosal membranes					
Normal		12	12	12	12
Lacrimation					
Normal		12	12	12	12
Piloerection					
Absent		12	12	12	12
Pupil size					
Normal		12	12	12	12
Salivation					
None		12	12	12	12
Abnormal respiration					
Absent		12	12	12	12
Vocalization					
None		12	9	11	9
Soft		0	3	1	3
Reactivity to handling					
Easy		12	12	12	12

R-944

Table 2-32 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
Easy		12	11	11	12
Some resistance/avoidance		0	1	1	0
Fur condition					
Normal		12	12	12	12
Skin					
Normal		12	12	12	12
Secretions-Eye, Nose					
Absent		12	12	12	12
Exophthalmos					
Absent		12	12	12	12
Palpebral closure					
Normal		12	12	12	12
Mucosal membranes					
Normal		12	12	12	12
Lacrimation					
Normal		12	12	12	12
Piloerection					
Absent		12	12	12	12
Pupil size					
Normal		12	12	12	12
Salivation					
None		12	12	12	12
Abnormal respiration					
Absent		12	12	12	12
Vocalization					
None		10	6	10	10
Soft		2	6	2	2
Reactivity to handling					
Easy		12	12	11	12
Slightly awkward		0	0	1	0

Table 2-33 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 4 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
	Easy	12	8	11	12
	Some resistance/avoidance	0	4	1	0
Fur condition					
	Normal	12	12	12	12
Skin					
	Normal	12	12	12	12
Secretions-Eye, Nose					
	Absent	12	12	12	12
Exophthalmos					
	Absent	12	12	12	12
Palpebral closure					
	Normal	12	12	12	12
Mucosal membranes					
	Normal	12	12	12	12
Lacrimation					
	Normal	12	12	12	12
Piloerection					
	Absent	12	12	12	12
Pupil size					
	Normal	12	12	12	12
Salivation					
	None	12	12	12	12
Abnormal respiration					
	Absent	12	12	12	12
Vocalization					
	None	10	8	7	10
	Soft	2	4	4	1
	Moderate	0	2	1	1
Reactivity to handling					
	Easy	11	10	10	11
	Slightly awkward	1	2	2	1

Table 2-34 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
	Easy	12	11	11	12
	Some resistance/avoidance	0	1	1	0
Fur condition					
	Normal	12	12	12	12
Skin					
	Normal	12	12	12	12
Secretions-Eye, Nose					
	Absent	12	12	12	12
Exophthalmos					
	Absent	12	12	12	12
Palpebral closure					
	Normal	12	12	12	12
Mucosal membranes					
	Normal	12	12	12	12
Lacrimation					
	Normal	12	12	12	12
Piloerection					
	Absent	12	12	12	12
Pupil size					
	Normal	12	12	12	12
Salivation					
	None	12	12	12	12
Abnormal respiration					
	Absent	12	12	12	12
Vocalization					
	None	9	9	10	11
	Soft	3	3	2	1
Reactivity to handling					
	Easy	12	12	12	12

Table 2-35 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Main group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 12
Ease of removal from cage					
	Easy	12	11	12	12
	Some resistance/avoidance	0	1	0	0
Fur condition					
	Normal	12	12	12	12
Skin					
	Normal	12	12	12	12
Secretions-Eye, Nose					
	Absent	12	12	12	12
Exophthalmos					
	Absent	12	12	12	12
Palpebral closure					
	Normal	12	12	12	12
Mucosal membranes					
	Normal	12	12	12	12
Lacrimation					
	Normal	12	12	12	12
Piloerection					
	Absent	12	12	12	12
Pupil size					
	Normal	12	12	12	12
Salivation					
	None	12	12	12	12
Abnormal respiration					
	Absent	12	12	12	12
Vocalization					
	None	10	8	10	11
	Soft	2	3	2	1
	Moderate	0	1	0	0
Reactivity to handling					
	Easy	12	12	12	12

R-944

Table 2-36 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 12
Ease of removal from cage					
	Easy	12	11	12	12
	Some resistance/avoidance	0	1	0	0
Fur condition					
	Normal	12	12	12	12
Skin					
	Normal	12	12	12	12
Secretions-Eye, Nose					
	Absent	12	12	12	12
Exophthalmos					
	Absent	12	12	12	12
Palpebral closure					
	Normal	12	12	12	12
Mucosal membranes					
	Normal	12	12	12	12
Lacrimation					
	Normal	12	12	12	12
Piloerection					
	Absent	12	12	12	12
Pupil size					
	Normal	12	12	12	12
Salivation					
	None	12	12	12	12
Abnormal respiration					
	Absent	12	12	12	12
Vocalization					
	None	10	11	10	9
	Soft	2	1	2	3
Reactivity to handling					
	Easy	10	10	10	11
	Slightly awkward	2	2	2	1

Table 2-37 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Ease of removal from cage					
	Easy	12	11	12	12
	Some resistance/avoidance	0	1	0	0
Fur condition					
	Normal	12	12	12	12
Skin					
	Normal	12	12	12	12
Secretions-Eye, Nose					
	Absent	12	12	12	12
Exophthalmos					
	Absent	12	12	12	12
Palpebral closure					
	Normal	12	12	12	12
Mucosal membranes					
	Normal	12	12	12	12
Lacrimation					
	Normal	12	12	12	12
Piloerection					
	Absent	12	12	12	12
Pupil size					
	Normal	12	12	12	12
Salivation					
	None	12	12	12	12
Abnormal respiration					
	Absent	12	12	12	12
Vocalization					
	None	12	11	12	11
	Soft	0	1	0	1
Reactivity to handling					
	Easy	12	11	12	12
	Slightly awkward	0	1	0	0

R-944

Table 2-38 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Day 1 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Ease of removal from cage					
	Easy	12	11	12	9
	Some resistance/avoidance	0	1	0	2
Fur condition					
	Normal	12	12	12	11
Skin					
	Normal	12	12	12	11
Secretions-Eye, Nose					
	Absent	12	12	12	11
Exophthalmos					
	Absent	12	12	12	11
Palpebral closure					
	Normal	12	12	12	11
Mucosal membranes					
	Normal	12	12	12	11
Lacrimation					
	Normal	12	12	12	11
Piloerection					
	Absent	12	12	12	11
Pupil size					
	Normal	12	12	12	11
Salivation					
	None	12	12	12	11
Abnormal respiration					
	Absent	12	12	12	11
Vocalization					
	None	11	11	12	10
	Soft	1	1	0	1
Reactivity to handling					
	Easy	10	11	12	9
	Slightly awkward	2	1	0	2

Table 2-39 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Day 7 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Ease of removal from cage					
Easy		12	12	12	11
Fur condition					
Normal		12	12	12	11
Skin					
Normal		12	12	12	11
Secretions-Eye, Nose					
Absent		12	12	12	11
Exophthalmos					
Absent		12	12	12	11
Palpebral closure					
Normal		12	12	12	11
Mucosal membranes					
Normal		12	12	12	11
Lacrimation					
Normal		12	12	12	11
Piloerection					
Absent		12	12	12	11
Pupil size					
Normal		12	12	12	11
Salivation					
None		12	12	12	11
Abnormal respiration					
Absent		12	12	12	11
Vocalization					
None		12	11	11	11
Soft		0	1	1	0
Reactivity to handling					
Easy		11	12	12	11
Slightly awkward		1	0	0	0

Table 2-40 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Day 14 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Ease of removal from cage					
Easy		12	12	12	11
Fur condition					
Normal		12	12	12	11
Skin					
Normal		12	12	12	11
Secretions-Eye, Nose					
Absent		12	12	12	11
Exophthalmos					
Absent		12	12	12	11
Palpebral closure					
Normal		12	12	12	11
Mucosal membranes					
Normal		12	12	12	11
Lacrimation					
Normal		12	12	12	11
Piloerection					
Absent		12	12	12	11
Pupil size					
Normal		12	12	12	11
Salivation					
None		12	12	12	11
Abnormal respiration					
Absent		12	12	12	11
Vocalization					
None		12	10	11	11
Soft		0	2	1	0
Reactivity to handling					
Easy		12	11	12	11
Slightly awkward		0	1	0	0

Table 2-41 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Day 20 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 11
Ease of removal from cage					
	Easy	12	10	12	11
	Some resistance/avoidance	0	2	0	0
Fur condition					
	Normal	12	12	12	11
Skin					
	Normal	12	12	12	11
Secretions-Eye, Nose					
	Absent	12	12	12	11
Exophthalmos					
	Absent	12	12	12	11
Palpebral closure					
	Normal	12	12	12	11
Mucosal membranes					
	Normal	12	12	12	11
Lacrimation					
	Normal	12	12	12	11
Piloerection					
	Absent	12	12	12	11
Pupil size					
	Normal	12	12	12	11
Salivation					
	None	12	12	12	11
Abnormal respiration					
	Absent	12	12	12	11
Vocalization					
	None	12	11	12	11
	Moderate	0	1	0	0
Reactivity to handling					
	Easy	12	10	12	10
	Slightly awkward	0	2	0	1

R-944

Table 2-42 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Main group, Day 4 of lactation)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 10
Ease of removal from cage					
	Easy	11	12	11	9
	Some resistance/avoidance	1	0	1	1
Fur condition					
	Normal	12	12	12	10
Skin					
	Normal	12	12	12	10
Secretions-Eye, Nose					
	Absent	12	12	12	10
Exophthalmos					
	Absent	12	12	12	10
Palpebral closure					
	Normal	12	12	12	10
Mucosal membranes					
	Normal	12	12	12	10
Lacrimation					
	Normal	12	12	12	10
Piloerection					
	Absent	12	12	12	10
Pupil size					
	Normal	12	12	12	10
Salivation					
	None	12	12	12	10
Abnormal respiration					
	Absent	12	12	12	10
Vocalization					
	None	12	11	12	10
	Soft	0	1	0	0
Reactivity to handling					
	Easy	12	11	11	9
	Slightly awkward	0	1	1	1

Table 2-43 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		2	5
Soft		3	0
Reactivity to handling			
Easy		4	5
Slightly awkward		1	0

Table 2-44 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		4	5
Soft		1	0
Reactivity to handling			
Easy		5	5

Table 2-45 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 3 of administration)

Dose (mg/kg)	0	1000
Parameter / No. of animals	5	5
Ease of removal from cage		
Easy	4	5
Some resistance/avoidance	1	0
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	3	4
Soft	1	1
Moderate	1	0
Reactivity to handling		
Easy	5	5

R-944

Table 2-46 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 4 of administration)

Dose (mg/kg)	0	1000
Parameter / No. of animals	5	5
Ease of removal from cage		
Easy	5	5
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	4	4
Soft	1	1
Reactivity to handling		
Easy	5	5

Table 2-47 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 5 of administration)

Dose (mg/kg)	0	1000
Parameter No. of animals	5	5
Ease of removal from cage		
Easy	5	5
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	5	5
Reactivity to handling		
Easy	5	5

Table 2-48 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 6 of administration)

Dose (mg/kg)	0	1000
Parameter No. of animals	5	5
Ease of removal from cage		
Easy	4	5
Some resistance/avoidance	1	0
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	2	4
Soft	3	1
Reactivity to handling		
Easy	5	5

Table 2-49 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 1 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Ease of removal from cage			
	Easy	3	5
	Some resistance/avoidance	2	0
Fur condition			
	Normal	5	5
Skin			
	Normal	5	5
Secretions-Eye, Nose			
	Absent	5	5
Exophthalmos			
	Absent	5	5
Palpebral closure			
	Normal	5	5
Mucosal membranes			
	Normal	5	5
Lacrimation			
	Normal	5	5
Piloerection			
	Absent	5	5
Pupil size			
	Normal	5	5
Salivation			
	None	5	5
Abnormal respiration			
	Absent	5	5
Vocalization			
	None	3	4
	Soft	0	1
	Moderate	2	0
Reactivity to handling			
	Easy	5	5

R-944

Table 2-50 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: in-the-hand observations (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Ease of removal from cage			
	Easy	3	5
	Some resistance/avoidance	2	0
Fur condition			
	Normal	5	5
Skin			
	Normal	5	5
Secretions-Eye, Nose			
	Absent	5	5
Exophthalmos			
	Absent	5	5
Palpebral closure			
	Normal	5	5
Mucosal membranes			
	Normal	5	5
Lacrimation			
	Normal	5	5
Piloerection			
	Absent	5	5
Pupil size			
	Normal	5	5
Salivation			
	None	5	5
Abnormal respiration			
	Absent	5	5
Vocalization			
	None	3	3
	Soft	1	2
	Moderate	1	0
Reactivity to handling			
	Easy	5	4
	Slightly awkward	0	1

Table 2-51 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	4
Slightly awkward		0	1

Table 2-52 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	5

Table 2-53 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 3 of administration)

Dose (mg/kg)	0	1000
Parameter No. of animals	5	5
Ease of removal from cage		
Easy	5	5
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	5	5
Reactivity to handling		
Easy	5	5

R-944

Table 2-54 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 4 of administration)

Dose (mg/kg)	0	1000
Parameter No. of animals	5	5
Ease of removal from cage		
Easy	5	5
Fur condition		
Normal	5	5
Skin		
Normal	5	5
Secretions-Eye, Nose		
Absent	5	5
Exophthalmos		
Absent	5	5
Palpebral closure		
Normal	5	5
Mucosal membranes		
Normal	5	5
Lacrimation		
Normal	5	5
Piloerection		
Absent	5	5
Pupil size		
Normal	5	5
Salivation		
None	5	5
Abnormal respiration		
Absent	5	5
Vocalization		
None	5	5
Reactivity to handling		
Easy	5	5

Table 2-55 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	5

Table 2-56 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	5

Table 2-57 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 1 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	5

R-944

Table 2-58 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: in-the-hand observations (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Ease of removal from cage			
Easy		5	5
Fur condition			
Normal		5	5
Skin			
Normal		5	5
Secretions-Eye, Nose			
Absent		5	5
Exophthalmos			
Absent		5	5
Palpebral closure			
Normal		5	5
Mucosal membranes			
Normal		5	5
Lacrimation			
Normal		5	5
Piloerection			
Absent		5	5
Pupil size			
Normal		5	5
Salivation			
None		5	5
Abnormal respiration			
Absent		5	5
Vocalization			
None		5	5
Reactivity to handling			
Easy		5	5

Table 2-59 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	1	2	1
Normal		12	11	10	11
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		3± 2	3± 2	3± 2	3± 2
Defecation count (Mean±S.D.)		1± 1	0± 0	0± 0	1± 2
Urination					
None		12	9	11	10
Small amount		0	3	1	2

No significant difference in any treated groups from control group.

Table 2-60 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	0	1	0
Normal		12	12	11	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		3± 2	3± 2	3± 2	4± 2
Defecation count (Mean±S.D.)		0± 0	0± 1	0± 1	0± 1
Urination					
None		12	10	12	11
Small amount		0	2	0	1

No significant difference in any treated groups from control group.

Table 2-61 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	2	2	0
Normal		12	10	10	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		3± 2	3± 3	3± 2	3± 2
Defecation count (Mean±S.D.)		0± 0	0± 1	0± 0	0± 0
Urination					
None		10	7	11	11
Small amount		2	3	1	1
Moderate amount		0	2	0	0

No significant difference in any treated groups from control group.

Table 2-62 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 4 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	1	0	0
Normal		12	11	12	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		5± 2	4± 2	4± 2	4± 1
Defecation count (Mean±S.D.)		0± 0	0± 1	0± 0	0± 0
Urination					
None		10	7	11	11
Small amount		2	4	1	1
Moderate amount		0	1	0	0

No significant difference in any treated groups from control group.

Table 2-63 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	1	0	0
Normal		12	11	12	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		4± 2	4± 2	5± 2	4± 1
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		11	7	11	12
Small amount		1	5	1	0

No significant difference in any treated groups from control group.

Table 2-64 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Main group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
No/minimal location		0	0	0	1
Normal		12	12	12	11
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		5± 2	4± 2	5± 2	4± 2
Defecation count (Mean±S.D.)		0± 0	0± 1	0± 0	0± 0
Urination					
None		10	7	9	12
Small amount		2	4	3	0
Moderate amount		0	1	0	0

No significant difference in any treated groups from control group.

Table 2-65 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
Normal		12	12	12	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		6± 2	5± 2	6± 3	6± 2
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	11	12
Small amount		0	0	1	0

No significant difference in any treated groups from control group.

Table 2-66 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 12
Arousal					
Normal		12	12	12	12
Convulsion					
None		12	12	12	12
Abnormal behavior					
None		12	12	12	12
Stereotypy					
None		12	12	12	12
Gait					
Normal		12	12	12	12
Posture					
Normal		12	12	12	12
Grooming					
None		12	12	12	12
Rearing (Mean±S.D.)		8± 3	7± 2	7± 2	7± 2
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	12

No significant difference in any treated groups from control group.

Table 2-67 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Day 1 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 11
Arousal					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11
Stereotypy					
None		12	12	12	11
Gait					
Normal		12	12	12	11
Posture					
Normal		12	12	12	11
Grooming					
None		12	12	12	11
Rearing (Mean±S.D.)		7± 2	6± 1	7± 2	7± 3
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	11

No significant difference in any treated groups from control group.

Table 2-68 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Day 7 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 / 12	100 / 12	300 / 12	1000 / 11
Arousal					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11
Stereotypy					
None		12	12	12	11
Gait					
Normal		12	12	12	11
Posture					
Normal		12	12	12	11
Grooming					
None		12	12	12	11
Rearing (Mean±S.D.)		6± 2	7± 2	8± 2	6± 2
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	10
Small amount		0	0	0	1

No significant difference in any treated groups from control group.

Table 2-69 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Day 14 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Arousal					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11
Stereotypy					
None		12	12	12	11
Gait					
Normal		12	12	12	11
Posture					
Normal		12	12	12	11
Grooming					
None		12	12	12	11
Rearing (Mean±S.D.)		5± 1	6± 2	5± 2	4± 1
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	11

No significant difference in any treated groups from control group.

R-944

Table 2-70 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Day 20 of gestation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 11
Arousal					
Normal		12	12	12	11
Convulsion					
None		12	12	12	11
Abnormal behavior					
None		12	12	12	11
Stereotypy					
None		12	12	12	11
Gait					
Normal		12	12	12	11
Posture					
Normal		12	12	12	11
Grooming					
None		12	12	12	11
Rearing (Mean±S.D.)		6± 2	5± 2	5± 1	4± 2*D
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	11

* : p<0.05 (Significant difference from control group)
D : Dunnett's test

Table 2-71 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Main group, Day 4 of lactation)

Parameter	Dose (mg/kg) / No. of animals	0 12	100 12	300 12	1000 10
Arousal					
Normal		12	12	12	10
Convulsion					
None		12	12	12	10
Abnormal behavior					
None		12	12	12	10
Stereotypy					
None		12	12	12	10
Gait					
Normal		12	12	12	10
Posture					
Normal		12	12	12	10
Grooming					
None		12	12	12	10
Rearing (Mean±S.D.)		7± 2	7± 1	6± 2	5± 2
Defecation count (Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Urination					
None		12	12	12	10

No significant difference in any treated groups from control group.

Table 2-72 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
No/minimal location		0	2
Normal		5	3
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		3± 1	2± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	4
Small amount		0	1

No significant difference between treated group and control group.

Table 2-73 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		5± 2	3± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	4
Small amount		0	1

No significant difference between treated group and control group.

Table 2-74 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		3± 1	3± 2
Defecation count (Mean±S.D.)		0± 0	0± 1
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-75 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 4 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		4± 0	4± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		4	4
Small amount		1	1

No significant difference between treated group and control group.

Table 2-76 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		3± 2	2± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		3	5
Small amount		1	0
Moderate amount		1	0

No significant difference between treated group and control group.

Table 2-77 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		5± 2	4± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		3	3
Small amount		1	2
Moderate amount		1	0

No significant difference between treated group and control group.

Table 2-78 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 1 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		3± 1	4± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	4
Small amount		0	1

No significant difference between treated group and control group.

Table 2-79 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in male rats: open field observation (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		4± 2	4± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		3	3
Small amount		0	1
Moderate amount		2	1

No significant difference between treated group and control group.

R-944

Table 2-80 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 1 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		6± 3	6± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-81 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 2 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		7± 2	7± 3
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-82 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 3 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		8± 3	7± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-83 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 4 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		10± 2	8± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-84 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 5 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		9± 2	8± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-85 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
	Normal	5	5
Convulsion			
	None	5	5
Abnormal behavior			
	None	5	5
Stereotypy			
	None	5	5
Gait			
	Normal	5	5
Posture			
	Normal	5	5
Grooming			
	None	5	5
Rearing (Mean±S.D.)		8± 2	7± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
	None	5	5

No significant difference between treated group and control group.

Table 2-86 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 1 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 / 5	1000 / 5
Arousal			
	Normal	5	5
Convulsion			
	None	5	5
Abnormal behavior			
	None	5	5
Stereotypy			
	None	5	5
Gait			
	Normal	5	5
Posture			
	Normal	5	5
Grooming			
	None	5	5
Rearing (Mean±S.D.)		9± 3	7± 2
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
	None	5	5

No significant difference between treated group and control group.

Table 2-87 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Detailed clinical signs in female rats: open field observation (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Arousal			
Normal		5	5
Convulsion			
None		5	5
Abnormal behavior			
None		5	5
Stereotypy			
None		5	5
Gait			
Normal		5	5
Posture			
Normal		5	5
Grooming			
None		5	5
Rearing (Mean±S.D.)		7± 2	7± 1
Defecation count (Mean±S.D.)		0± 0	0± 0
Urination			
None		5	5

No significant difference between treated group and control group.

Table 2-88 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of male rats (Main group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	100 5	300 5	1000 5
Auditory response					
Normal		5	5	5	5
Approach response					
Normal		5	5	5	5
Touch response					
Normal		5	5	5	5
Tail pinch response					
Normal		5	5	5	5
Pupillary reflex					
Pass, both		5	5	5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		83±16	87±13	73±18	76±14

No significant difference in any treated groups from control group.

Table 2-89 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of female rats (Main group, Day 4 of lactation)

Parameter	Dose (mg/kg) / No. of animals	0 5	100 5	300 5	1000 5
Auditory response Normal		5	5	5	5
Approach response Normal		5	5	5	5
Touch response Normal		5	5	5	5
Tail pinch response Normal		5	5	5	5
Pupillary reflex Pass, both		5	5	5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0	0± 0	0± 0
Landing foot splay (mm; Mean±S.D.)		56±11	58±18	66±18	83±11*D

* : p<0.05 (Significant difference from control group)
D : Dunnett's test

Table 2-90 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of male rats (Recovery group, Week 6 of administration)

Parameter	Dose (mg/kg) / No. of animals	0 5	1000 5
Auditory response Normal		5	5
Approach response Normal		5	5
Touch response Normal		5	5
Tail pinch response Normal		5	5
Pupillary reflex Pass, both		5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		82±20	80±17

No significant difference between treated group and control group.

Table 2-91 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of female rats (Recovery group, Week 6 of administration)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Auditory response			
Normal		5	5
Approach response			
Normal		5	5
Touch response			
Normal		5	5
Tail pinch response			
Normal		5	5
Pupillary reflex			
Pass, both		5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		60±19	72±13

No significant difference between treated group and control group.

R-944

Table 2-92 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of male rats (Recovery group, Week 2 of recovery)

	Dose (mg/kg)	0	1000
Parameter	No. of animals	5	5
Auditory response			
Normal		5	5
Approach response			
Normal		5	5
Touch response			
Normal		5	5
Tail pinch response			
Normal		5	5
Pupillary reflex			
Pass, both		5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		90±13	83±12

No significant difference between treated group and control group.

Table 2-93 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Manipulative test of female rats (Recovery group, Week 2 of recovery)

Parameter	Dose (mg/kg)	0	1000
	No. of animals	5	5
Auditory response			
Normal		5	5
Approach response			
Normal		5	5
Touch response			
Normal		5	5
Tail pinch response			
Normal		5	5
Pupillary reflex			
Pass, both		5	5
Aerial righting reflex (Total score: Mean±S.D.)		0± 0	0± 0
Landing foot splay (mm: Mean±S.D.)		70±14	61± 4

No significant difference between treated group and control group.

Table 2-94 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of male rats (Main group, Week 6 of administration)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1601	894
	S.D.	115	77
100	No.	5	5
	Mean	1579	812
	S.D.	176	50
300	No.	5	5
	Mean	1563	823
	S.D.	172	122
1000	No.	5	5
	Mean	1359	707*
	S.D.	130	96D

* : p<0.05 (Significant difference from control group)
D: Dunnett's test

Table 2-95 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of female rats (Main group, Day 4 of lactation)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1288	822
	S.D.	166	108
100	No.	5	5
	Mean	1335	754
	S.D.	193	113
300	No.	5	5
	Mean	1133	682
	S.D.	90	97
1000	No.	5	5
	Mean	1145	654
	S.D.	202	125

No significant difference in any treated groups from control group.

Table 2-96 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of male rats (Recovery group, Week 6 of administration)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1624	902
	S.D.	139	70
1000	No.	5	5
	Mean	1549	914
	S.D.	173	141

No significant difference between treated group and control group.

Table 2-97 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of female rats (Recovery group. Week 6 of administration)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1096	635
	S.D.	96	226
1000	No.	5	5
	Mean	999	675
	S.D.	152	157

No significant difference between treated group and control group.

R-944

Table 2-98 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of male rats (Recovery group. Week 2 of recovery)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1490	948
	S.D.	202	51
1000	No.	5	5
	Mean	1621	1010
	S.D.	158	125

No significant difference between treated group and control group.

Table 2-99 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Grip strength of female rats (Recovery group, Week 2 of recovery)

Dose mg/kg		Fore limb g	Hind limb g
0	No.	5	5
	Mean	1054	791
	S.D.	157	85
1000	No.	5	5
	Mean	1110	704
	S.D.	217	164

No significant difference between treated group and control group.

R-944

Table 2-100 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of male rats (Main group, Week 6 of administration)

Dose mg/kg		Interval (minutes)						
		0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	390	185	77	118	100	63	934
	S.D.	40	69	51	67	169	111	416
100	No.	5	5	5	5	5	5	5
	Mean	362	236	169	171	111	87	1135
	S.D.	56	111	139	89	87	88	294
300	No.	5	5	5	5	5	5	5
	Mean	327	256	110	83	58	44	878
	S.D.	114	101	91	52	44	47	247
1000	No.	5	5	5	5	5	5	5
	Mean	343	276	146	126	59	26	977
	S.D.	45	58	57	62	51	12	138

No significant difference in any treated groups from control group.

Table 2-101 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of female rats (Main group, Day 4 of lactation)

Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	176	59	30	13	37	39	354
	S.D.	109	104	45	16	67	52	362
100	No.	5	5	5	5	5	5	5
	Mean	168	52	22	13	54	44	353
	S.D.	60	63	17	9	41	88	118
300	No.	5	5	5	5	5	5	5
	Mean	261	112	124*	103*	114	20	733
	S.D.	75	84	79D	61DT	56	11	267
1000	No.	5	5	5	5	5	5	5
	Mean	108	17	38	17	11	39	230
	S.D.	70	12	39	10	14	45	102

* : $p<0.05$ (Significant difference from control group)
D: Dunnett's test
DT: Dunnett-type rank test

R-944

Table 2-102 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of male rats (Recovery group, Week 6 of administration)

Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	265	208	64	35	48	50	670
	S.D.	171	148	65	22	71	39	334
1000	No.	5	5	5	5	5	5	5
	Mean	386	254	93	26	75	34	867
	S.D.	42	93	74	27	100	27	149

No significant difference between treated group and control group.

Table 2-103 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of female rats (Recovery group, Week 6 of administration)

Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	365	271	62	35	51	149	932
	S.D.	57	87	74	42	79	151	208
1000	No.	5	5	5	5	5	5	5
	Mean	363	273	222*	245*	227**	232	1562**
	S.D.	53	133	88T	127AT	71T	132	343T

* : p<0.05 ; ** : p<0.01 (Significant difference from control group)
T: Student's t-test
AT: Aspin-Welch t-test

Table 2-104 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of male rats (Recovery group, Week 2 of recovery)

Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	311	217	224	123	127	110	1112
	S.D.	87	96	88	92	99	87	223
1000	No.	5	5	5	5	5	5	5
	Mean	305	283	265	205	146	144	1349
	S.D.	89	78	98	170	162	141	639

No significant difference between treated group and control group.

Table 2-105 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Motor activity of female rats (Recovery group, Week 2 of recovery)

Dose mg/kg		Interval (minutes) 0-10	10-20	20-30	30-40	40-50	50-60	Total(0-60)
0	No.	5	5	5	5	5	5	5
	Mean	265	228	168	183	126	107	1057
	S.D.	91	117	128	123	122	129	513
1000	No.	5	5	5	5	5	5	5
	Mean	271	210	177	185	165	86	1092
	S.D.	54	65	56	53	133	85	336

No significant difference between treated group and control group.

Table 3-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of male rats (Main group)

Dose mg/kg		Pre-mating period 1	4	8	11	15	Mating period 18	22	25	29	Post-mating period 32	36	39	42a)	Gain 1-42
0	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	361.8	374.8	390.4	405.5	416.1	424.8	439.4	449.8	461.9	474.4	486.6	496.6	502.9	141.1
	S.D.	15.9	17.4	20.8	23.1	27.2	28.2	29.6	30.2	31.6	32.7	31.0	33.1	33.9	23.3
100	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	360.9	375.6	390.3	402.3	414.3	419.3	433.4	442.1	457.3	468.3	481.5	490.8	495.3	134.4
	S.D.	12.6	13.9	17.5	20.0	23.1	24.3	25.6	27.0	28.7	31.0	33.2	32.5	33.1	26.4
300	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	359.6	371.0	385.1	398.5	411.3	417.8	431.8	444.8	457.1	465.6	480.3	487.8	493.3	133.7
	S.D.	15.1	16.4	18.8	19.5	19.8	22.7	23.6	21.5	23.5	25.9	26.9	27.3	27.0	19.2
1000	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	360.8	382.4	371.0*	383.2*	389.3*	392.6*	406.8*	415.1**	424.7**	432.3**	441.5**	442.7**	447.5**	86.7**
	S.D.	14.8	17.3	20.0D	23.4D	26.0D	28.6D	28.3D	28.0D	29.0D	30.7D	33.4D	34.9D	35.0D	26.1D

Unit: g
No.: No. of animals
a): Day of administration
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 3-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of female rats during the pre-mating period (Main group)

Dose mg/kg		Administration 1	4	8	11	15a)	Gain 1-15
0	No.	12	12	12	12	12	12
	Mean	224.3	233.2	240.9	246.6	252.8	28.5
	S.D.	8.9	9.6	9.6	10.8	11.1	6.9
100	No.	12	12	12	12	12	12
	Mean	225.6	233.7	244.8	250.7	257.3	31.8
	S.D.	7.3	7.0	9.5	11.7	12.7	7.4
300	No.	12	12	12	12	12	12
	Mean	223.7	230.5	236.2	239.8	244.4	20.8
	S.D.	12.3	11.3	12.7	14.0	15.6	9.1
1000	No.	12	12	12	12	12	12
	Mean	223.8	224.1*	224.1**	223.7**	240.2	16.4**
	S.D.	9.8	7.6D	14.5D	17.5D	12.4	8.7D

Unit: g
No.: No. of animals
a): Day of administration
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 3-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of dams during the gestation period (Main group)

Dose mg/kg		Administration 0	4	7	11	14	17	20a)	Gain 0-20
0	No.	12	12	12	12	12	12	12	12
	Mean	254.8	277.9	290.8	314.3	329.0	360.6	407.3	152.5
	S.D.	10.9	13.8	17.6	20.1	24.0	23.0	24.8	19.8
100	No.	12	12	12	12	12	12	12	12
	Mean	259.3	278.7	291.2	313.7	329.8	361.0	408.2	148.9
	S.D.	13.8	14.2	15.3	17.2	17.3	19.1	22.3	16.2
300	No.	12	12	12	12	12	12	12	12
	Mean	251.7	273.2	281.1	304.0	318.4	348.6	396.6	144.9
	S.D.	18.2	16.3	18.7	18.0	19.5	23.0	26.3	11.0
1000	No.	11	11	11	11	11	11	11	11
	Mean	236.1*	254.2**	265.7**	280.8**	289.6**	317.6**	351.5**	115.4**
	S.D.	14.8D	20.7D	18.2D	21.1D	27.2D	29.3D	33.9D	23.5D

Unit: g
No.: No. of dams
a): Day of gestation
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 3-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of dams during the lactation period (Main group)

Dose mg/kg		Administration 0	Administration 4a)	Gain 0-4
0	No.	12	12	12
	Mean	309.9	328.8	18.8
	S.D.	31.8	26.0	19.3
100	No.	12	12	12
	Mean	316.4	325.3	8.9
	S.D.	21.3	20.9	13.1
300	No.	12	12	12
	Mean	300.2	299.8*	-0.4
	S.D.	27.6	28.1D	22.4
1000	No.	11	10[b)]	10
	Mean	268.8**	274.4**	3.2
	S.D.	33.0D	35.3D	26.7

Unit: g
No.: No. of dams
a): Day of lactation
b): One dam died on day 0 of lactation.
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 3-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of male rats during the administration period (Recovery group)

Dose mg/kg		Day of administration 1	4	8	11	15	18	22	25	29	32	36	39	42	Gain 1-42
0	No.	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	355.0	363.6	377.2	390.8	400.8	408.6	422.8	430.8	444.2	454.8	469.0	479.6	476.4	121.4
	S.D.	13.5	17.9	20.1	26.0	26.8	29.8	32.6	37.9	38.0	39.1	40.0	40.3	45.9	38.2
1000	No.	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	357.4	356.4	363.6	377.4	386.2	393.0	405.0	414.4	422.2	430.0	438.0	442.8	447.8	90.4
	S.D.	17.9	18.9	17.7	24.2	25.7	28.6	29.6	28.3	29.8	28.1	32.2	35.9	31.6	16.6

Unit: g
No.: No. of animals
No significant difference between treated group and control group.

Table 3-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of female rats during the administration period (Recovery group)

Dose mg/kg		Day of administration 1	4	8	11	15	18	22	25	29	32	36	39	42	Gain 1-42
0	No.	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	218.8	230.6	239.2	241.8	248.0	248.4	250.2	259.0	263.6	266.8	272.0	274.4	270.4	51.6
	S.D.	8.3	9.0	10.4	13.5	19.0	20.1	18.9	25.6	23.4	18.3	21.7	22.0	21.1	17.9
1000	No.	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	224.6	226.2	229.8	231.8	234.6	240.4	246.0	249.8	253.8	253.4	259.2	257.4	258.2	33.6
	S.D.	11.4	15.7	14.4	16.9	17.7	12.8	17.9	19.6	16.2	24.1	20.8	19.7	21.8	12.7

Unit: g
No.: No. of animals
No significant difference between treated group and control group.

R-944

Table 3-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of male rats during the recovery period (Recovery group)

Dose mg/kg		Day of recovery 1	4	8	11	14	Gain 1-14
0	No.	5	5	5	5	5	5
	Mean	482.2	491.4	496.8	501.4	504.8	22.6
	S.D.	42.5	44.1	44.3	44.5	48.4	7.2
1000	No.	5	5	5	5	5	5
	Mean	450.0	456.4	471.8	486.0	486.6	36.6**
	S.D.	32.6	30.1	35.5	29.7	32.2	5.9T

Unit: g
No.: No. of animals
**: $p<0.01$ (Significant difference from control group)
T: Student's t-test

Table 3-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of female rats during the recovery period (Recovery group)

Dose mg/kg		Day of recovery 1	4	8	11	14	Gain 1-14
0	No.	5	5	5	5	5	5
	Mean	278.6	275.2	277.0	282.2	279.4	0.8
	S.D.	24.1	21.5	25.4	25.4	23.6	6.7
1000	No.	5	5	5	5	5	5
	Mean	259.4	261.0	268.2	275.8	272.4	13.0*
	S.D.	25.0	19.6	20.9	23.1	27.9	8.6T

Unit: g
No.: No. of animals
*: p<0.05 (Significant difference from control group)
T: Student's t-test

Table 4-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of male rats (Main group)

Dose mg/kg		Pre-mating period 1	4	8	11	15	Post-mating period 32	36	39	42a)
0	No.	12	12	12	12	12	12	12	12	12
	Mean	26.8	24.9	25.0	21.5	24.1	23.9	23.6	22.7	25.3
	S.D.	2.8	1.9	2.9	2.5	2.7	2.2	1.7	2.8	2.0
100	No.	12	12	12	12	12	12	12	12	12
	Mean	25.3	24.2	22.5	21.2	23.6	22.5	22.2	22.4	22.9
	S.D.	2.1	2.7	2.8	2.7	2.4	4.1	2.0	2.4	2.6
300	No.	12	12	12	12	12	12	12	12	12
	Mean	25.7	22.3	23.8	20.8	25.1	23.2	23.9	21.9	25.4
	S.D.	4.1	2.7	2.6	2.7	2.5	4.5	3.7	3.7	2.2
1000	No.	12	12	12	12	12	12	12	12	12
	Mean	26.1	16.8**	24.1	21.6	26.5	24.7	23.7	22.1	26.6
	S.D.	3.2	5.4DT	3.7	2.8	4.3	4.5	4.3	4.1	4.4

Unit: g/rat/day
No.: No. of animals
a): Day of administration
**: p<0.01 (Significant difference from control group)
DT: Dunnett-type rank test

Table 4-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of female rats during the pre-mating period (Main group)

Dose mg/kg		Administration 1	4	8	11	15a)
0	No.	12	12	12	12	12
	Mean	17.7	18.8	18.6	14.3	19.4
	S.D.	3.1	2.2	1.6	2.4	3.1
100	No.	12	12	12	12	12
	Mean	18.8	17.9	18.0	15.0	19.4
	S.D.	2.4	1.5	2.3	1.4	2.7
300	No.	12	12	12	12	12
	Mean	17.3	16.5*	18.5	14.7	18.8
	S.D.	2.1	1.8D	2.7	3.6	3.1
1000	No.	12	12	12	12	12
	Mean	18.5	10.8**	14.7	12.4	24.9*
	S.D.	3.1	3.0D	7.9	5.4	7.8DT

Unit: g/rat/day
No.: No. of animals
a): Day of administration
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test
DT: Dunnett-type rank test

Table 4-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of dams during the gestation period (Main group)

Dose mg/kg		Administration 1	4	7	11	14	17	20a)
0	No.	12	12	12	12	12	12	12
	Mean	17.4	20.1	21.1	23.2	22.7	24.7	21.2
	S.D.	2.2	3.0	3.3	3.3	3.1	4.1	3.6
100	No.	12	12	12	12	12	12	12
	Mean	17.8	20.8	21.2	21.9	22.4	24.8	20.1
	S.D.	2.6	3.1	2.3	2.4	3.3	2.4	2.5
300	No.	12	12	12	12	12	12	12
	Mean	17.2	22.1	20.4	22.2	21.9	25.5	19.9
	S.D.	2.6	3.4	3.7	3.0	3.3	3.6	3.8
1000	No.	11	11	11	11	11	11	11
	Mean	18.2	22.1	20.5	18.8	19.0	23.8	14.0**
	S.D.	4.9	4.7	4.9	6.5	7.7	4.5	4.8D

Unit: g/rat/day
No.: No. of dams
a): Day of gestation
**: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 4-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of dams during the lactation period (Main group)

Dose mg/kg		Administration 2	4a)
0	No.	12	12
	Mean	22.7	37.3
	S.D.	6.5	8.1
100	No.	12	12
	Mean	19.2	38.2
	S.D.	3.9	6.7
300	No.	12	12
	Mean	13.5**	35.0
	S.D.	6.2D	11.7
1000	No.	10[b)]	10
	Mean	12.7**	31.4
	S.D.	8.8D	12.6

Unit: g/rat/day
No.: No. of dams
a): Day of lactation
b): One dam died on day 0 of lactation.
**: p<0.01 (Significant difference from control group)
D: Dunnett's test

Table 4-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of male rats during the administration period (Recovery group)

Dose mg/kg		Day of administration 1	4	8	11	15	32	36	39	42
0	No.	5	5	5	5	5	5	5	5	5
	Mean	24.0	22.4	24.0	19.8	25.0	22.6	23.0	23.6	22.0
	S.D.	3.2	2.6	2.7	0.8	2.3	3.1	2.1	1.9	2.8
1000	No.	5	5	5	5	5	5	5	5	5
	Mean	25.6	14.0**	23.4	22.0	25.0	22.4	23.4	24.2	25.8*
	S.D.	4.6	3.7T	6.1	3.7	2.8	2.2	2.5	4.6	1.8T

Unit: g/rat/day
No.: No. of animals
*: p<0.05; **: p<0.01 (Significant difference from control group)
T: Student's t-test

Table 4-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of female rats during the administration period (Recovery group)

Dose mg/kg		Day of administration 1	4	8	11	15	32	36	39	42
0	No.	5	5	5	5	5	5	5	5	5
	Mean	15.8	18.2	19.4	14.6	18.4	16.6	15.8	17.4	11.4
	S.D.	2.8	0.4	1.9	3.8	2.8	3.1	1.9	2.8	2.7
1000	No.	5	5	5	5	5	5	5	5	5
	Mean	19.0	12.0*	19.4	10.2	19.6	16.4	16.0	16.4	16.0
	S.D.	3.5	4.4AT	3.6	3.4	3.8	8.4	3.2	1.8	4.6

Unit: g/rat/day
No.: No. of animals
*: $p<0.05$ (Significant difference from control group)
AT: Aspin-Welch t-test

R-944

Table 4-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of male rats during the recovery period (Recovery group)

Dose mg/kg		Day of recovery 1	4	8	11	14
0	No.	5	5	5	5	5
	Mean	22.6	27.0	25.6	27.6	26.4
	S.D.	2.1	2.8	1.9	1.9	3.4
1000	No.	5	5	5	5	5
	Mean	22.2	30.6	32.0*	33.0*	27.8
	S.D.	5.2	3.2	4.2T	3.1T	2.8

Unit: g/rat/day
No.: No. of animals
*: $p<0.05$ (Significant difference from control group)
T: Student's t-test

Table 4-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Food consumption of female rats during the recovery period (Recovery group)

Dose mg/kg		Day of recovery 1	4	8	11	14
0	No.	5	5	5	5	5
	Mean	15.4	14.4	18.2	21.0	17.6
	S.D.	2.7	1.3	2.4	2.1	0.9
1000	No.	5	5	5	5	5
	Mean	15.8	23.0**	19.8	23.2	18.8
	S.D.	4.0	3.1T	2.2	1.8	5.4

Unit: g/rat/day
No.: No. of animals
**: p<0.01 (Significant difference from control group)
T: Student's t-test

R-944

Table 5-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2.3.4.4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 6 of administration)

Dose mg/kg	No.	pH 5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	1) Protein -	+-	+	++	+++	++++	2) Ketone body -	+-	+	++	+++	++++	3) Glucose -	+-	+	++	+++	++++
0	17	0	0	0	1	0	1	4	10	1	3	6	8	0	0	0	5	5	7	0	0	0	17	0	0	0	0	0
100	12	0	0	0	0	1	1	4	6	0	0	1	11	0	0	0	0	3	9	0	0	0	12	0	0	0	0	0
300	12	0	0	0	0	2	3	4	3	0	3	4	5	0	0	0	2	3	7	0	0	0	12	0	0	0	0	0
1000	17	0	0	0	3	5	7	2	0	0	4	9	4	0	0	0	3	7	7	0	0	0	17	0	0	0	0	0

1) - : <10 mg/dL +- : 10 - 25 mg/dL + : 26 - 85 mg/dL ++ : 86 - 250 mg/dL +++ : 251 - 600 mg/dL ++++ : >600 mg/dL
2) - : <5 mg/dL +- : 5 - 7.5 mg/dL + : 7.6 - 30 mg/dL ++ : 31 - 70 mg/dL +++ : 71 - 125 mg/dL ++++ : >125 mg/dL
3) - : <30 mg/dL +- : 30 - 60 mg/dL + : 61 - 125 mg/dL ++ : 126 - 250 mg/dL +++ : 251 - 750 mg/dL ++++ : >750 mg/dL

Table 5-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 6 of administration)

Dose mg/kg	No.	4) Occult blood					5) Bilirubin					6) Urobilinogen					7) Color		
		-	+-	+	++	+++	-	+	++	+++	++++	+-	+	++	+++	++++	LY	Y	DY
0	17	16	1	0	0	0	16	1	0	0	0	15	2	0	0	0	0	17	0
100	12	0	0	5	7	0	11	1	0	0	0	10	2	0	0	0	0	10	2
300	12	0	0	5	6	1	12	0	0	0	0	11	1	0	0	0	0	9	3
1000	17	0	0	0	11	6	16	1	0	0	0	14	3	0	0	0	0	14	3

4) - : <0.03 mg/dL +- : 0.03 - 0.05 mg/dL + : 0.06 - 0.15 mg/dL ++ : 0.16 - 0.75 mg/dL +++ : >0.75 mg/dL
5) - : <0.5 mg/dL + : 0.5 - 1.5 mg/dL ++ : 1.6 - 5.0 mg/dL +++ : 5.1 - 10.0 mg/dL ++++ : >10.0 mg/dL
6) +- : <2.0 mg/dL + : 2.0 - 3.5 mg/dL ++ : 3.6 - 7.0 mg/dL +++ : 7.1 - 12.0 mg/dL ++++ : >12.0 mg/dL
7) LY : Light yellow Y : Yellow DY : Dark yellow

Table 5-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 6 of administration)

Dose mg/kg	No.	URINE SEDIMENT																														
		RBC					WBC					SEC					SREC					Cast			CRYSTALLIZATION PS					CRYSTALLIZATION CO				
		-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	-	+-	+	++	+++	-	+-	+	++	+++
0	17	15	2	0	0	0	16	1	0	0	0	0	16	1	0	0	17	0	0	0	0	17	0	0	8	9	0	0	0	17	0	0	0	0
100	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	12	0	0	0	0	12	0	0	4	8	0	0	0	12	0	0	0	0
300	12	12	0	0	0	0	11	1	0	0	0	0	12	0	0	0	11	1	0	0	0	12	0	0	4	7	1	0	0	12	0	0	0	0
1000	17	17	0	0	0	0	17	0	0	0	0	0	17	0	0	0	17	0	0	0	0	17	0	0	7	10	0	0	0	17	0	0	0	0

SEC : Squamous Epithelial Cell
SREC : Small Round Epithelial Cell
PS : Phosphate Salts
CO : Calcium Oxalate

- : Negative
+- : Slight
+ : Mild
++ : Moderate
+++ : Severe

Table 5-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Water intake and urinalysis (Week 6 of administration)

Male

Dose mg/kg	No.		Water intake mL/24h	Urine volume mL/24h	Osmolality mOsm/kg
0	17	Mean	41	14.7	1791
		S.D.	11	5.5	433
100	12	Mean	40	11.0	1927
		S.D.	7	3.2	330
300	12	Mean	49	14.3	1783
		S.D.	11	6.1	451
1000	17	Mean	51*	14.0	1524
		S.D.	12D	4.4	425

* : p<0.05 (Significant difference from control group)
D : Dunnett's test

R-944

Table 5-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 2 of recovery)

Dose mg/kg	No.	pH									1) Protein						2) Ketone body						3) Glucose					
		5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++	-	+-	+	++	+++	++++
0	5	0	0	0	0	0	2	2	1	0	0	1	4	0	0	0	0	2	3	0	0	0	5	0	0	0	0	0
1000	5	0	0	0	0	0	1	3	1	0	2	2	1	0	0	0	4	1	0	0	0	0	5	0	0	0	0	0

1) - : <10 mg/dL +- : 10 - 25 mg/dL + : 26 - 85 mg/dL ++ : 86 - 250 mg/dL +++ : 251 - 600 mg/dL ++++ : >600 mg/dL
2) - : <5 mg/dL +- : 5 - 7.5 mg/dL + : 7.6 - 30 mg/dL ++ : 31 - 70 mg/dL +++ : 71 - 125 mg/dL ++++ : >125 mg/dL
3) - : <30 mg/dL +- : 30 - 60 mg/dL + : 61 - 125 mg/dL ++ : 126 - 250 mg/dL +++ : 251 - 750 mg/dL ++++ : >750 mg/dL

Table 5-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 2 of recovery)

Dose mg/kg	No.	4) Occult blood					5) Bilirubin					6) Urobilinogen					7) Color		
		-	+-	+	++	+++	-	+	++	+++	++++	+-	+	++	+++	++++	LY	Y	DY
0	5	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	5	0
1000	5	5	0	0	0	0	5	0	0	0	0	4	1	0	0	0	0	5	0

4) - : <0.03 mg/dL +- : 0.03 - 0.05 mg/dL + : 0.06 - 0.15 mg/dL ++ : 0.16 - 0.75 mg/dL +++ : >0.75 mg/dL
5) - : <0.5 mg/dL + : 0.5 - 1.5 mg/dL ++ : 1.6 - 5.0 mg/dL +++ : 5.1 - 10.0 mg/dL ++++ : >10.0 mg/dL
6) +- : <2.0 mg/dL + : 2.0 - 3.5 mg/dL ++ : 3.6 - 7.0 mg/dL +++ : 7.1 - 12.0 mg/dL ++++ : >12.0 mg/dL
7) LY : Light yellow Y : Yellow DY : Dark yellow

R-944

Table 5-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Urinalysis of male rats (Week 2 of recovery)

Dose mg/kg	No.	URINE SEDIMENT																											
		RBC					WBC					SEC					SREC					Cast			CRYSTALLIZATION PS					CRYSTALLIZATION CO				
		-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	++	+++	-	+-	+	-	+-	+	++	+++	-	+-	+	++	+++
0	5	5	0	0	0	0	5	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5	0	0	1	3	1	0	0	5	0	0	0	0
1000	5	5	0	0	0	0	5	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5	0	0	2	3	0	0	0	5	0	0	0	0

SEC : Squamous Epithelial Cell
SREC : Small Round Epithelial Cell
PS : Phosphate Salts
CO : Calcium Oxalate

- : Negative
+- : Slight
+ : Mild
++ : Moderate
+++ : Severe

Table 5-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Water intake and urinalysis (Week 2 of recovery)

Male

Dose mg/kg	No.		Water intake mL/24h	Urine volume mL/24h	Osmolality mOsm/kg
0	5	Mean	41	16.6	1895
		S.D.	10	3.9	472
1000	5	Mean	54	21.4	1760
		S.D.	16	9.3	331

No significant difference between treated group and control group.

Table 6-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Week 6 of administration)

Male

Dose mg/kg	No.		RBC X10⁴/μL	Hb g/dL	Ht %	MCV fL	MCH pg	MCHC g/dL	Reticulocyte %	Platelet X10⁴/μL	PT s	APTT s	Fibrinogen mg/dL
0	5	Mean	890	15.8	42.5	47.8	17.8	37.2	1.7	112.2	15.6	19.7	257
		S.D.	39	0.3	0.7	2.7	1.1	0.6	0.3	15.6	0.9	1.4	22
100	5	Mean	851	15.7	42.7	50.2	18.4	36.7	1.7	100.1	16.1	18.3	270
		S.D.	31	0.7	1.9	2.4	0.8	0.2	0.3	8.3	0.9	1.0	9
300	5	Mean	842*	15.8	42.8	50.8	18.8	36.9	1.8	114.3	15.4	18.0	291**
		S.D.	18D	0.6	1.6	1.4	0.4	0.3	0.2	12.1	0.4	0.6	8D
1000	5	Mean	793**	14.3**	39.5*	49.7	18.1	36.3*	1.9	134.7*	15.8	19.6	241
		S.D.	26D	0.6D	2.2D	1.5	0.6	0.7D	0.3	14.5D	0.8	1.5	14

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
D : Dunnett's test

Table 6-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Week 6 of administration)

Male

Dose mg/kg	No.		WBC	Differential leukocyte counts (%)						Differential leukocyte counts ($X10^2/\mu L$)					
			$X10^2/\mu L$	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC
0	5	Mean	89.7	76.5	19.2	1.3	0.3	2.0	0.7	68.9	17.1	1.1	0.3	1.7	0.7
		S.D.	10.4	4.4	4.1	0.5	0.1	0.5	0.4	11.2	3.2	0.3	0.2	0.3	0.4
100	5	Mean	91.7	79.1	16.7	1.6	0.4	1.7	0.5	72.9	14.9	1.5	0.3	1.6	0.5
		S.D.	26.9	3.7	3.4	0.3	0.1	0.6	0.3	23.4	3.2	0.5	0.2	0.7	0.3
300	5	Mean	101.2	74.4	22.1	1.0	0.3	1.6	0.5	75.5	22.2	1.1	0.3	1.7	0.5
		S.D.	19.9	4.5	4.5	0.4	0.1	0.4	0.1	16.3	5.7	0.5	0.1	0.7	0.1
1000	5	Mean	134.7	73.5	22.4	1.0	0.3	2.3	0.5	100.2	29.4**	1.3	0.4	2.8*	0.6
		S.D.	47.7	4.5	3.7	0.4	0.1	0.7	0.2	41.1	8.3D	0.3	0.2	0.4D	0.3

LUC : Large unstained cells
* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
D : Dunnett's test

Table 6-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 4 of lactation)

Female

Dose mg/kg	No.		RBC	Hb	Ht	MCV	MCH	MCHC	Reticulocyte	Platelet	PT	APTT	Fibrinogen
			$X10^4/\mu L$	g/dL	%	fL	pg	g/dL	%	$X10^4/\mu L$	s	s	mg/dL
0	5	Mean	682	13.0	36.5	53.6	19.1	35.5	6.6	125.0	15.3	19.3	330
		S.D.	44	0.6	1.1	2.6	0.9	0.8	1.8	7.7	0.7	4.5	109
100	5	Mean	671	13.3	35.3	52.6	19.7	37.5*	5.4	130.4	15.6	19.7	299
		S.D.	46	0.8	1.5	1.8	0.6	0.7D	1.0	24.2	0.5	6.6	38
300	5	Mean	681	13.1	36.1	53.1	19.2	36.3	4.5	161.0	14.7	23.3	370
		S.D.	27	0.2	0.8	1.9	0.7	0.3	2.4	43.0	0.7	9.1	76
1000	5	Mean	659	12.1	34.1	52.0	18.4	35.6	7.2	184.8**	15.4	24.7	296
		S.D.	50	0.8	2.4	3.8	0.7	1.8	5.9	19.6D	0.5	10.8	75

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
D : Dunnett's test

Table 6-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 4 of lactation)

Female

Dose mg/kg	No.		WBC	Differential leukocyte counts (%)						Differential leukocyte counts (X10²/μL)					
			X10²/μL	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC
0	5	Mean	138.7	59.3	37.0	0.7	0.2	2.2	0.5	82.4	51.1	1.1	0.3	3.0	0.8
		S.D.	33.1	11.2	11.0	0.3	0.1	0.7	0.3	25.6	16.9	0.6	0.2	1.4	0.5
100	5	Mean	193.6	60.5	35.9	0.6	0.3	2.1	0.5	118.1	68.7	1.1	0.6	4.0	1.1
		S.D.	54.5	4.3	3.8	0.2	0.1	0.5	0.2	38.6	16.9	0.4	0.3	0.7	0.7
300	5	Mean	181.1	57.3	38.9	0.4	0.2	2.5	0.7	104.1	69.9	0.8	0.4	4.5	1.2
		S.D.	29.3	5.0	4.6	0.2	0.1	1.4	0.1	21.4	10.8	0.3	0.3	2.7	0.3
1000	5	Mean	165.5	60.2	35.8	0.4	0.2	2.8	0.6	101.4	57.7	0.7	0.4	4.5	1.0
		S.D.	23.8	14.7	13.2	0.2	0.1	1.7	0.4	35.7	17.9	0.2	0.2	2.5	0.7

LUC : Large unstained cells
No significant difference in any treated groups from control group.

Table 6-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 14 of recovery)

Male

Dose mg/kg	No.		RBC	Hb	Ht	MCV	MCH	MCHC	Reticu-locyte	Plate-let	PT	APTT	Fibri-nogen
			X10⁴/μL	g/dL	%	fL	pg	g/dL	%	X10²/μL	s	s	mg/dL
0	5	Mean	892	16.0	42.7	47.9	17.9	37.4	1.9	99.7	17.4	21.9	288
		S.D.	27	0.6	1.8	1.5	0.5	0.3	0.4	9.4	0.7	3.5	22
1000	5	Mean	822**	15.0*	40.8	49.6	18.3	36.9	2.8*	111.5	17.3	19.1	309
		S.D.	35T	0.6T	2.0	0.9	0.2	0.5	0.5T	7.7	1.4	2.0	33

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
T : Student's t-test

Table 6-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 14 of recovery)

Male

Dose mg/kg	No.		WBC	Differential leukocyte counts (%)						Differential leukocyte counts ($X10^2/\mu L$)					
			$X10^2/\mu L$	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC
0	5	Mean	109.5	78.0	17.2	1.5	0.4	2.4	0.6	85.5	18.9	1.6	0.4	2.6	0.6
		S.D.	16.1	3.3	2.5	0.4	0.1	0.9	0.2	12.9	4.0	0.4	0.2	1.0	0.2
1000	5	Mean	90.7	77.9	18.3	0.9*	0.3	1.9	0.7	70.9	16.3	0.8**	0.3	1.7	0.7
		S.D.	14.6	2.5	2.5	0.3T	0.1	0.5	0.2	13.3	1.0	0.2T	0.1	0.6	0.3

LUC : Large unstained cells
* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
T : Student's t-test

Table 6-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 14 of recovery)

Female

Dose mg/kg	No.		RBC	Hb	Ht	MCV	MCH	MCHC	Reticulocyte	Platelet	PT	APTT	Fibrinogen
			$X10^4/\mu L$	g/dL	%	fL	pg	g/dL	%	$X10^4/\mu L$	s	s	mg/dL
0	5	Mean	827	15.9	41.8	50.6	19.3	38.1	1.5	114.5	15.7	24.3	224
		S.D.	35	0.4	1.7	1.2	0.4	0.6	0.3	18.3	0.6	9.1	19
1000	5	Mean	811	15.2	40.6	50.1	18.8	37.5	2.0	117.5	15.8	16.2	241
		S.D.	34	0.6	1.3	1.1	0.7	0.6	0.6	15.9	0.7	1.5	24

No significant difference between treated group and control group.

Table 6-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Hematology (Day 14 of recovery)

Female

Dose mg/kg	No.		WBC	Differential leukocyte counts (%)						Differential leukocyte counts ($X10^3/\mu L$)					
			$X10^3/\mu L$	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC	Lymph.	Neut.	Eosino.	Baso.	Mono.	LUC
0	5	Mean	50.5	69.4	25.5	2.0	0.2	2.4	0.5	35.2	12.6	1.0	0.1	1.3	0.3
		S.D.	12.8	6.0	6.5	0.7	0.1	1.0	0.4	9.8	3.8	0.5	0.1	0.7	0.2
1000	5	Mean	59.6	67.1	28.5	1.8	0.3	1.9	0.4	39.5	17.5	1.0	0.2	1.2	0.2
		S.D.	14.8	10.9	11.1	0.5	0.1	0.3	0.2	9.5	8.6	0.2	0.1	0.3	0.1

LUC : Large unstained cells
No significant difference between treated group and control group.

R-944

Table 7-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Week 6 of administration)

Male

Dose mg/kg	No.		AST (GOT) IU/L	ALT (GPT) IU/L	LDH IU/L	γ-GTP IU/L	AlP IU/L	T.cho mg/dL	TG mg/dL	PL mg/dL	T.bili-rubin mg/dL	Glucose mg/dL	BUN mg/dL	Crea-tinine mg/dL
0	5	Mean	65	33	51	1	452	46	41	86	0.1	128	12	0.28
		S.D.	3	3	6	0	77	7	20	8	0.0	3	1	0.02
100	5	Mean	62	26	44	1	422	47	29	80	0.1	144*	12	0.28
		S.D.	6	4	7	0	48	2	13	5	0.0	12D	1	0.03
300	5	Mean	68	36	77*	1	425	52	36	88	0.1	134	12	0.28
		S.D.	11	7	22D	0	73	10	19	13	0.0	11	3	0.03
1000	5	Mean	57	35	53	1	504	38	48	81	0.1	111*	14	0.29
		S.D.	4	4	18	0	135	7	9	12	0.0	10D	1	0.04

* : $p<0.05$ (Significant difference from control group)
D : Dunnett's test

Table 7-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Week 6 of administration)

Male

Dose mg/kg	No.		Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	Albumin g/dL	A/G
0	5	Mean	144	4.7	108	9.3	6.1	6.0	2.6	0.75
		S.D.	1	0.3	2	0.2	0.5	0.2	0.1	0.02
100	5	Mean	144	4.9	107	9.7*	6.5	6.0	2.6	0.77
		S.D.	1	0.1	1	0.2D	0.4	0.3	0.1	0.04
300	5	Mean	144	5.3*	108	9.7	7.0**	6.2	2.7	0.75
		S.D.	1	0.2D	1	0.3	0.3D	0.2	0.2	0.04
1000	5	Mean	143	5.1	108	9.7	7.4**	6.0	2.6	0.77
		S.D.	1	0.4	1	0.2	0.3D	0.1	0.1	0.04

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
D : Dunnett's test

R-944

Table 7-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 4 of lactation)

Female

Dose mg/kg	No.		AST (GOT) IU/L	ALT (GPT) IU/L	LDH IU/L	γ-GTP IU/L	AlP IU/L	T.cho mg/dL	TG mg/dL	PL mg/dL	T.bili-rubin mg/dL	Glucose mg/dL	BUN mg/dL	Crea-tinine mg/dL
0	5	Mean	111	69	43	1	345	59	44	115	0.1	135	14	0.35
		S.D.	15	8	10	1	149	5	24	7	0.0	8	2	0.03
100	5	Mean	108	52	56	1	251	58	39	114	0.1	134	13	0.36
		S.D.	53	10	23	0	104	10	17	13	0.0	6	2	0.02
300	5	Mean	116	70	51	1	241	66	40	124	0.1	123	14	0.37
		S.D.	17	22	17	0	25	15	12	20	0.0	36	2	0.04
1000	5	Mean	103	59	56	1	300	65	40	129	0.1	120	18	0.32
		S.D.	34	13	7	1	84	19	13	35	0.0	17	7	0.04

No significant difference in any treated groups from control group.

Table 7-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 4 of lactation)

Female

Dose mg/kg	No.		Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	Albumin g/dL	A/G
0	5	Mean	141	4.2	107	9.8	7.0	6.1	2.7	0.79
		S.D.	1	0.6	2	0.3	0.7	0.2	0.1	0.03
100	5	Mean	141	4.2	106	10.0	7.3	6.4	2.8	0.79
		S.D.	2	0.2	1	0.2	0.6	0.3	0.1	0.03
300	5	Mean	141	4.3	104	10.1	7.8	5.8	2.5	0.77
		S.D.	2	0.4	4	0.3	0.7	0.4	0.2	0.05
1000	5	Mean	141	4.7	105	10.1	7.5	5.9	2.6	0.80
		S.D.	1	0.4	4	0.4	0.6	0.4	0.2	0.02

No significant difference in any treated groups from control group.

Table 7-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 14 of recovery)

Male

Dose mg/kg	No.		AST (GOT) IU/L	ALT (GPT) IU/L	LDH IU/L	γ-GTP IU/L	AlP IU/L	T.cho mg/dL	TG mg/dL	PL mg/dL	T.bili-rubin mg/dL	Glucose mg/dL	BUN mg/dL	Crea-tinine mg/dL
0	5	Mean	68	31	46	1	404	49	43	88	0.1	148	15	0.31
		S.D.	9	8	8	0	45	11	15	12	0.0	10	2	0.03
1000	5	Mean	87	37	54	1	329*	54	37	91	0.1	144	15	0.30
		S.D.	28	9	23	0	51T	7	11	7	0.0	12	3	0.03

* : $p<0.05$ (Significant difference from control group)
T : Student's t-test

Table 7-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 14 of recovery)

Male

Dose mg/kg	No.		Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	Albumin g/dL	A/G
0	5	Mean	143	4.8	107	9.4	6.4	6.3	2.6	0.73
		S.D.	0	0.4	2	0.3	0.5	0.2	0.1	0.05
1000	5	Mean	143	4.9	106	9.3	6.8	6.1	2.6	0.74
		S.D.	1	0.5	0	0.3	0.4	0.1	0.1	0.03

No significant difference between treated group and control group.

Table 7-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 14 of recovery)

Female

Dose mg/kg	No.		AST (GOT) IU/L	ALT (GPT) IU/L	LDH IU/L	γ-GTP IU/L	AlP IU/L	T.cho mg/dL	TG mg/dL	PL mg/dL	T.bili-rubin mg/dL	Glucose mg/dL	BUN mg/dL	Crea-tinine mg/dL
0	5	Mean	70	31	46	1	224	65	16	129	0.1	131	16	0.35
		S.D.	19	14	8	0	60	5	9	8	0.0	12	1	0.04
1000	5	Mean	62	33	47	1	176	80	20	143	0.1	120	18	0.32
		S.D.	10	9	5	0	26	21	11	28	0.0	22	4	0.06

No significant difference between treated group and control group.

Table 7-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Blood chemistry (Day 14 of recovery)

Female

Dose mg/kg	No.		Na mmol/L	K mmol/L	Cl mmol/L	Ca mg/dL	P mg/dL	TP g/dL	Albumin g/dL	A/G
0	5	Mean	142	4.4	110	9.5	4.2	6.7	2.9	0.77
		S.D.	1	0.2	2	0.1	0.9	0.2	0.1	0.02
1000	5	Mean	144*	4.3	110	9.5	5.1	6.7	2.8*	0.71**
		S.D.	1T	0.2	1	0.2	0.5	0.2	0.0T	0.03T

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control group)
T : Student's t-test

R-944

Table 8-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of male rats (Main group)

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thyroid (R+L) mg(mg/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)
Absolute	0	No.	5	5	5	5	5	5
		Mean	469	2.06	22.7	265	1.31	12.34
		S.D.	42	0.07	6.1	128	0.10	1.69
	100	No.	5	5	5	5	5	5
		Mean	488	2.06	25.6	320	1.34	13.34
		S.D.	25	0.17	2.6	98	0.06	1.27
	300	No.	5	5	5	5	5	5
		Mean	461	2.06	22.9	261	1.36	12.14
		S.D.	27	0.08	4.0	72	0.10	1.13
	1000	No.	5	5	5	5	5	5
		Mean	392**	2.07	19.1	182	1.20	11.87
		S.D.	20D	0.04	4.4	33	0.07	0.91
Relative	0	No.		5	5	5	5	5
		Mean		0.44	4.9	56	0.28	2.62
		S.D.		0.03	1.4	22	0.01	0.14
	100	No.		5	5	5	5	5
		Mean		0.42	5.3	66	0.28	2.73
		S.D.		0.05	0.6	19	0.02	0.16
	300	No.		5	5	5	5	5
		Mean		0.45	5.0	56	0.30	2.63
		S.D.		0.03	0.7	14	0.02	0.11
	1000	No.		5	5	5	5	5
		Mean		0.53**	4.9	47	0.31	3.02**
		S.D.		0.03D	1.1	10	0.02	0.12D

**: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 8-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of male rats (Main group)

	Dose mg/kg		Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)	Body weight g	Testis (R+L) g(g/100g BW)	Epididymis (R+L) mg(mg/100g BW)
Absolute	0	No.	5	5	5	12	12	12
		Mean	0.68	3.12	60	478	3.31	1242
		S.D.	0.08	0.38	13	32	0.33	117
	100	No.	5	5	5	12	12	12
		Mean	0.77	3.22	60	474	3.16	1206
		S.D.	0.08	0.14	4	32	0.44	109
	300	No.	5	5	5	12	12	12
		Mean	0.66	3.11	56	469	3.27	1254
		S.D.	0.04	0.13	5	26	0.21	68
	1000	No.	5	5	5	12	12	12
		Mean	0.69	2.94	56	417**	3.17	1210
		S.D.	0.04	0.20	6	35D	0.20	79
Relative	0	No.	5	5	5		12	12
		Mean	0.15	0.66	12		0.69	260
		S.D.	0.01	0.05	2		0.07	25
	100	No.	5	5	5		12	12
		Mean	0.16	0.66	12		0.67	256
		S.D.	0.02	0.03	1		0.10	32
	300	No.	5	5	5		12	12
		Mean	0.14	0.68	12		0.70	268
		S.D.	0.01	0.04	1		0.07	23
	1000	No.	5	5	5		12	12
		Mean	0.17*	0.75*	14		0.76	292*
		S.D.	0.02D	0.07D	2		0.05	24D

*: p<0.05; **: p<0.01 (Significant difference from control group)
D: Dunnett's test

Table 8-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of female rats (Main group)

	Dose mg/kg		Body weight g	Brain g(g/100g BW)	Thyroid (R+L) mg(mg/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)
Absolute	0	No.	5	5	5	5	5	5
		Mean	299	1.90	15.2	213	0.91	9.71
		S.D.	9	0.07	2.3	65	0.04	0.71
	100	No.	5	5	5	5	5	5
		Mean	290	1.91	16.9	192	0.91	10.11
		S.D.	14	0.10	3.6	48	0.04	0.82
	300	No.	5	5	5	5	5	5
		Mean	269*	1.89	14.6	105*	0.86	9.20
		S.D.	27D	0.10	2.7	57D	0.06	1.46
	1000	No.	5	5	5	5	5	5
		Mean	238**	1.89	14.5	76**	0.78**	9.88
		S.D.	15D	0.09	2.7	54D	0.05D	0.87
Relative	0	No.		5	5	5	5	5
		Mean		0.64	5.1	71	0.31	3.25
		S.D.		0.02	0.8	21	0.02	0.16
	100	No.		5	5	5	5	5
		Mean		0.66	5.8	67	0.31	3.49
		S.D.		0.02	1.1	18	0.02	0.20
	300	No.		5	5	5	5	5
		Mean		0.71	5.4	38*	0.32	3.40
		S.D.		0.07	0.7	17D	0.02	0.35
	1000	No.		5	5	5	5	5
		Mean		0.80**	6.1	31*	0.33	4.15**
		S.D.		0.06D	0.8	21D	0.02	0.31D

*: p<0.05; **: p<0.01 (Significant difference from control group)
D: Dunnett's test

Table 8-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of female rats (Main group)

	Dose mg/kg		Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
Absolute	0	No.	5	5	5
		Mean	0.65	1.84	80
		S.D.	0.07	0.10	8
	100	No.	5	5	5
		Mean	0.63	1.91	80
		S.D.	0.11	0.12	8
	300	No.	5	5	5
		Mean	0.55	1.87	69
		S.D.	0.12	0.21	6
	1000	No.	5	5	5
		Mean	0.58	1.86	62**
		S.D.	0.23	0.18	6D
Relative	0	No.	5	5	5
		Mean	0.22	0.62	27
		S.D.	0.03	0.02	3
	100	No.	5	5	5
		Mean	0.22	0.66	27
		S.D.	0.04	0.03	3
	300	No.	5	5	5
		Mean	0.20	0.70	26
		S.D.	0.03	0.08	2
	1000	No.	5	5	5
		Mean	0.24	0.78**	26
		S.D.	0.09	0.08D	3

**: p<0.01 (Significant difference from control group)
D: Dunnett's test

Table 8-5 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of male rats (Recovery group)

	Dose mg/kg	No. of animals		Body weight g	Brain g(g/100g BW)	Thyroid (R+L) mg(mg/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)
Absolute	0	5	Mean	479	2.11	21.6	266	1.37	12.46
			S.D.	42	0.12	2.6	33	0.18	1.21
	1000	5	Mean	459	2.11	24.6	258	1.42	12.82
			S.D.	27	0.10	3.7	92	0.12	1.47
Relative	0	5	Mean		0.44	4.5	56	0.28	2.60
			S.D.		0.04	0.5	8	0.03	0.10
	1000	5	Mean		0.46	5.3	56	0.31	2.79
			S.D.		0.03	0.7	18	0.01	0.20

No significant difference between treated group and control group.

Table 8-6 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of male rats (Recovery group)

	Dose mg/kg	No. of animals		Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)	Testis (R+L) g(g/100g BW)	Epididymis (R+L) mg(mg/100g BW)
Absolute	0	5	Mean	0.76	3.21	61	3.15	1297
			S.D.	0.16	0.38	7	0.27	131
	1000	5	Mean	0.75	3.14	62	3.40	1340
			S.D.	0.07	0.31	8	0.41	89
Relative	0	5	Mean	0.16	0.67	13	0.66	272
			S.D.	0.03	0.05	1	0.06	33
	1000	5	Mean	0.16	0.68	14	0.74	292
			S.D.	0.01	0.04	2	0.05	9

No significant difference between treated group and control group.

Table 8-7 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of female rats (Recovery group)

	Dose mg/kg	No. of animals		Body weight g	Brain g(g/100g BW)	Thyroid (R+L) mg(mg/100g BW)	Thymus mg(mg/100g BW)	Heart g(g/100g BW)	Liver g(g/100g BW)
Absolute	0	5	Mean	264	1.92	16.7	253	0.83	6.42
			S.D.	24	0.05	2.6	73	0.08	0.55
	1000	5	Mean	256	1.93	19.0	218	0.84	7.29
			S.D.	23	0.11	2.7	43	0.04	1.09
Relative	0	5	Mean		0.73	6.4	94	0.32	2.44
			S.D.		0.06	1.5	19	0.03	0.18
	1000	5	Mean		0.76	7.5	86	0.33	2.84*
			S.D.		0.10	1.3	20	0.02	0.21T

*: $p<0.05$ (Significant difference from control group)
T: Student's t-test

Table 8-8 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Organ weight of female rats (Recovery group)

	Dose mg/kg	No. of animals		Spleen g(g/100g BW)	Kidney (R+L) g(g/100g BW)	Adrenal (R+L) mg(mg/100g BW)
Absolute	0	5	Mean	0.47	1.75	67
			S.D.	0.03	0.16	6
	1000	5	Mean	0.56	1.86	74
			S.D.	0.16	0.19	9
Relative	0	5	Mean	0.18	0.66	25
			S.D.	0.01	0.03	3
	1000	5	Mean	0.22	0.73	29
			S.D.	0.04	0.08	3

No significant difference between treated group and control group.

Table 9-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Gross pathological findings (Dead animal)

Organs / Findings	Sex:	F
	Dose(mg/kg):	1000
	Number:	1
Spleen		
Small		1
Thymus		
Small		1

Table 9-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Gross pathological findings (Main group)

Organs / Findings	Sex: / Dose(mg/kg): / Number:	M 0 12	M 100 12	M 300 12	M 1000 12	F 0 12	F 100 12	F 300 12	F 1000 11
General descriptions									
Undernourishment		0	0	0	0	0	0	0	1
Cerebrum									
Focus,depressed		0	0	0	0	0	1	0	0
Epididymis									
Small		0	1	0	0	-	-	-	-
Focus,yellow		0	0	0	1	-	-	-	-
Liver									
Discoloration,dark		0	0	0	6	0	0	0	0
Adhesion		0	1	0	0	0	0	0	0
Stomach									
Focus,white,glandular stomach		1	0	0	0	0	0	0	0
Focus,depressed,forestomach		0	0	0	0	0	0	1	0
Focus,dark red,glandular stomach		0	0	0	0	0	0	1	1
Focus,dark red,forestomach		0	0	0	0	0	0	1	0
Thickening,limiting ridge		0	0	0	0	0	0	1	0
Testis									
Small		0	1	0	0	-	-	-	-
Thymus									
Small		0	0	0	0	0	0	1	3
Uterus									
Hypoplasia		-	-	-	-	0	1	0	0

- : Not applicable

R-944

Table 9-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Gross pathological findings (Recovery group)

Organs / Findings	Sex: / Dose(mg/kg): / Number:	M 0 5	M 1000 5	F 0 5	F 1000 5
All tissues					
Not remarkable		5	5	5	5

Table 10-1 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Histopathological findings (Dead animal)

Organs / Findings	Sex: / Dose(mg/kg): / Number: F 1000 1
Bone+Bone marrow,sternal	
Number examined	1
Degeneration,chondromucinous	1
minimal	1
Liver	
Number examined	1
Vacuolation,hepatocyte,periportal	1
minimal	1
Spleen	
Number examined	1
Atrophy,white pulp	1
minimal	1
Thymus	
Number examined	1
Atrophy	1
moderate	1

Table 10-2 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Histopathological findings (Main group)

Organs / Findings	Sex: / Dose(mg/kg): / Number: M 0 12	M 100 12	M 300 12	M 1000 12	F 0 12	F 100 12	F 300 12	F 1000 11
Bone+Bone marrow,sternal								
Number examined	5	0	0	5	5	0	0	5
Degeneration,chondromucinous	5	0	0	5	5	0	0	4
minimal	5	0	0	5	5	0	0	4
Cerebrum								
Number examined	5	0	0	5	5	1	0	5
Malformation	0	0	0	0	0	1	0	0
minimal	0	0	0	0	0	1	0	0
Epididymis								
Number examined	5	1	0	5	-	-	-	-
Hypospermia	0	1	0	0	-	-	-	-
severe	0	1	0	0	-	-	-	-
Cell debris,ductal	0	1	0	0	-	-	-	-
minimal	0	1	0	0	-	-	-	-
Heart								
Number examined	5	0	0	5	5	0	0	5
Cardiomyopathy	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Intestine,cecum								
Number examined	12	12	12	12	12	12	12	11
Cell infiltration,mucosal	3	1	1	5	1	0	1	3
minimal	2	1	1	5	1	0	1	3
mild	1	0	0	0	0	0	0	0
Cell infiltration,serosal	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Necrosis,single cell,mucosal	0	4	3	8	0	2	3	7
minimal	0	4	3	7	0	2	3	7
mild	0	0	0	1	0	0	0	0
Hyperplasia,mucosal,diffuse	0	1	3	7	0	1	4	6
minimal	0	1	3	7	0	1	4	6
Intestine,colon								
Number examined	5	0	0	5	5	0	0	5
Cell infiltration,serosal	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Kidney								
Number examined	5	0	0	5	5	0	0	5
Vacuolation,tubular cell	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Regeneration,tubular	4	0	0	2	1	0	0	0
minimal	4	0	0	2	1	0	0	0
Mineralization	0	0	0	0	0	0	0	2
minimal	0	0	0	0	0	0	0	2
Hyperplasia,transitional cell	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Liver								
Number examined	12	12	12	12	12	12	12	11
Vacuolation,hepatocyte,periportal	11	10	7	0	5	4	1	0
minimal	8	9	7	0	5	4	1	0
mild	3	1	0	0	0	0	0	0
Necrosis,focal	0	0	0	0	0	0	1	0
minimal	0	0	0	0	0	0	1	0
Hematopoiesis,extramedullary	0	0	0	0	1	0	0	1
minimal	0	0	0	0	1	0	0	1
Microgranuloma	10	10	8	8	2	2	2	3
minimal	10	10	8	8	2	2	2	3

- : Not applicable

Table 10-3 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Histopathological findings (Main group)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg):	0	100	300	1000	0	100	300	1000
	Number:	12	12	12	12	12	12	12	11
Liver (continued)									
Fibrosis,capsular		0	1	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0
Altered cell focus,eosinophilic		0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	1	0	0
Lung(bronchus)									
Number examined		5	0	0	5	5	0	0	5
Mineralization,arterial wall		1	0	0	0	2	0	0	0
minimal		1	0	0	0	2	0	0	0
Accumulation,foamy cell		2	0	0	2	0	0	0	1
minimal		1	0	0	2	0	0	0	1
mild		1	0	0	0	0	0	0	0
Inflammatory change,focal		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Spleen									
Number examined		5	0	0	5	12	12	12	11
Hematopoiesis,extramedullary		2	0	0	3	12	12	11	8
minimal		2	0	0	3	5	6	9	3
mild		0	0	0	0	7	6	2	5
Stomach									
Number examined		5	0	0	5	5	0	4	5
Inflammation,muscular layer/serosa		0	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	0	1
Erosion,glandular stomach		2	0	0	0	0	0	1	1
minimal		2	0	0	0	0	0	1	1
Ulcer,forestomach		0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	1	0
Testis									
Number examined		5	1	0	5	-	-	-	-
Atrophy,seminiferous tubular		1	1	0	0	-	-	-	-
minimal		1	0	0	0	-	-	-	-
severe		0	1	0	0	-	-	-	-
Thymus									
Number examined		5	0	0	5	12	12	12	11
Atrophy		0	0	0	0	1	1	4	5
minimal		0	0	0	0	0	1	2	1
mild		0	0	0	0	1	0	1	1
moderate		0	0	0	0	0	0	1	2
severe		0	0	0	0	0	0	0	1
Thyroid									
Number examined		5	0	0	5	5	0	0	5
Cyst,ultimobranchial		1	0	0	0	1	0	0	1
minimal		1	0	0	0	1	0	0	1
Urinary bladder									
Number examined		5	0	0	5	5	0	0	5
Cell infiltration,mucosal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Hyperplasia,mucosal,diffuse		0	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	0	1
Uterus									
Number examined		-	-	-	-	5	1	0	5
Hypoplasia		-	-	-	-	0	1	0	0
mild		-	-	-	-	0	1	0	0

- : Not applicable

Table 10-4 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Histopathological findings (Recovery group)

Organs / Findings	Sex:	M	M	F	F
	Dose(mg/kg):	0	1000	0	1000
	Number:	5	5	5	5
Intestine,cecum					
Number examined		5	5	5	5
Cell infiltration,mucosal		1	2	0	2
minimal		1	2	0	2
Hyperplasia,mucosal,diffuse		0	1	0	0
minimal		0	1	0	0
Liver					
Number examined		5	5	5	5
Vacuolation,hepatocyte,periportal		1	0	1	0
minimal		1	0	1	0
Microgranuloma		4	4	5	5
minimal		4	4	5	5
Spleen					
Number examined		0	0	5	5
Hematopoiesis,extramedullary		0	0	2	2
minimal		0	0	2	2

Table 11 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Estrous cycle in female rats during the pre-mating period (Main group)

Dose mg/kg	No. of animals	Count of estrus						Mean duration of cycles Mean±S.D.
		0	1	2	3	4	Mean±S.D.	
0	12	0	0	0	5	7	3.6±0.5	4.1±0.3
100	12	0	0	0	7	5	3.4±0.5	4.4±0.5
300	12	0	0	0	6	6	3.5±0.5	4.1±0.2
1000	12	0	0	0	8	4	3.3±0.5	4.4±0.5

No significant difference in any treated groups from control group.

R-944

Table 12 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Mating and fertility of animals

Dose mg/kg	Male				Female			
	No. of males	Days until copulation Mean±S.D.	Copulation index (%) a)	Insemination index (%) b)	No. of females	Days until copulation Mean±S.D.	Copulation index (%) a)	Fertility index (%) c)
0	12	2.8±1.1	12/12(100.0)	12/12(100.0)	12	2.8±1.1	12/12(100.0)	12/12(100.0)
100	12	3.0±1.0	12/12(100.0)	12/12(100.0)	12	3.0±1.0	12/12(100.0)	12/12(100.0)
300	12	2.4±1.3	12/12(100.0)	12/12(100.0)	12	2.4±1.3	12/12(100.0)	12/12(100.0)
1000	12	2.7±1.2	12/12(100.0)	11/12(91.7)	12	2.7±1.2	12/12(100.0)	11/12(91.7)

a): (No. of copulated animals / No. of mated animals) X 100
b): (No. of pregnant females / No. of copulated males) X 100
c): (No. of pregnant animals / No. of copulated females) X 100
No significant difference in any treated groups from control group.

Table 13 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Delivery data on dams

Dose mg/kg		No. of pregnant females	No. of females with live pups	Delivery index % a)	Gestation period	No. of corpora lutea	No. of implantation sites	Implantation index % b)	No. of stillborn pups (%)c)	No. of liveborn pups	Live birth index % d)
0	Total	12	12	100.0		193	181		2	166	
	Mean				22.2	16.1	15.1	93.8	(1.0)	13.8	99.0
	S.D.				0.2	1.0	1.7	8.8	(2.4)	2.2	2.4
100	Total	12	12	100.0		195	183		2	173	
	Mean				22.0	16.3	15.3	93.9	(1.1)	14.4	98.9
	S.D.				0.3	1.7	2.3	10.6	(3.8)	2.4	3.8
300	Total	12	12	100.0		209	191		2	175	
	Mean				22.0	17.4	15.9	91.5	(1.4)	14.6	98.7
	S.D.				0.3	1.5	1.6	6.8	(3.2)	1.9	3.2
1000	Total	11	11	100.0		172	162		2	151	
	Mean				22.1	15.6	14.7	94.7	(1.3)	13.7	98.7
	S.D.				0.4	1.9	1.5	6.9	(3.0)	1.4	3.0

a): (No. of females which delivered live pups / No. of pregnant females) × 100
b): (No. of implantation sites / No. of corpora lutea) × 100
c): (No. of stillborn pups / No. of stillborn and liveborn pups) × 100
d): (No. of liveborn pups / No. of stillborn and liveborn pups) × 100
No significant difference in any treated groups from control group.

Table 14 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

External examination of liveborn pups

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Body weight(g) Male	Body weight(g) Female	External b) abnormalities(%)c)
0	12	Total	82	84	0.49			0
		Mean	6.8	7.0		6.5	6.3	(0.0)
		S.D.	2.4	2.0		0.5	0.4	(0.0)
100	12	Total	80	93	0.46			0
		Mean	6.7	7.8		6.5	6.1	(0.0)
		S.D.	1.5	1.9		0.5	0.5	(0.0)
300	12	Total	95	80	0.54			0
		Mean	7.9	6.7		6.2	6.1	(0.0)
		S.D.	2.9	1.4		0.4	0.4	(0.0)
1000	11	Total	81	70	0.54			0
		Mean	7.4	6.4		5.5**	5.1**	(0.0)
		S.D.	3.0	2.1		0.6D	0.6D	(0.0)

a): No. of males / (No. of males + No. of females)
b): No. of liveborn pups with external abnormalities
c): (No. of liveborn pups with external abnormalities / No. of liveborn pups) × 100
**: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 15 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Viability index of pups

Dose mg/kg		No. of dams	No. of live pups Day 0	No. of live pups Day 4	Viability index on day 4 after birth % a)
0	Total	12	166	166	
	Mean		13.8	13.8	100.0
	S.D.		2.2	2.2	0.0
100	Total	12	173	173	
	Mean		14.4	14.4	100.0
	S.D.		2.4	2.4	0.0
300	Total	12	175	172	
	Mean		14.6	14.3	98.4
	S.D.		1.9	1.8	2.8
1000	Total	10	136	131	
	Mean		13.6	13.1	96.7
	S.D.		1.4	1.2	6.5

a): (No. of live pups on day 4 / No. of liveborn pups on day 0) × 100
No significant difference in any treated groups from control group.

R-944

Table 16 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Body weight of pups

Dose mg/kg		Male 0	Male 4	Female 0	Female 4a)
0	No.	12	12	12	12
	Mean	6.5	10.1	6.3	9.8
	S.D.	0.5	1.4	0.4	1.3
100	No.	12	12	12	12
	Mean	6.5	10.1	6.1	9.5
	S.D.	0.5	1.1	0.5	1.1
300	No.	12	12	12	12
	Mean	6.2	8.6*	6.1	8.3*
	S.D.	0.4	1.1D	0.4	1.1D
1000	No.	11[b)]	10	11	10
	Mean	5.5**	7.1**	5.1**	6.7**
	S.D.	0.6D	1.7D	0.6D	1.8D

Unit: g
No.: No. of dams
a): Day after birth
b): One dam died on day 0 of lactation.
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 17 A combined repeated-dose/reproductive-developmental toxicity study in rats treated orally with 2,3,4,4'-Tetrahydroxybenzophenone

Gross pathological findings in pups on day 4 after birth

Dose (mg/kg)	0	100	300	1000
Male				
No. of pups examined	82	80	93	68
No. of pups with abnormal findings	0	1	0	9
Thymic remnant in neck	0	1	0	0
Diaphragmatic hernia	0	0	0	1
Undernourishment	0	0	0	8
Female				
No. of pups examined	84	93	79	63
No. of pups with abnormal findings	1	0	0	5
Thymic remnant in neck	1	0	0	1
Undernourishment	0	0	0	4

[要　　約]

イソシアヌル酸（ICA）は、CHL/IU細胞（チャイニーズ・ハムスター、肺）に染色体異常を誘発しなかった。

ICAはCHL/IU細胞に対して、連続処理（新鮮培地中で24時間処理）および短時間処理のS9 mix存在下および非存在下（それぞれS9反応液およびMEM培地中で6時間処理後18時間の回復時間）で、最高処理濃度である1.3 mg/ml（10 mM）においても50%を越える増殖抑制は認められなかった。

このことから染色体異常試験において、連続処理（24時間および48時間処理）および短時間処理（S9 mix存在下および非存在下）ともに1.3 mg/ml（10 mM）を最高処理濃度とし、公比2で各濃度を設定した。染色体分析は、全ての系列で1.3 mg/ml（10 mM）の濃度含む3濃度群を観察対象とした。

ICAはいずれの処理条件下においても、染色体の構造異常および倍数性細胞を誘発しなかった。

溶媒の背景データ（Appendix 2）と被験物質処理群間で、フィッシャーの直接確率法[2]により、familywiseの有意水準を5%として有意差検定を実施した。直接確率法で有意差がある場合、用量依存性の有無をコクラン・アーミテッジの傾向性検定[3]（$p < 0.05$）により判定した。両検定でともに有意差が認められた場合を陽性とし、直接確率法でのみ有意差が認められた場合は疑陽性とした。

[結　　果]

ICAは連続処理および短時間処理した場合、処理限界濃度の1.3 mg/ml（10 mM）を含むいずれの処理濃度においても、染色体の構造異常および倍数性細胞を誘発しなかった（Table 1、2）。

一方、陽性対照物質として用いたMCは、連続処理において染色体の構造異常を誘発し（Table 1）、CPAは短時間処理のS9 mix存在下において染色体の構造異常を誘発した（Table 2）。これらの陽性対照物質の結果より、本実験系の成立が確認された。

[特記事項]

本試験の実施にあたり、試験の信頼性に悪影響を及ぼす疑いのある予期し得なかった事態および試験計画書からの逸脱は無かった。

[参考文献]

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3) 吉村　功, 大橋靖夫編:「毒性試験講座 14、毒性試験データの統計解析」, 地人書館, 東京 (1992)

Table 1 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with isocyanuric acid (ICA)* without S9 mix

Group	Concentration (mg/ml)	Time of exposure (h)	No. of cells analysed	No. of structural aberrations							Others[3]	No. of cells with aberrations		Polyploid[4] (%)	Trend test[5]		Concurrent[6] cytotoxicity (%)
				gap	ctb	cte	csb	cse	mul[2]	total		TAG (%)	TA (%)		SA	NA	
Control[1]			200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.63			——
Solvent[1]	0	24	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.25			100.0
ICA	0.33	24	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.50			95.0
ICA	0.65	24	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.63	NT	NT	95.0
ICA	1.3	24	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.50			106.5
MC	0.00005	24	200	20	65	134	1	1	10	231	1	116 (58.0)	105 (52.5)	0.25			——
Solvent[1]	0	48	200	0	1	0	0	0	0	1	0	1 (0.5)	1 (0.5)	0.13			100.0
ICA	0.33	48	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.38			101.5
ICA	0.65	48	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.50	NT	NT	100.0
ICA	1.3	48	200	1	0	1	0	0	0	2	0	2 (1.0)	1 (0.5)	0.13			109.0
MC	0.00005	48	200	6	44	135	6	6	40	237	2	96 (48.0)	93 (46.5)	0.38			——

Abbreviations, gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring), mul : multiple aberrations, TAG : total no.of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, MC : mitomycin C, NT: not tested.
1) Dimethyl sulfoxide was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Cochran • Armitage's trend test was done ($p<0.05$) when the incidence of TAG and polyploid in the treatment groups was significantly different from historical solvent control ($p<0.05$) by Fisher's exact test. 6) Cell confluency, representing cytotoxicity, was measured with Monocellater™.* : Purity was 99.5 wt%. Water (0.3%) and urea (0.2%) were contained as impurities.

Table 2 Chromosome analysis of Chinese hamster cells (CHL/IU) treated with isocyanuric acid (ICA)* with and without S9 mix

Group	Concentration (mg/ml)	S9 mix	Time of exposure (h)	No. of cells analysed	No. of structural aberrations gap	ctb	cte	csb	cse	mul[2]	total	Others[3]	No. of cells with aberrations TAG (%)	TA (%)	Polyploid[4] (%)	Trend test[5] SA	NA	Concurrent[6] cytotoxicity (%)
Control[1]				200	1	2	2	0	0	0	5	0	3 (1.5)	3 (1.5)	1.38			———
Solvent[1]	0	−	6 - (18)	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.38			100.0
ICA	0.33	−	6 - (18)	200	0	1	1	0	0	0	2	0	2 (1.0)	2 (1.0)	0.75			109.0
ICA	0.65	−	6 - (18)	200	0	1	1	0	0	0	2	0	2 (1.0)	2 (1.0)	0.63	NT	NT	106.5
ICA	1.3	−	6 - (18)	200	2	0	0	0	0	0	2	0	2 (1.0)	0 (0.0)	0.63			95.5
CPA	0.005	−	6 - (18)	200	3	2	0	0	0	0	5	0	5 (2.5)	2 (1.0)	0.88			———
Solvent[1]	0	+	6 - (18)	200	1	1	1	0	0	0	3	0	3 (1.5)	2 (1.0)	0.75			100.0
ICA	0.33	+	6 - (18)	200	2	0	1	0	0	0	3	0	3 (1.5)	1 (0.5)	0.25			99.5
ICA	0.65	+	6 - (18)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.25	NT	NT	104.5
ICA	1.3	+	6 - (18)	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.63			107.0
CPA	0.005	+	6 - (18)	200	7	33	34	0	2	0	76	0	51 (25.5)	48 (24.0)	0.75			———

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), mul : multiple aberrations, TAG : total no.of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, CPA : cyclophosphamide, NT: not tested.
1) Dimethyl sulfoxide was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group.
5) Cochran • Armitage's trend test was done ($p<0.05$) when the incidence of TAG and polyploid in the treatment groups was significantly different from historical solvent control ($p<0.05$) by Fisher's exact test. 6) Cell confluency, representing cytotoxicity, was measured with Monocellater™.* : Purity was 99.5 wt%. Water (0.3%) and urea (0.2%) were contained as impurities.

要約

プール等での殺菌用塩素の安定剤として広く用いられている高生産量既存化学物質イソシアヌル酸について、反復経口投与毒性・生殖発生毒性併合試験をSD系〔Crj:CD(SD)〕ラットを用い、0、10、40、150および600mg/kg/day用量で実施した。動物は1群雌雄各10匹とし、被験物質は交配開始14日前から雄は44日間、雌は分娩後哺育3日（41～48日間）まで投与した。

1．反復投与毒性

雌雄の親動物とも、600mg/kg群で毒性影響と考えられる変化が認められた。雄親について、赤色尿の排泄および体重増加の抑制が認められた。尿検査で、尿の混濁、被験物質と類似した板状の結晶物質の析出、赤血球および白血球の出現率の増加が認められた。血液学検査で、赤血球数、血色素量およびヘマトクリット値の減少、血液生化学検査で、尿素窒素およびクレアチニンの増加ならびにナトリウムの減少が認められた。病理組織学検査で、腎臓に尿細管の拡張、尿細管上皮の壊死および過形成、好塩基性尿細管の増加、好中球の浸潤、鉱質沈着、線維化などの変化、膀胱に粘膜上皮の過形成、副腎に皮質束状帯細胞の空胞化が認められ、腎臓の絶対および相対重量ならびに副腎の相対重量は増加した。一方、雌親について、雄親と同様の赤色尿の排泄および腎臓、膀胱および副腎の病理学的変化が認められたほか、胸腺の萎縮例の増加が認められた。

以上の結果から、イソシアヌル酸のラットへの反復投与による主な毒性影響は腎臓および膀胱に認められ、副腎および胸腺に対する影響も認められた。無影響量は、雌雄とも150mg/kg/dayと推定された。

2．生殖発生毒性

親動物の交尾率、受胎率、妊娠期間、黄体数、着床数、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600mg/kg/dayと推定された。

(2) 外表異常および一般状態観察

分娩完了後、新生児について口腔内を含む外表の異常を観察した。また、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〕および新生児生存率〔（哺育４日生児数／出産確認時生児数）×100〕を求めた。

(3) 体重測定

新生児について哺育０日および４日に雌雄別に各腹ごとの総体重を測定し、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、生存例は雌親の解剖時(哺育４日）にエーテル・クロロホルムで麻酔死させ、胸腹部における主要器官を肉眼的に観察した。

６．統計処理

得られた平均値あるいは頻度について、対照群との間の有意差（危険率５％以下）を次の方法で検定した。

体重、摂餌量、血液学および血液生化学データ、器官重量、黄体数、着床数、妊娠期間、産児数などのパラメトリックデータは、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 法またはScheff6法（群の大きさが異なる場合）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ならびに着床率、出生率、分娩率、新生児生存率、尿検査の定性的データなどのノンパラメトリックデータはKruskal-Wallisの順位検定を行い、その結果有意差を認めた場合、Dunnett法またはScheff6法（群の大きさが異なる場合）により対照群に対する各群の比較検定を行った。親動物の生存率、交尾率、受胎率、出産率、出産児の性比、一般状態の変化および病理学的異常例の出現率などのカテゴリカルデータは、χ^2 検定を行った。なお、病理学的異常が対照群にも認められ、被験物質の影響が変化の程度の差として現れる所見については、データを適宜併合して2つのカテゴリーとし、検定した。

試験結果

１．反復投与毒性

１）死亡動物（Tables 1, 2, Appendices 9, 10）

死亡は各群の雌雄とも認められなかった。

２）一般状態（Tables 3, 4, Appendices 11, 12）

妊娠を成立させた雄において、赤色尿の排泄が600mg/kg群の10匹中９匹に認められた。分娩し哺育も順調であった雌においても600mg/kg群で、赤色尿の排泄が10匹中３匹に認められたほ

か削痩が４匹、被毛の汚れが２匹に認められた。これら以外にも雌雄に変化が認められたが、発現率が低く、用量依存的な変化でもなかった。10、40および150mg/kg群で認められた交配不成立を含む妊娠不成立の対あるいは出産後全児死亡の雌には、変化は認められなかった。

３）体重（Figures 1,2, Tables 5,6, Appendices 13,14）

雄において、600mg/kg群の投与22日以降の体重は対照群と比べて有意に低値を示し、投与期間中の体重増加量は有意に減少した。

雌においては、体重に有意な変化は認められなかったが、600mg/kg群で哺育期間中に体重が著しく減少する例が認められた。

４）摂餌量（Figures 3,4, Tables 7,8, Appendices 15,16）

600mg/kg群で、雄は投与29日まで、雌は投与1日、妊娠0日および哺育3日の摂餌量が対照群を下回る傾向にあったが、有意な変化ではなかった。

５）雄の尿所見（Table 9, Appendix 17）

150および600mg/kg群で、尿の混濁する例が有意に増加した。また、600mg/kg群で、沈渣中赤血球および白血球の有意な増加が認められた。さらに、40,150および600mg/kg群の沈渣中には多くは板状を呈する結晶物質が認められ、その形態は水中で析出した被験物質と類似したものであった。

６）雄の血液学所見（Table 10, Appendix 18, 背景データ：Appendix 30）

600mg/kg群で、赤血球数、血色素量およびヘマトクリット値の有意な減少が認められた。平均赤血球容積、平均赤血球血色素量および平均赤血球血色素濃度には変化は認められず、網状赤血球数は増加傾向にあったが、有意な変化ではなかった。

７）雄の血液生化学所見（Table 11, Appendix 19, 背景データ：Appendix 30）

600mg/kg群で、尿素窒素およびクレアチニンの有意な増加ならびにナトリウムの有意な減少が認められた。なお、GPT、γ-GTPおよびグルコースにも有意差が認められたが、いずれも用量依存的な変化でなく、また背景データにおける正常範囲内の変動であった。

８）剖検所見（Tables 12, 13, Appendices 20, 21）

妊娠を成立させた雄において、600mg/kg群で10匹中腎臓の腫大／退色が7匹、副腎の退色が6匹に認められた。分娩し哺育も順調であった雌においても、600mg/kg群で10匹中腎臓の腫大／

退色が全例、副腎の退色が5匹に認められた。さらに、胸腺の萎縮は対照群を含む150mg/kg以下の群で0～1匹に認められたのに対し、600mg/kg群では4匹と増加する傾向にあった。交配不成立を含む妊娠不成立の対においては、雌雄とも変化は認められなかった。40および150mg/kg群の各1匹に認められた分娩後全児死亡の雌においては、副腎の退色が共通して認められたほか、40mg/kg群の例で捕食した児動物の肉片と思われる多量の内容物による胃の膨満および胸腺の萎縮が認められた。以上の所見以外にも変化は認められたが、用量依存的でなく発現率も低かった。

9）器官重量（Tables 14, 15, Appendices 22～25）

600mg/kg群で雌雄に腎臓の絶対および相対重量、副腎の相対重量、雄に脾臓の相対重量、雌に脳の相対重量の有意な増加が認められた。また、雄の下垂体は150mg/kg群で絶対および相対重量、600mg/kg群で相対重量の有意な増加が認められた。なお、雄の肝臓、雌の甲状腺および下垂体にも有意差が認められたが、用量依存的な変化ではなかった。

10）病理組織学所見（Tables 16, 17, Appendices 20, 21, Photos 1～14）

被験物質の投与に起因すると考えられる変化が、600mg/kg群で雌雄の腎臓、膀胱、副腎および雌の胸腺に認められた。

妊娠を成立させた雄において、腎臓には10匹中全例に、ネフロン単位でび漫性の尿細管拡張が認められた。尿細管の拡張は遠位尿細管、集合管および乳頭管に認められ、近位尿細管も拡張する傾向にあった。多くの例で尿細管上皮の壊死および過形成、再生像と考えられる好塩基性尿細管の増加、間質の線維化、髄質に好中球の浸潤などを伴っており、拡張した尿細管には脱落した上皮細胞や浸潤細胞の集塊が認められた。また、皮質から皮髄境界部にかけて、鉱質沈着がみられる例もあった。膀胱には粘膜の過形成が2匹に認められ、その内の1匹の粘膜下織には好中球の浸潤が認められた。副腎では皮質束状帯細胞の空胞化が、対照群の1匹に対し6匹と増加した。分娩し哺育も順調であった雌においても、腎臓に雄と同様の変化が10匹中全例に認められたほか、近位尿細管上皮の空胞変性が8匹に認められた。また、膀胱には粘膜の過形成、副腎には束状帯細胞の空胞化がいずれも4匹に認められた。さらに、胸腺には皮質の萎縮が対照群にも2匹認られたが600mg/kgでは5匹に認められ、発現率の増加傾向が認められたほか、600mg/kg群の5匹中2匹の変化は、対照群の例に比べて強かった。交配不成立の40mg/kg群の1対では雄に変化は認められず、雌には肺に出血を伴う炎症性細胞浸潤巣が認められた。妊娠不成立の10mg/kg群の2対においては、雌の1匹に肺胞内水腫が認められたがごく軽度な変化で、その他の雌雄には異常は認められなかった。分娩後全児死亡の40および150mg/kg群の雌各1匹では、副腎皮質束状帯細胞の空胞化が共通して認められたほか、40mg/kg群の例で肝細胞および腎臓近位尿

細管上皮の脂肪変性、腺胃粘膜のびらん、胸腺皮質の萎縮が、150mg/kg群の例で前胃扁平上皮の過形成が認められた。雌雄の下垂体、生殖器系器官、雌の乳腺には異常は認められなかった。以上の所見以外にも変化は認められたが、用量依存性は認められなかった。

2．生殖発生毒性

1）親動物に及ぼす影響（Table 18, Appendix 26）

(1) 交尾率および受胎率

交尾は40mg/kg群の1対を除いて各群の全例に成立し、成立に要する期間にも有意な変化は認められなかった。受胎率も10mg/kg群は80%であったが、対照を含む他の群は全て100%であった。

(2) 黄体数、着床数および着床率

被験物質投与各群において、黄体数、着床数および着床率は対照群と類似した値を示し、有意な変化は認められなかった。

(3) 出産率および妊娠期間

出産率は、対照群および被験物質投与各群とも100%であった。なお、150mg/kg群の1匹は分娩確認時に全児が死亡していたが、その大部分の例の肺は吸気肺でしかも体表には咬傷が認められたことから、多くは出産後死亡あるいは食殺されたものと判断した。妊娠期間にも有意な変化は認められなかった。

(4) 分娩および哺育状態

分娩状態については、各群のいずれの動物にも異常は認められなかった。哺育状態についても、前述の分娩直後に全児が死亡あるいは食殺されたと思われる150mg/kg群の1匹および哺育3日までに全児が死亡した40mg/kg群の1匹が認められたが、用量依存的な変化ではなく、被験物質の投与の影響を示唆する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 19, Appendix 27）

被験物質投与各群の1腹当たりの総出産児数、新生児数、出生率、性比、哺育0日の体重ならびに哺育4日の生存率および体重には、いずれも対照群と比べて有意な変化が認められず、新生児の一般状態にも異常は認められなかった。

（２）形態（Tables 20, 21, Appendices 28, 29）

外表異常について、痕跡尾が対照群および150mg/kg群の各1匹に認められたが、被験物質の投与に起因すると考えられる異常は認められなかった。内臓異常はいずれの児動物にも認められなかった。内臓変異についても、胸腺の頸部遺残、左腎動脈遺残あるいは腎盂の拡張が総計対照群で5匹(3.1%)に対し被験物質投与各群で2～6匹(1.7～4.2%)の範囲で、有意な変化は認められなかった。

考察および結論

１．反復投与毒性

雌雄の親動物とも、腎臓および膀胱に対する毒性影響ならびにそれとの関連性が考えられる変化が600mg/kg群に認められた。150mg/kg以下の群では、被験物質の投与による毒性影響と考えられる変化は、認められなかった。析出した被験物質と思われる尿中の結晶物質は40および150mg/kg群にも認められたが、これらの用量では生体に有害と思われる変化を伴っていなかった。

すなわち、600mg/kg群で、腎臓においては、剖検で腫大、退色および重量の増加が認められた。組織学的には、尿細管上皮の壊死、脱落およびそれによる尿の停滞を示唆する尿細管の拡張を特徴とする変化であった。

腎臓および膀胱の組織標本では結晶物質の存在は確認できなかったが、尿中には析出した被験物質と思われる結晶物質が認められた。Cascieriら[5]はシアヌル酸ナトリウムのラットおよびマウスへの投与により発現する腎障害は、腎臓で析出したイソシアヌル酸の結晶による物理的な影響によることを報告している。本試験において認められた腎臓および膀胱の変化も、尿細管内で水分の再吸収に伴って析出した被験物質の結晶が起炎物質として作用して発現したものと推察される。類似した変化はサルファ剤、メチシリンなどでも報告されている[6]。

また、膀胱においても、刺激に対する反応性増殖と考えられる粘膜上皮の過形成が認められたが、変化は腎臓に比べて軽度なものであった。

雌雄の親動物で認められた赤色尿の排泄、雄親の検査で認められた尿沈渣中赤血球および白血球の増加、血液尿素窒素およびクレアチニンの増加、ナトリウムの減少は、いずれも主に腎臓の変化と関連する所見と考えられる。また、雄親の貧血所見も、骨髄および脾臓に造血能に対する影響や赤血球破壊亢進を示唆する変化が認められなかったことから、障害されたおそらく腎臓からの出血によるものと推察される。

雌雄の親動物とも、副腎の退色および相対重量の増加が認められ、皮質束状帯細胞に脂質の増加を示唆する空胞化が組織学的に観察された。また、雌親では胸腺皮質の萎縮する例が増加する傾向にあった。副腎および胸腺の変化は、イソシアヌル酸の毒性影響に対するストレスと

関連した二次的な変化と判断される。

これらの変化に加えて、雄親では体重増加の抑制が、雌親においても体重の平均値では有意な変化は認められなかったものの削痩する例が認められた。

なお、最終体重が対照群と比べて小さかった600mg/kg群で、雄は下垂体および脾臓、雌は脳のいずれも相対重量が増加し、下垂体重量の変化は150mg/kg群の雄にも認められたが、これらの器官に病理組織学的変化は認められなかった。したがって、下垂体、脾臓および脳の重量変化は主に体重の変化に伴う所見で、毒性影響を示唆する変化ではないと判断された。

以上の結果から、イソシアヌル酸のラットへの反復投与による主な毒性影響は腎臓および膀胱に認められ、副腎および胸腺に対する影響も認められた。無影響量は雌雄とも150mg/kg/dayと推定された。

2．生殖発生毒性

雄親および雌親の生殖能に対する被験物質の投与による影響について、観察した各指標とも対照群と比べ有意な変化は認められなかった。また、児動物の発生に関する指標に対しても、影響は認められなかった。

交配および妊娠の不成立の対、分娩後全児が死亡した雌親が投与量とは無関係に散発したが、いずれにも生殖能の異常を示唆する病理学的な異常は認められず、偶発的な変化と考えられた。

以上の結果から、雌雄親動物の生殖能および児動物の発生に対する影響は600mg/kg/day投与によっても認められず、無影響量はいずれも600mg/kg/dayと推定された。

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Table 1 Mortality rate of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	0	10	40	150	600
No. of animals	10	10	10	10	10
No. of animals that died	0	0	0	0	0
Mortality (%)	0	0	0	0	0

Table 2 Mortality rate of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	0	10	40	150	600
No. of animals	10	10	10	10	10
No. of animals that died	0	0	0	0	0
Mortality (%)	0	0	0	0	0

Table 3 Incidence of clinical signs of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Clinical sign	Dose(mg/kg)	0		10			40			150		600	
	Fate	TK	(Total)	TK	FP	(Total)	TK	UC	(Total)	TK	(Total)	TK	(Total)
	No. of animals	10	(10)	8	2	(10)	9	1	(10)	10	(10)	10	(10)
Reddish urine		0	(0)	0	0	(0)	0	0	(0)	0	(0)	9	(9)**
Chromodacryorrhea		0	(0)	0	0	(0)	1	0	(1)	1	(1)	0	(0)
Ptosis		0	(0)	0	0	(0)	0	0	(0)	1	(1)	0	(0)
Alopecia		0	(0)	0	0	(0)	0	0	(0)	1	(1)	0	(0)
Loss of upper incisors		0	(0)	0	0	(0)	1	0	(1)	0	(0)	0	(0)

TK : Terminal kill

UC : Animal with unsuccessful copulation

FP : Failed to cause pregnancy, killed at the termination

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

Table 4 Incidence of clinical signs of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Clinical sign	Dose(mg/kg)	0		10			40				150			600	
	Fate	TK	(Total)	TK	NP	(Total)	TK	UC	KL	(Total)	TK	KL	(Total)	TK	(Total)
	No. of animals	10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	10	(10)
Emaciation		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	4	(4)*
Reddish urine		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	3	(3)
Decrease in locomotor activity/ piloerection/hypothermia		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	1	(1)
Soiled fur		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	2	(2)
Alopecia/scabbing		0	(0)	0	0	(0)	0	0	0	(0)	1	0	(1)	1	(1)

TK : Terminal kill

NP : Non-pregnant, killed on 26 days after copulation

UC : Animal with unsuccessful copulation

KL : Killed because all pups died after delivery

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

Table 5 Body weights of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

(g)

Dose (mg/kg)	Days of treatment								
	1	8	15	22	29	36	43	44	Gain 1~44
0	343 ± 13 (10)	376 ± 21 (10)	408 ± 28 (10)	435 ± 30 (10)	463 ± 35 (10)	490 ± 40 (10)	503 ± 45 (10)	505 ± 44 (10)	163 ± 33 (10)
10	343 ± 13 (10)	390 ± 15 (10)	431 ± 14 (10)	458 ± 15 (10)	490 ± 16 (10)	511 ± 15 (10)	525 ± 22 (10)	528 ± 22 (10)	185 ± 20 (10)
40	343 ± 12 (10)	383 ± 21 (10)	420 ± 26 (10)	449 ± 26 (10)	478 ± 27 (10)	502 ± 29 (10)	511 ± 36 (10)	514 ± 36 (10)	171 ± 27 (10)
150	343 ± 11 (10)	385 ± 11 (10)	422 ± 19 (10)	443 ± 26 (10)	475 ± 27 (10)	499 ± 36 (10)	514 ± 40 (10)	518 ± 42 (10)	174 ± 34 (10)
600	344 ± 12 (10)	358 ± 30 (10)	391 ± 25 (10)	402* ± 20 (10)	425** ± 18 (10)	453* ± 25 (10)	461* ± 30 (10)	464* ± 33 (10)	120** ± 27 (10)

Each value is expressed as mean±S.D. and (number of animals examined).
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 6 Body weights of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

(g)

Dose (mg/kg)	Days of premating				Days of pregnancy					Days of lactation		
	1	8	15	Gain 1~15	0	7	14	20	Gain 0~20	0	4	Gain 0~4
0	223 ± 5 (10)	246 ± 10 (10)	265 ± 13 (10)	43 ± 11 (10)	271 ± 11 (10)	311 ± 11 (10)	353 ± 12 (10)	451 ± 16 (10)	180 ± 10 (10)	330 ± 22 (10)	345 ± 16 (10)	15 ± 16 (10)
10	223 ± 5 (10)	240 ± 10 (10)	258 ± 11 (10)	36 ± 10 (10)	265 ± 16 (8)	302 ± 13 (8)	344 ± 15 (8)	436 ± 18 (8)	171 ± 12 (8)	316 ± 24 (8)	336 ± 17 (8)	21 ± 17 (8)
40	222 ± 5 (10)	247 ± 9 (10)	263 ± 11 (10)	41 ± 9 (10)	273 ± 7 (9)	314 ± 6 (9)	359 ± 8 (9)	456 ± 13 (9)	182 ± 16 (9)	338 ± 28 (9)	359 ± 14 (8)	16 ± 20 (8)
150	223 ± 4 (10)	245 ± 7 (10)	262 ± 9 (10)	39 ± 6 (10)	274 ± 13 (10)	308 ± 10 (10)	346 ± 15 (10)	432 ± 34 (10)	157 ± 31 (10)	343 ± 18 (10)	350 ± 16 (9)	4 ± 10 (9)
600	222 ± 5 (10)	227 ± 28 (10)	252 ± 19 (10)	30 ± 17 (10)	261 ± 17 (10)	291 ± 23 (10)	337 ± 19 (10)	429 ± 26 (10)	168 ± 18 (10)	309 ± 27 (10)	307 ± 38 (10)	-2 ± 25 (10)

Each value is expressed as mean±S.D. and (number of animals available).

Table 7 Food consumption of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

(g/rat/day)

Dose (mg/kg)	Days of treatment					
	1	8	22	29	36	43
0	24 ± 2 (10)	27 ± 4 (10)	28 ± 3 (10)	28 ± 3 (10)	29 ± 3 (10)	27 ± 2 (10)
10	25 ± 2 (10)	30 ± 2 (10)	27 ± 1 (10)	28 ± 3 (10)	27 ± 3 (10)	26 ± 2 (10)
40	25 ± 3 (10)	28 ± 3 (10)	27 ± 2 (9)	27 ± 2 (10)	24 ± 8 (10)	26 ± 4 (10)
150	24 ± 2 (10)	28 ± 4 (10)	28 ± 2 (10)	28 ± 4 (10)	27 ± 3 (10)	28 ± 4 (10)
600	18 ±10 (10)	24 ± 6 (10)	24 ± 7 (10)	24 ± 5 (10)	28 ± 3 (10)	27 ± 5 (10)

Each value is expressed as mean±S.D. and (number of animals examined).

Table 8 Food consumption of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

(g/rat/day)

Dose (mg/kg)	Days of premating		Days of pregnancy				Days of lactation	
	1	8	0	7	14	20	0	3
0	19 ± 3 (10)	20 ± 3 (10)	20 ± 3 (10)	25 ± 3 (10)	28 ± 3 (10)	23 ± 3 (10)	14 ±10 (10)	52 ± 8 (10)
10	17 ± 3 (10)	20 ± 3 (10)	19 ± 2 (8)	25 ± 3 (8)	25 ± 3 (8)	23 ± 4 (8)	17 ±11 (8)	49 ± 3 (8)
40	17 ± 3 (10)	21 ± 3 (10)	20 ± 4 (9)	26 ± 3 (9)	27 ± 3 (9)	25 ± 4 (9)	15 ±10 (9)	49 ± 6 (8)
150	18 ± 3 (10)	20 ± 4 (10)	20 ± 2 (10)	24 ± 3 (10)	26 ± 2 (10)	25 ± 3 (10)	11 ± 7 (9)	42 ±12 (9)
600	16 ± 5 (10)	19 ± 6 (10)	15 ± 4 (10)	23 ± 7 (10)	27 ± 4 (10)	22 ± 7 (10)	12 ±11 (10)	37 ±22 (10)

Each value is expressed as mean±S.D. and (number of animals available).

Table 9 - 1 Urinary findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	No. of animals	Color				Cloudy		Specific gravity	pH							Protein					
		C	PY	Y	PB	−	+		5.0	6.0	6.5	7.0	7.5	8.0	8.5	−	±	+	++	+++	++++
0	10		4	3	3	7	3	1.050 ± 0.021 a)			1	1	1	2	5		2	6	2		
10	10		3	5	2	5	5	1.068 ± 0.031			1		4	5				5	4	1	
40	10		4	5	1	3	7	1.051 ± 0.019			2	2		4	2			10			
150	10	1	5	2	2		10**	1.056 ± 0.018				2	3	2	3			5	5		
600	10		9		1	1	9*	1.033 ± 0.012		1	1	4	1	3			1	9			

Dose (mg/kg)	No. of animals	Glucose					Ketone body					Occult blood					Urobilinogen				Bilirubin			
		−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	0.1	1	2	4	−	+	++	+++
0	10	10					4	4	1	1		7	3				10				10			
10	10	10					1	6	3			9	1				10				10			
40	10	10					5	5				8	2				10				10			
150	10	10					2	7	1			9	1				10				10			
600	10	10					6	4				7		1	1	1	10				10			

a) : Mean±S.D.
Color : C(colorless), PY(pale yellow), Y(yellow), PB(pale brown)
Cloudy : −(negligible), +(cloudy)
Protein : −(negligible), ±(15～30mg/dl), +(30mg/dl), ++(100mg/dl), +++(300mg/dl), ++++(1000mg/dl)
Glucose : −(negligible), ±(0.1g/dl), +(0.25g/dl), ++(0.5g/dl), +++(1g/dl)
Ketone body : −(negligible), ±(5mg/dl), +(15mg/dl), ++(40mg/dl), +++(80mg/dl)
Occult blood : −(negligible), ±(trace), +(slight), ++(moderate), +++(marked)
Urobilinogen : Ehrlich unit/dl
Bilirubin : −(negligible), +(slight), ++(moderate), +++(marked)
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 9 - 2 Urinary findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	No. of animals	Erythrocytes				Leukocytes				Crystals: Mg				Ca		Ams		Others			
		−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	−	+	−	+	++	+++
0	10	10				10				1	4	4	1	10		10		10			
10	10	10				9	1			2	2	5	1	10		10		10			
40	10	10				10				4		4	2	10		10		6	3	1	
150	10	10				10				1	3	5	1	10		10			5	3	2**
600	10	7	1	1	1*	5	1	4**		4	3	3		10		10		2	5	2	1**

Dose (mg/kg)	No. of animals	Epithelial cells: Sq				R			S			Casts: G		H		W		Fat globules		
		−	+	++	+++	−	+	++	−	+	++	−	+	−	+	−	+	−	+	++
0	10	1	8	1		10			10			10		10		10		10		
10	10	1	4	5		10			10			10		10		10		10		
40	10	1	9			10			10			10		10		10		10		
150	10		9	1		10			10			10		10		10		10		
600	10		10			10			10			10		10		10		10		

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

Crystals
- Mg(ammonium magnesium phosphate)
- Ca(calcium phosphate)
- Ams(amorphous)
- Others(crystals considered to be the test substance precipitated from urine)

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

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

* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 10 Hematological findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	No. of animals	RBC ($10^4/\mu l$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (%)	Ret. (%.)	WBC ($10^2/\mu l$)	Plat. ($10^4/\mu l$)	PT (sec)	APTT (sec)
0	10	805 ± 47	14.9 ± 0.4	43.7 ± 0.9	55 ± 3	18.5 ± 0.8	34.0 ± 0.4	26 ± 10	69 ± 14	133 ± 18	13.0 ± 0.3	19.4 ± 1.5
10	10	806 ± 29	14.9 ± 0.6	43.9 ± 1.3	54 ± 2	18.5 ± 0.9	33.9 ± 0.5	26 ± 7	71 ± 16	131 ± 15	13.3 ± 0.4	18.5 ± 0.6
40	10	821 ± 29	15.0 ± 0.5	44.2 ± 1.4	54 ± 2	18.3 ± 0.7	33.9 ± 0.5	23 ± 7	79 ± 34	137 ± 9	13.3 ± 0.3	19.6 ± 0.6
150	10	804 ± 28	15.0 ± 0.4	44.0 ± 1.0	55 ± 1	18.6 ± 0.3	34.0 ± 0.4	21 ± 5	59 ± 12	136 ± 9	13.2 ± 0.9	19.4 ± 1.0
600	10	752** ± 32	13.6** ± 0.5	40.5** ± 1.4	54 ± 1	18.1 ± 0.4	33.7 ± 0.5	32 ± 18	72 ± 20	147 ± 10	13.3 ± 0.2	18.9 ± 0.8

Each value is expressed as mean±S.D.
** : Significantly different from control at 1% level of probability

Table 11 Blood biochemical findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	No. of animals	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	γ-GTP (IU/l)	T.P. (g/dl)	Alb. (g/dl)	A/G	T-Cho. (mg/dl)	T.G. (mg/dl)
0	10	57 ± 5	33 ± 5	257 ± 68	0.34 ± 0.14	6.21 ± 0.15	3.14 ± 0.12	1.03 ± 0.10	70 ± 16	73 ± 31
10	10	52 ± 4	27** ± 3	261 ± 47	0.25 ± 0.20	6.27 ± 0.24	3.20 ± 0.21	1.04 ± 0.12	83 ± 17	83 ± 40
40	10	50 ± 4	27** ± 3	240 ± 50	0.70 ± 0.78	6.33 ± 0.17	3.26 ± 0.16	1.07 ± 0.08	71 ± 10	83 ± 34
150	10	53 ± 10	28* ± 5	262 ± 57	0.50 ± 0.43	6.35 ± 0.23	3.25 ± 0.10	1.06 ± 0.07	76 ± 14	88 ± 37
600	10	55 ± 7	27** ± 5	254 ± 38	0.68* ± 0.21	6.21 ± 0.26	3.18 ± 0.14	1.05 ± 0.10	85 ± 11	69 ± 30

Dose (mg/kg)	No. of animals	Glu. (mg/dl)	T-Bil. (mg/dl)	BUN (mg/dl)	Crea. (mg/dl)	Ca (mg/dl)	P (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
0	10	141 ± 14	0.30 ± 0.02	14.2 ± 2.8	0.57 ± 0.05	10.1 ± 0.3	7.3 ± 0.4	142.9 ± 0.9	4.20 ± 0.25	101 ± 1
10	10	156* ± 11	0.28 ± 0.03	13.8 ± 1.4	0.57 ± 0.05	10.2 ± 0.3	7.2 ± 0.6	142.4 ± 0.8	4.36 ± 0.22	101 ± 1
40	10	151 ± 9	0.28 ± 0.02	12.0 ± 1.0	0.57 ± 0.05	10.3 ± 0.2	7.5 ± 0.6	143.0 ± 1.1	4.13 ± 0.19	101 ± 1
150	10	155 ± 17	0.31 ± 0.03	13.3 ± 1.1	0.58 ± 0.05	10.3 ± 0.3	7.3 ± 0.7	143.2 ± 0.9	4.22 ± 0.31	101 ± 1
600	10	140 ± 6	0.29 ± 0.04	38.2** ± 12.8	1.08** ± 0.37	10.4 ± 0.2	8.5 ± 1.4	141.6* ± 1.6	4.46 ± 0.44	100 ± 1

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 12 Incidence of necropsy findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose(mg/kg) 0	10	40	150	600
		Fate: TK (T)	TK FP (T)	TK UC (T)	TK (T)	TK (T)
		No. of animals: 10 (10)	8 2 (10)	9 1 (10)	10 (10)	10 (10)
Liver : Diaphragmatic nodule	-	10 (10)	7 2 (9)	9 1 (10)	10 (10)	10 (10)
	+	0 (0)	1 0 (1)	0 0 (0)	0 (0)	0 (0)
Kidney : Enlargement	-	10 (10)	8 2 (10)	9 1 (10)	10 (10)	3 (3)
	+	0	0 0	0 0	0	4
	++	0)(0)	0 0)(0)	0 0)(0)	0) (0)	3)(7)**
Decoloration	-	10 (10)	8 2 (10)	9 1 (10)	10 (10)	3 (3)
	+	0	0 0	0 0	0	5
	++	0)(0)	0 0)(0)	0 0)(0)	0) (0)	2)(7)**
Adrenal : Decoloration	-	10 (10)	8 2 (10)	9 1 (10)	10 (10)	4 (4)
	+	0 (0)	0 0 (0)	0 0 (0)	0 (0)	6 (6)**
Skin : Alopecia	-	10 (10)	8 2 (10)	9 1 (10)	9 (9)	10 (10)
	+	0 (0)	0 0 (0)	0 0 (0)	1 (1)	0 (0)

- : Negative; + : Slight; ++ : Moderate; TK : Terminal kill; FP : Failed to cause pregnancy, killed at the termination;
UC : Animal with unsuccessful copulation, killed at the termination; T : Total
** : Significantly different from control at 1% level of probability

Table 13 Incidence of necropsy findings of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose(mg/kg)	0	10	40	150	600
		Fate	TK (T)	TK NP (T)	TK UC KL (T)	TK KL (T)	TK (T)
		No. of animals	10 (10)	8 2 (10)	8 1 1 (10)	9 1 (10)	10 (10)
Stomach : Distention	-		10 (10)	8 2 (10)	8 1 0 (9)	9 1 (10)	10 (10)
	++		0 (0)	0 0 (0)	0 0 1 (1)	0 0 (0)	0 (0)
Kidney : Enlargement	-		10 (10)	8 2 (10)	8 1 1 (10)	9 1 (10)	0 (0)
	+		0	0 0	0 0 0	0 0	4
	++		0 (0)	0 0 (0)	0 0 0 (0)	0 0 (0)	6 (10)**
Decoloration	-		10 (10)	8 2 (10)	8 1 1 (10)	9 1 (10)	1 (1)
	+		0	0 0	0 0 0	0 0	6
	++		0 (0)	0 0 (0)	0 0 0 (0)	0 0 (0)	2 (9)**
	+++		0	0 0	0 0 0	0 0	1
Adrenal : Decoloration	-		10 (10)	8 2 (10)	8 1 0 (9)	9 0 (9)	5 (5)
	+		0	0 0	0 0 1	0 1	2
	++		0 (0)	0 0 (0)	0 0 0 (1)	0 0 (1)	3 (5)**
Thymus : Atrophy	-		9 (9)	7 2 (9)	8 1 0 (9)	9 1 (10)	6 (6)
	+		1 (1)	1 0 (1)	0 0 1 (1)	0 0 (0)	4 (4)
Skin : Alopecia	-		10 (10)	8 2 (10)	8 1 1 (10)	9 1 (10)	9 (9)
	+		0 (0)	0 0 (0)	0 0 0 (0)	0 0 (0)	1 (1)

- : Negative; + : Slight; ++ : Moderate; +++ : Marked; TK : Terminal kill; NP : Non-pregnant; UC : Animal with unsuccessful copulation; KL : Killed because all pups died after delivery; T : Total
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 14 Absolute and relative organ weights of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

	Dose (mg/kg)	No. of animals	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyr. (mg)	Pitui. (mg)	Adrenal (mg)	Testis (g)	Epidid. (g)
Absolute	0	10	483 ± 41	2.14 ±0.08	13.68 ± 2.09	3.01 ±0.34	0.74 ±0.09	1.49 ±0.08	0.35 ±0.06	30.3 ± 7.9	15.6 ± 2.2	63.8 ± 8.9	3.60 ±0.26	1.45 ±0.17
	10	10	508 ± 19	2.10 ±0.07	15.71* ± 1.33	3.36 ±0.37	0.79 ±0.08	1.48 ±0.06	0.33 ±0.07	34.2 ± 5.0	16.6 ± 2.0	63.2 ± 6.2	3.47 ±0.24	1.37 ±0.11
	40	10	492 ± 32	2.07 ±0.06	14.35 ± 1.47	3.06 ±0.29	0.81 ±0.11	1.58 ±0.15	0.34 ±0.08	36.3 ± 4.9	16.3 ± 0.8	63.2 ± 7.5	3.40 ±0.14	1.36 ±0.09
	150	10	495 ± 38	2.06 ±0.07	14.76 ± 1.48	3.12 ±0.15	0.78 ±0.06	1.48 ±0.12	0.31 ±0.08	38.2 ± 6.3	19.8** ± 2.8	66.4 ±14.5	3.41 ±0.27	1.41 ±0.13
	600	10	444* ± 31	2.08 ±0.08	12.08 ± 1.62	4.62** ±0.96	0.83 ±0.10	1.38 ±0.11	0.28 ±0.07	33.6 ± 6.4	17.4 ± 2.7	72.1 ±10.8	3.32 ±0.24	1.32 ±0.10
Relative@	0	10	483 ± 41	0.45 ±0.03	2.82 ±0.26	0.62 ±0.03	0.16 ±0.01	0.31 ±0.02	0.07 ±0.02	6.32 ± 1.71	3.23 ± 0.42	13.18 ± 1.19	0.75 ±0.06	0.30 ±0.04
	10	10	508 ± 19	0.41 ±0.02	3.09* ±0.22	0.66 ±0.08	0.16 ±0.02	0.29 ±0.02	0.07 ±0.01	6.75 ± 1.01	3.29 ± 0.49	12.46 ± 1.27	0.68 ±0.04	0.27 ±0.02
	40	10	492 ± 32	0.42 ±0.03	2.92 ±0.19	0.62 ±0.05	0.16 ±0.01	0.32 ±0.02	0.07 ±0.02	7.38 ± 0.98	3.33 ± 0.22	12.88 ± 1.55	0.69 ±0.03	0.28 ±0.02
	150	10	495 ± 38	0.42 ±0.04	2.98 ±0.17	0.63 ±0.05	0.16 ±0.02	0.30 ±0.02	0.06 ±0.01	7.80 ± 1.71	4.02* ± 0.61	13.39 ± 2.64	0.69 ±0.07	0.28 ±0.03
	600	10	444* ± 31	0.47 ±0.03	2.71 ±0.21	1.04** ±0.21	0.19** ±0.02	0.31 ±0.02	0.07 ±0.02	7.61 ± 1.62	3.95* ± 0.68	16.23** ± 2.14	0.75 ±0.07	0.30 ±0.03

Each value is expressed as mean ± S.D.
@ : Relative organ weight per 100g body weight
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 15 Absolute and relative organ weights of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

	Dose (mg/kg)	No. of animals	B.W. (g)	Brain (g)	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Thymus (g)	Thyr. (mg)	Pitui. (mg)	Adrenal (mg)
Absolute	0	10	345 ± 16	1.88 ±0.06	13.93 ± 1.19	1.89 ±0.13	0.64 ±0.06	1.04 ±0.06	0.20 ±0.06	25.4 ± 3.5	18.6 ± 2.0	73.8 ± 10.1
	10	8	336 ± 17	1.90 ±0.06	14.20 ± 1.22	1.89 ±0.13	0.64 ±0.09	1.01 ±0.06	0.22 ±0.09	28.1 ± 3.8	21.6 ± 3.5	77.2 ± 13.3
	40	8	359 ± 14	1.94 ±0.05	14.55 ± 1.10	1.83 ±0.11	0.71 ±0.10	1.07 ±0.09	0.26 ±0.06	30.7* ± 3.2	19.5 ± 1.7	76.5 ± 6.6
	150	9	350 ± 16	1.91 ±0.08	13.87 ± 1.46	1.93 ±0.10	0.66 ±0.07	1.07 ±0.10	0.25 ±0.09	26.2 ± 3.7	22.0* ± 2.0	74.0 ± 10.0
	600	10	307 ± 38	1.88 ±0.09	12.33 ± 2.00	2.97* ±0.41	0.63 ±0.13	0.98 ±0.17	0.15 ±0.08	24.4 ± 3.9	17.7 ± 3.0	80.9 ± 10.3
Relative@	0	10	345 ± 16	0.55 ±0.02	4.04 ±0.32	0.55 ±0.04	0.19 ±0.02	0.30 ±0.02	0.06 ±0.02	7.36 ± 0.90	5.42 ± 0.64	21.47 ± 3.29
	10	8	336 ± 17	0.57 ±0.03	4.22 ±0.28	0.56 ±0.04	0.19 ±0.02	0.30 ±0.01	0.06 ±0.02	8.37 ± 1.09	6.39* ± 0.78	23.02 ± 4.17
	40	8	359 ± 14	0.54 ±0.03	4.06 ±0.35	0.51 ±0.03	0.20 ±0.03	0.30 ±0.03	0.07 ±0.02	8.58 ± 0.96	5.46 ± 0.61	21.36 ± 2.17
	150	9	350 ± 16	0.55 ±0.03	3.96 ±0.44	0.55 ±0.03	0.19 ±0.03	0.31 ±0.03	0.07 ±0.02	7.50 ± 1.28	6.31* ± 0.69	21.23 ± 3.45
	600	10	307 ± 38	0.62* ±0.06	4.02 ±0.44	0.99** ±0.22	0.20 ±0.03	0.32 ±0.02	0.05 ±0.02	7.94 ± 0.81	5.76 ± 0.53	26.69* ± 4.65

Each value is expressed as mean ± S.D.
@ : Relative organ weight per 100g body weight
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 16 - 1 Incidence of histopathological findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose(mg/kg) 0		10			40			150		600	
		Fate	TK	(T)	TK	FP	(T)	TK	UC	(T)	TK	(T)	TK	(T)
		No. of animals	10	(10)	8	2	(10)	9	1	(10)	10	(10)	10	(10)
Kidney : Necrosis, tubular epithelium	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	2	(2)
	+		0	(0)	0	0	(0)	0	0	(0)	0	(0)	8	(8)**
Mineralization, cortex/cortico-medullary junction	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	6	(6)
	+		0	(0)	0	0	(0)	0	0	(0)	0	(0)	4	(4)*
Cellular infiltration, lymphocyte, cortex	-		9	(9)	8	2	(10)	9	1	(10)	10	(10)	10	(10)
	++		1	(1)	0	0	(0)	0	0	(0)	0	(0)	0	(0)
Cellular infiltration, neutrophile, medulla	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	0	(0)
	+		0		0	0		0	0		0		7	
	++		0	(0)	0	0	(0)	0	0	(0)	0	(0)	3	(10)**
Eosinophilic body, proximal tubular epithelium	-		8	(8)	4	1	(5)	7	1	(8)	8	(8)	10	(10)
	+		1		2	0		1	0		1		0	
	++		1	(2)	2	1	(5)	1	0	(2)	1	(2)	0	(0)
Dilatation, distal/collecting tubules, focal	-		10	(10)	6	2	(8)	9	1	(10)	9	(9)	10	(10)
	+		0	(0)	2	0	(2)	0	0	(0)	1	(1)	0	(0)
Dilatation, renal tubule, diffuse	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	0	(0)
	+		0		0	0		0	0		0		1	
	++		0	(0)	0	0	(0)	0	0	(0)	0	(0)	6	(10)**
	+++		0		0	0		0	0		0		3	
Basophilic tubules	-		5		2	1		6	1		5		0	
	+		5	(10)	5	1	(9)	3	0	(10)	5	(10)	1	(1)
	++		0		1	0		0	0		0		7	
	+++		0	(0)	0	0	(1)	0	0	(0)	0	(0)	2	(9)**
Hyperplasia, tubular epithelium	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	2	(2)
	+		0	(0)	0	0	(0)	0	0	(0)	0	(0)	8	(8)**
Fibrosis	-		9	(9)	7	2	(9)	9	1	(10)	9	(9)	3	(3)
	+		0		1	0		0	0		1		7	
	++		1	(1)	0	0	(1)	0	0	(0)	0	(1)	0	(7)**
Cyst	-		8	(8)	8	2	(10)	9	1	(10)	8	(8)	10	(10)
	+		1		0	0		0	0		2		0	
	++		1	(2)	0	0	(0)	0	0	(0)	0	(2)	0	(0)
Heart : Myocardial degeneration/fibrosis, focal	-		9	(9)	—	2	(2)	—	1	(1)	—	—	10	(10)
	+		1	(1)	—	0	(0)	—	0	(0)	—	—	0	(0)

— : Not examined; - : Negative; + : Slight; ++ : Moderate; +++ : Marked; TK : Terminal kill; FP : Failed to cause pregnancy, killed at the termination; UC : Animal with unsuccessful copulation, killed at the termination; T : Total

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

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

Table 15 - 2 Incidence of histopathological findings of male rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose(mg/kg) 0		10			40			150		600	
		Fate	TK	(T)	TK	FP	(T)	TK	UC	(T)	TK	(T)	TK	(T)
		No. of animals	10	(10)	8	2	(10)	9	1	(10)	10	(10)	10	(10)
Lung : Mineralization, artery	-		8	(8)	—	1	(1)	—	1	(1)	—	—	10	(10)
	+		2	(2)	—	1	(1)	—	0	(0)	—	—	0	(0)
Metaplasia, osseous	-		8	(8)	—	2	(2)	—	1	(1)	—	—	7	(7)
	+		2	(2)	—	0	(0)	—	0	(0)	—	—	3	(3)
Accumulation, foam cell	-		9	(9)	—	2	(2)	—	1	(1)	—	—	8	(8)
	+		1	(1)	—	0	(0)	—	0	(0)	—	—	2	(2)
Liver : Microgranuloma	-		6	(6)	1*	2	(3)	—	1	(1)	—	—	6	(6)
	+		4	(4)	0	0	(0)	—	0	(0)	—	—	4	(4)
Fibrosis, capsule	-		10	(10)	0	2	(2)	—	1	(1)	—	—	10	(10)
	+		0	(0)	1*	0	(1)	—	0	(0)	—	—	0	(0)
Hyperplasia, bile duct	-		10	(10)	0	2	(2)	—	1	(1)	—	—	10	(10)
	+		0	(0)	1*	0	(1)	—	0	(0)	—	—	0	(0)
Hemorrhage	-		10	(10)	0	2	(2)	—	1	(1)	—	—	10	(10)
	+		0	(0)	1*	0	(1)	—	0	(0)	—	—	0	(0)
Pancreas: Proliferation, ductule	-		8	(8)	—	1	(1)	—	1	(1)	—	—	9	(9)
	+		2	(2)	—	1	(1)	—	0	(0)	—	—	1	(1)
Stomach : Hyperplasia, squamous, limiting ridge	-		9	(9)	—	2	(2)	—	1	(1)	—	—	10	(10)
	+		1	(1)	—	0	(0)	—	0	(0)	—	—	0	(0)
Urinary bladder : Hyperplasia, mucosal epithelium	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	8	(8)
	+		0	(0)	0	0	(0)	0	0	(0)	0	(0)	2	(2)
Cellular infiltration, neutrophile, submucosa	-		10	(10)	8	2	(10)	9	1	(10)	10	(10)	9	(9)
	+		0	(0)	0	0	(0)	0	0	(0)	0	(0)	1	(1)
Testis : Atrophy, seminiferous tubule, focal	-		9	(9)	—	2	(2)	—	1	(1)	—	—	10	(10)
	+		1	(1)	—	0	(0)	—	0	(0)	—	—	0	(0)
Prostate: Cellular infiltration, lymphocyte, interstitium	-		9	(9)	—	2	(2)	—	1	(1)	—	—	9	(9)
	+		1	(1)	—	0	(0)	—	0	(0)	—	—	1	(1)
Pituitary : Cyst, Rathke's pouch, anterior lobe	-		10	(10)	—	2	(2)	—	1	(1)	—	—	9	(9)
	+		0	(0)	—	0	(0)	—	0	(0)	—	—	1	(1)
Adrenal : Vacuolization, zona fasciculata	-		9	(9)	8	2	(10)	8	1	(9)	9	(9)	4	(4)
	+		1		0	0		1	0		1		5	
	++		0	(1)	0	0	(0)	0	0	(1)	0	(1)	1	(6)*

— : Not examined; - : Negative; + : Slight; ++ : Moderate; TK : Terminal kill; FP : Failed to cause pregnancy, killed at the termination; UC : Animal with unsuccessful copulation, killed at the termination; T : Total

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

The organs of the heart, lung, liver, pancreas, stomach, intestine, kidney, urinary bladder, testis, epididymis, seminal vesicle, prostate, pituitary, thyroid, parathyroid, adrenal, thymus, spleen, bone marrow, lymph node and brain were examined from animals of the control and 600 mg/kg groups, and UC and FP animals. The skin from an animal of the 150 mg/kg group, which had a macroscopic skin lesion, was also examined.

* : The liver with diaphragmatic nodule from one animal was examined.

Table 17 - 1 Incidence of histopathological findings of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose(mg/kg) 0		10			40				150			600	
		Fate	TK	(T)	TK	NP	(T)	TK	UC	KL	(T)	TK	KL	(T)	TK	(T)
		No. of animals	10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	10	(10)
Kidney : Degeneration, fatty, proximal tubular epithelium	-		10	(10)	8	2	(10)	8	1	0	(9)	9	1	(10)	10	(10)
	++		0	(0)	0	0	(0)	0	0	1	(1)	0	0	(0)	0	(0)
Degeneration, vacuolar, proximal tubular epithelium	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	2	(2)
	+		0		0	0		0	0	0		0	0		3	
	++		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	5	(8)**
Necrosis, tubular epithelium	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	5	(5)
	+		0		0	0		0	0	0		0	0		4	
	++		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	1	(5)**
Mineraiization, cortex	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	8	(8)
	+		0		0	0		0	0	0		0	0		1	
	++		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	1	(2)
Cellular infiltration, neutrophile, medulla	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	5	(5)
	+		0		0	0		0	0	0		0	0		2	
	++		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	3	(5)**
Dilatation, distal tubule, focal/multifocal	-		9	(9)	8	2	(10)	8	1	0	(9)	8	1	(9)	10	(10)
	+		1	(1)	0	0	(0)	0	0	1	(1)	1	0	(1)	0	(0)
Dilatation renal tubule, diffuse	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	0	(0)
	+		0		0	0		0	0	0		0	0		5	
	++		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	3	(10)**
	+++		0		0	0		0	0	0		0	0		2	
Basophilic tubules	-		9	(9)	7	2	(9)	7	1	0	(8)	6	1	(7)	0	(0)
	+		1		1	0		1	0	1		3	0		1	
	++		0	(1)	0	0	(1)	0	0	0	(2)	0	0	(3)	8	(10)**
	+++		0		0	0		0	0	0		0	0		1	
Hyperplasia, tubular epithelium	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	3	(3)
	+		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	7	(7)**
Fibrosis	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	7	(7)
	+		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	3	(3)
Cast hyaline/proteinous/granular	-		10	(10)	7	2	(9)	8	1	0	(9)	9	0	(9)	10	(10)
	+		0	(0)	1	0	(1)	0	0	1	(1)	0	1	(1)	0	(0)
Heart : Myocardial degeneration/fibrosis, focal	-		9	(9)	—	2	(2)	—	1	1	(2)	—	0	(0)	10	(10)
	+		1	(1)	—	0	(0)	—	0	0	(0)	—	1	(1)	0	(0)
Lung : Inflammatory cell infiltration, focal	-		10	(10)	—	2	(2)	—	0	1	(1)	—	1	(1)	10	(10)
	+		0	(0)	—	0	(0)	—	1	0	(1)	—	0	(0)	0	(0)
Edema, alveolar	-		10	(10)	—	1	(1)	—	1	1	(2)	—	1	(1)	10	(10)
	+		0	(0)	—	1	(1)	—	0	0	(0)	—	0	(0)	0	(0)
Hemorrhage	-		10	(10)	—	2	(2)	—	0	1	(1)	—	1	(1)	10	(10)
	+		0	(0)	—	0	(0)	—	1	0	(1)	—	0	(0)	0	(0)

— : Not examined; - : Negative; + : Slight; ++ : Moderate; +++ : Marked; TK : Terminal kill; NP : Non-pregnant; UC : Animal with unsuccessful copulation; KL : Killed because all pups died after delivery; T : Total
* : Significantly different from control at 5% level of probability
** : Significantly different from control at 1% level of probability

Table 17 - 2 Incidence of histopathological findings of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Organ : Findings	Degree	Dose (mg/kg) 0		10			40				150			600	
		Fate	TK	(T)	TK	NP	(T)	TK	UC	KL	(T)	TK	KL	(T)	TK	(T)
		No. of animals	10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	10	(10)
Lung : Mineralization, artery	-		9	(9)	—	2	(2)	—	1	1	(2)	—	1	(1)	10	(10)
	+		1	(1)	—	0	(0)	—	0	0	(0)	—	0	(0)	0	(0)
Metaplasia, osseous	-		10	(10)	—	2	(2)	—	1	1	(2)	—	1	(1)	8	(8)
	+		0	(0)	—	0	(0)	—	0	0	(0)	—	0	(0)	2	(2)
Accumulation, foam cell	-		8	(8)	—	2	(2)	—	1	0	(1)	—	0	(0)	8	(8)
	+		2	(2)	—	0	(0)	—	0	1	(1)	—	1	(1)	2	(2)
Liver : Degeneration, fatty, hepatocyte, periportal	-		10	(10)	8	2	(10)	7	1	0	(8)	9	1	(10)	8	(8)
	+		0		0	0		1	0	0		0	0		2	
	++		0	)(0)	0	0	)(0)	0	0	1	)(2)	0	0	)(0)	0	)(2)
Necrosis, focal	-		10	(10)	7	2	(9)	7	1	1	(9)	9	1	(10)	9	(9)
	+		0	(0)	1	0	(1)	1	0	0	(1)	0	0	(0)	1	(1)
Microgranuloma	-		10	(10)	7	1	(8)	8	1	1	(10)	6	1	(7)	10	(10)
	+		0	(0)	1	1	(2)	0	0	0	(0)	3	0	(3)	0	(0)
Pancreas: Proliferation, ductule	-		8	(8)	—	2	(2)	—	1	1	(2)	—	1	(1)	9	(9)
	+		2	(2)	—	0	(0)	—	0	0	(0)	—	0	(0)	1	(1)
Hypertrophic foci, acinar cell	-		10	(10)	—	2	(2)	—	1	1	(2)	—	1	(1)	9	(9)
	+		0	(0)	—	0	(0)	—	0	0	(0)	—	0	(0)	1	(1)
Stomach : Hyperplasia, squamous, forestomach	-		10	(10)	—	2	(2)	—	1	1	(2)	—	0	(0)	10	(10)
	+		0	(0)	—	0	(0)	—	0	0	(0)	—	1	(1)	0	(0)
Erosion, glandular stomach	-		10	(10)	—	2	(2)	—	1	0	(1)	—	1	(1)	10	(10)
	++		0	(0)	—	0	(0)	—	0	1	(1)	—	0	(0)	0	(0)
Dilatation, gastric glandular lumen	-		9	(9)	—	2	(2)	—	1	1	(2)	—	1	(1)	10	(10)
	+		1	(1)	—	0	(0)	—	0	0	(0)	—	0	(0)	0	(0)
Urinary bladder : Hyperplasia, mucosal epithelium	-		10	(10)	8	2	(10)	8	1	1	(10)	9	1	(10)	6	(6)
	+		0	(0)	0	0	(0)	0	0	0	(0)	0	0	(0)	4	(4)*
Pituitary : Cyst, Rathke's pouch, anterior lobe	-		9	(9)	—	2	(2)	—	1	1	(2)	—	1	(1)	10	(10)
	+		1	(1)	—	0	(0)	—	0	0	(0)	—	0	(0)	0	(0)
Adrenal : Vacuolization, zona fasciculata	-		10	(10)	8	2	(10)	8	1	0	(9)	9	0	(9)	6	(6)
	+		0		0	0		0	0	1		0	1		3	
	++		0	)(0)	0	0	)(0)	0	0	0	)(1)	0	0	)(1)	1	)(4)*
Hyperplasia, nodular, cortical cell	-		10	(10)	8	2	(10)	8	1	0	(9)	9	1	(10)	10	(10)
	++		0	(0)	0	0	(0)	0	0	1	(1)	0	0	(0)	0	(0)
Thymus : Atrophy, cortical	-		8	(8)	6	2	(8)	8	1	0	(9)	9	1	(10)	5	(5)
	+		2		2	0		0	0	0		0	0		3	
	++		0	)(2)	0	0	)(2)	0	0	1	)(1)	0	0	)(0)	2	)(5)
Hemorrhage	-		10	(10)	7	2	(9)	8	1	1	(10)	9	1	(10)	10	(10)
	+		0	(0)	1	0	(1)	0	0	0	(0)	0	0	(0)	0	(0)
Skin : Cellular infiltration, neutrophile, focal	-		—	—	—	—	—	—	—	—	—	—	—	—	0	(0)
	+		—	—	—	—	—	—	—	—	—	—	—	—	1*	(1)

— : Not examined; - : Negative; + : Slight; ++ : Moderate; TK : Terminal kill; NP : Non-pregnant; UC : Animal with unsuccessful copulation; KL : Killed because all pups died after delivery; T : Total
The organs of the heart, lung, liver, pancreas, stomach, intestine, kidney, urinary bladder, overy, uterus, vagina, mammary gland, pituitary, thyroid, parathyroid, adrenal, thymus, spleen, bone marrow, lymph node and brain were examined from animals of the control and 600 mg/kg groups, and NP, UC and KL animals.
* : Animal with macroscopic skin lisions

Table 1 8 Reproduction results of rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	0	10	40	150	600
No. of pairs mated	10	10	10	10	10
No. of pairs with successful copulation	10	10	9	10	10
Copulation index (%)	100	100	90	100	100
Pairing days until copulation(days, Mean±S.D.)	2.0±0.9	2.2±1.2	2.7±0.9	3.0±1.9	2.3±0.9
No. of pregnant females	10	8	9	10	10
Fertility index (%)	100	80	100	100	100
No. of corpora lutea (Mean±S.D.)	18.4±1.4	18.5±2.7	18.4±1.8	17.7±1.8	18.5±1.9
No. of implantation sites (Mean±S.D.)	17.8±1.8	17.4±1.3	17.1±1.2	16.2±3.6	16.8±1.2
Implantation index (%, Mean±S.D.)	96.7±4.8	94.8±8.5	93.2±7.4	90.6±17.3	91.6±10.1
No. of pregnant females with parturition	10	8	9	10	10
Gestation length (days, Mean±S.D.)	22.5±0.5	22.9±0.4	22.9±0.6	22.4±0.5	22.7±0.5
No. of pregnant females with live pups	10	8	9	10	10
Gestation index (%)	100	100	100	100	100
No. of pregnants killed[a)]	0	0	1	1	0
No. of pregnant females with live pups on day 4	10	8	8	9	10

Copulation index = (No. of pairs with successful copulation/No. of pairs mated)×100
Fertility index = (No. of pregnant animals/No. of pairs with successful copulation)×100
Gestation index = (No. of females with live pups/No. of living pregnant females) ×100
a) : All pups died after delivery, killed during the study for pathological examination

Table 19 Litter results of female rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Dose (mg/kg)	0	10	40	150	600
No. of pups born	16.8± 2.3	15.4± 1.2	16.1± 1.3	14.1± 5.2	15.4± 2.2
Delivery index (%)	94.1± 6.8	89.0±10.2	94.3± 6.2	88.1±25.4	91.6±10.2
No. of pups alive on day 0 of lactation					
Total	16.6± 2.2	14.8± 1.9	15.0± 2.3	11.9± 6.4	15.1± 2.0
Male	8.6± 2.8	6.6± 2.4	9.0± 2.8	5.8± 3.9	7.2± 2.4
Female	8.0± 2.8	8.1± 2.6	6.0± 1.7	6.1± 4.3	7.9± 2.6
Live birth index (%)	98.9± 2.4	95.7± 7.5	92.9±10.7	86.9±30.9	98.2± 4.1
Sex ratio (Male/Female)	1.10	0.81	1.46	1.04	0.95
No. of pups alive on day 4 of lactation					
Total	16.5± 2.2	14.8± 1.9	12.8± 5.2	13.1± 5.1	14.0± 1.7
Male	8.5± 2.9	6.6± 2.4	7.8± 3.7	6.4± 3.5	7.0± 2.2
Female	8.0± 2.8	8.1± 2.6	5.0± 2.7	6.7± 3.9	7.0± 1.8
Viability index (%)	99.4± 1.9	100 ± 0	86.2±32.8	99.4± 1.9	93.3± 9.4
Body weight of live pups (g)					
on day 0					
Male	7.0± 0.4	7.6± 0.7	7.1± 0.6	7.3± 0.9	6.9± 0.8
Female	6.8± 0.6	7.0± 0.7	6.7± 0.4	6.8± 0.8	6.6± 0.7
on day 4					
Male	11.1± 1.8	11.9± 2.3	11.5± 1.7	12.0± 2.5	10.0± 2.3
Female	10.7± 1.8	11.3± 2.2	11.2± 1.6	11.3± 2.4	9.8± 2.1

Delivery index = (No. of pups born / No. of implantation sites)x100
Live birth index = (No. of live pups on day 0 / No. of pups born)x100
Viability index = (No. of live pups on day 4 / No. of live pups on day 0)x100
Sex ratio = Total No. of male pups / Total No. of female pups
Each value is expressed as Mean ± SD., except sex ratio

Table 20 Incidence of external findings of rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Findings	Dose (mg/kg)	0	10	40	150	600
External	No. of pups examined	168	123	145	124	154
	No. of pups with external anomalies[a]	1 (0.6±1.9)	0 (0)	0 (0)	1 (0.7±2.1)	0 (0)
	External anomalies[a] Vestigial tail	1 (0.6±1.9)	0 (0)	0 (0)	1 (0.7±2.1)	0 (0)

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

Table 2 1 Incidence of visceral findings of rats treated orally with isocyanuric acid in the combined repeat dose and reproductive/developmental toxicity screening test

Findings	Dose (mg/kg)	0	10	40	150	600
Visceral	No. of pups examined	167	123	142	123	151
	No. of pups with visceral anomalies[a]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	No. of pups with visceral variations[a]	5 (3.1±4.5)	2 (1.7±3.2)	3 (2.2±6.7)	6 (4.2±8.3)	4 (2.8±4.8)
	Visceral variations[a]					
	Thymic remnant in neck	1 (0.6±1.8)	2 (1.7±3.2)	3 (2.2±6.7)	6 (4.2±8.3)	4 (2.8±4.8)
	Persistent left umbilical artery	3 (2.0±4.4)	0 (0)	0 (0)	0 (0)	0 (0)
	Dilatation of renal pelvis	1 (0.6±1.8)	0 (0)	0 (0)	0 (0)	0 (0)

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

FOREWORD INTRODUCTION

ISOCYANURIC ACID
CAS N°: 108-80-5

SIDS Initial Assessment Report
for
9th SIAM
(France, June 29-July 1, 1999)

Chemical Name: Isocyanuric acid
CAS No: 108-80-5
Sponsor Country: Japan

National SIDS Contact Point in Sponsor Country:
Mr. Kazuhide Ishikawa
Ministry of Foreign Affairs, Japan

HISTORY:

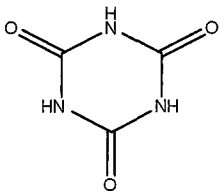
SIDS Testing Plan were reviewed in SIDS Review Process, where the following SIDS Testing Plan was agreed:

no testing ( )
testing (X) Water solubility, Vapour pressure, Octanol/water partition coefficient, Stability in water Biodegradation

Chronic toxicity to daphnia
Combined repeat dose and reproductive toxicity,
Chromosomal aberration test in vitro

Deadline for circulation: March 31, 1999
Date of Circulation: March 30, 1999
(To all National SIDS Contact Points and the OECD Secretariat)

SIDS INITIAL ASSESSMENT PROFILE

CAS NO.	108-80-5
CHEMICAL NAME	Isocyanuric acid
Structural formula	

RECOMMENDATIONS OF THE SPONSOR COUNTRY

The chemical is currently of low priority for further work.

SHORT SUMMARY WHICH SUPPORTS THE REASONS FOR THE RECOMMENDATIONS

Isocyanuric acid is not readily biodegradable (OECD 301C: 0% after 14-day) and stable in water. Bioconcentration factor to fish is low (<0.5, in Carp for 6 weeks).

Toxicity of this chemical to aquatic organisms seems to be low because all toxicity data are higher than 32 mg/l (NOEC for reproduction of *Daphnia magna*). 48-EC_{50} for immobilisation of *Daphnia magna* was 1000 mg/l. For testing in fish, Medaka (*Oryzias latipes*), both 96-h LC_{50} and 14-day LC_{50} were more than 100 mg/l. For algal test (*Selenastrum capricornutum*), 72-h EC_{50} and 72-h NOEC were 620.0 mg/l and 62.5 mg/l, respectively. No data are available for effects on terrestrial organisms.

Isocyanuric acid is lowly toxic in acute toxicity studies. This chemical is considered to be slightly irritating to eyes, but not to the skin. Several subchronic oral toxicity studies demonstrated renal damages, such as dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis. These changes were probably caused by crystal of this chemical in renal tubules. The mechanism of this renal toxicity is supported by the toxicokinetics studies in animals and humans, showing that this chemical is quickly absorbed and excreted to urine within a few hours as an unchanged form. NOAEL is considered to be 150 mg/kg/day. In a developmental toxicity study, reduction of fetal body weights and crown/rump lengths was observed and NOAEL was 200 mg/kg/day, but this most likely reflects toxicty to the dams. No reproductive toxicity was observed (NOAEL: 600 mg/kg/day). A variety of *in vitro* and *in vivo* genotoxicity studies show this chemical is not genotoxic. Two years studies of rats and mice indicate this chemical has no carcinogenic potential.

The production volume is ca. 20,000 tons/year in Japan in 1995. This chemical is used as an intermediate of chemical products in a closed system at industries. A generic fugacity model (Mackey level III) shows that this chemical will be distributed mainly (99.9%) in water phase after it is discharged into water.

As for consumer exposure, this chemical is used in the form of chlorides for disinfection of water. In Japan, trichloroisocyanurate is mainly used in swimming pool, and the average concentration of isocyanuric acid is estimated as 50 to 100 μg/ml.

IF FURTHER WORK IS RECOMMENDED, SUMMARISE ITS NATURE

FULL SIDS SUMMARY

CAS NO: 108-80-5		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point			330 °C
2.2	Boiling Point			Decomposed
2.3	Density			
2.4	Vapour Pressure		OECD TG 104	< 5.0 x 10^{-3} Pa at 25 °C
2.5	Partition Coefficient (Log Pow)		OECD TG 107	< 0.3
2.6 A.	Water Solubility		OECD TG 105	2.7 g/L at 25 °C
B.	pH			
	pKa			
2.12	Oxidation: Reduction Potential			
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation			
3.1.2	Stability in Water		OECD TG 111	Stable at pH 4,7 and 9 pK_1 = 6.88, pK_2 = 11.40, pK_3 = 13.5
3.2	Monitoring Data			In surface water = not detected In soil/sediment = not detected
3.3	Transport and Distribution		Calculated (Fugacity Level III type)	Release: 100% to Water In Air 0.0 % In Water 99.6% In Sediment 0.0 % In Soil 0.4 %
			(local exposure)	0.19 mg/L (Japan)
3.5	Biodegradation		OECD 301C	Not readily biodegradable 0% in 28 days
3.7	Bioaccumulation		OECD 305C	BCF: < 0.5
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	LC_{50}(96hr) > 100 mg/l LC_{50}(14 d) > 100 mg/l
4.2	Acute Toxicity to Aquatic Invertebrates *Daphnia*	*Daphnia magna*	OECD TG 202	EC_{50}(48hr): 1000 mg/l
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201	EC_{50}(72hr) = 620 mg/l NOEC= 62.5 mg/l
4.5.2	Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)	*Daphnia magna*	OECD TG 202	EC_{50}(21d, Repro)= 65.9 mg/l NOEC= 32.0 mg/l
4.6.1	Toxicity to Soil Dwelling Organisms			None
4.6.2	Toxicity to Terrestrial Plants			None
4.6.3	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)			None

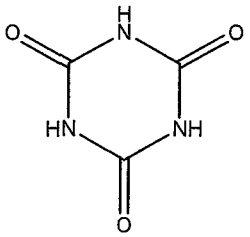
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Other (unknown)	LD_{50} = 7700 mg/kg
5.1.2	Acute Inhalation Toxicity	Rat	Other (unknown)	Minimum toxic concentration = 612 mg/m^3
5.1.3	Acute Dermal Toxicity	Rabbit	Other (unknown)	LD_{50} = > 7940 mg/kg
5.2.1	Skin Irritation/Corrosion	Rabbit	FHSA test	Not irritating
5.2.2	Eye Irritation/Corrosion	Rabbit	FHSA test	Slightly irritating
5.4	Repeated Dose Toxicity	Rat	OECD Combined	NOAEL = 150 mg/kg/day
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*S. typhimurium*	Other (unknown)	- (With metabolic activation) - (Without metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	Chinese hamster CHL cells	Japanese TG and OECD TG 473	- (With metabolic activation) - (Without metabolic activation)
5.6	Genetic Toxicity In Vivo (Chromosomal aberrations)	Rat	Other	-
5.7	Carcinogenicity	Rat	Other	Not carcinogenic
5.8	Toxicity to Reproduction	Rat	OECD combined	NOAEL = 600 mg/kg/day
5.9	Developmental Toxicity/ Teratogenicity	Rabbit	Other	NOAEL = 200 mg/kg/day
5.11	Experience with Human Exposure		Other (Toxicokinetics)	

[Note] Data beyond SIDS requirements can be added if the items are relevant to the assessment of the chemical, e.g. corrosiveness/irritation, carcinogenicity.

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

- OECD Name: Isocyanuric acid
- Synonym: sym-Triazine-2,4,6-triol; sym-Triazinetriol; normal Cyanuric acid; 2,4,6-Trihydroxy-1,3,5-triazine; Trihydroxycyanidine; Tricyanic acid; Isocyanuric acid; Pseudocyanuric acid; 1,3,5-Triazine-2,4,6(1H,3H,5H)-trione; 1,3,5-Triazine-2,4,6-triol; 1,3,5-Triazinetriol; 1,3,5-Triazinetrione; Tricarbimide; Trihydroxy-1,3,5-triazine
- CAS Number: 108-80-5
- Empirical Formula: $C_3H_3N_3O_3$
- Structural Formula:



- Degree of Purity: 99.7 %
- Major Impurity: None
- Essential Additives: None
- Physical-chemical properties
  - Melting Point: 330 °C
  - Vapour pressure: < 5.0 x 10^{-3} Pa at 25 °C
  - Water solubility: 2.7 g/L
  - Log Pow: < 0.3

2. GENERAL INFORMATION ON EXPOSURE

2.1 Production and import

The production volume of isocyanuric acid in Japan is 20,000 tonnes/year in 1995.

2.2 Use pattern

All of isocyanuric acid produced in Japan is used as intermediate of chemical products, and no consumer use is reported.

2.3 Other information

None

3. ENVIRONMENT

3.1 Environmental Exposure

3.1.1 General Discussion

Isocyanuric acid is not readily biodegradable (OECD 301C: 0 % after 14d) and stable in water. Direct photodegradation is not expected because isocyanuric acid has not absorption band in UV and VIS region.

Isocyanuric acid is low bioaccumulative (BCF < 0.5, Carp).

The potential environmental distributions of isocyanuric acid obtain from a generic Mackay level III fugacity model is shown in Table 1. Parameters used for this model are shown as Annex to this report. The results show that, if isocyanuric acid is released into water, it is unlikely to be distributed into other compartments. If isocyanuric acid is released into air and soil, it is likely to be distributed in other compartments.

Table 1
Environmental distribution of isocyanuric acid
Using a generic level III fugacity model.

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	0.1 %	0.0 %	0.0 %
Water	46.5 %	99.6 %	40.5 %
Soil	53.3 %	0.0 %	59.3 %
Sediment	0.2 %	0.4 %	0.2 %

As this chemical is used in closed system as an intermediate of chemical products and is not included in consumer products, its release to the environment may occur only from the production cite.

3.1.2 Predicted Environmental Concentration

As isocyanuric acid is produced under the well-controlled closed system, amount of release to air phase is negligibly small. The waste of isocyanuric acid from the production system is released to water phase after treated its own wastewater treatment plant. Therefore, Predicted Environmental Concentration (PEC) will be calculated only for the water environment.

a. Regional exposure

According to report from a Japanese manufacturer, 407.7 tonnes/year (measured) of isocyanuric acid are released with 2.19 x 10^{10} L/year of effluent into river. Local Predicted Environmental Concentration (PEC_{local}) is calculated to be 0.186 mg/L as a worst case scenario, employing the following calculation model and dilution factor of 100.

$$\frac{\text{Amount of release } (4.08 \times 10^{11} \text{ mg/y})}{\text{Volume of effluent } (2.19 \times 10^{10} \text{ L/y}) \times \text{Dilution Factor } (100)}$$

3.2 Effects on the Environments

3.2.1 Effects on aquatic organisms

Acute and chronic toxicity data of isocyanuric acid to aquatic organisms are summarized below (Table 2). Toxicity of this chemical to aquatic organisms seems low because all toxicity data are higher than 32 mg/l (NOEC of reproduction of *Daphnia magna*). Predicted No Effect Concentration (PNEC) of this chemical was determined based mainly on the toxicity data obtained by the Environment Agency of Japan through a GLP-laboratory. Toxicity data by different organizations were few. As the lowest acute and chronic toxicity data, 96 h LC_{50} of *Oryzias latipes* and 21 d NOEC (reproduction) of *D. magna* were used, respectively (Table 2). All toxicity in Table 2 were calculated based on the nominal concentration as the measured concentrations were kept within 95 to 102 % of the nominal concentrations.

The assessment factors of 100 were used to both acute and chronic toxicity data to determine PNEC, according to the OECD Provisional Guidance for Initial Assessment of Aquatic Effects (EXCH/MANUAL/96-4-5.DOC/May 1996), because chronic toxicity data for fish was absent.

From chronic toxicity data (21 d NOEC of *Daphnia*):

PNEC = 32/100 = 0.32 mg/l

Thus, PNEC of isocyanuric acid is 0.32 mg/l.

Table 2

Acute and chronic toxicity data of isocyanuric acid to aquatic organisms at different trophic levels. The data were obtained by the Environmental Agency of Japan based on the OECD Test Guide Lines.

Species	Endpoint	Conc. (mg/l)	Remarks
Selenastrum capricornutum (algae)	Bms 72 h EC50 Bms. 72 h NOEC	620.0 62.5	a, 1) c, 1),
Daphnia magna (Water flea)	Imm 48 h EC50 Rep 21 d EC50 Rep 21 d NOEC	1000 65.9 32.0	a, 1), c, 1) c, 1), C
Oryzias latipes (fish, Medaka)	Mor 96 h LC50 Mor 14 d LC50	> 100 > 100	a, 1), A a, 1)

Notes: Bms; biomass, Mor; mortality, Rep; reproduction, NR; not recorded.
A), C); the lowest values among the acute or chronic toxicity data of algae, Cladocera (water flea) and fishes to determine PNEC of isocyanuric acid.

1) Toxicity data were obtained by the Environment Agency of Japan based on OECD Test Guidelines and GLP.

3.2.2 Terrestrial effects

No data available

3.2.3 Other effects

No data available

3.3 Initial Assessment for the Environment

Predicted No Effect Concentration (PNEC) of this chemical has been calculated as 0.32 mg/l.

PEC from Japanese local exposure scenario is 0.186 mg/l.

PEC_{local} / PNEC = 0.186/0. 32 = 0.58 < 1

Therefore, it is currently considered of low potential risk for environments and low priority for further work.

4. HUMAN HEALTH

4.1 Human Exposure

4.1.1 Occupational exposure

Isocyanuric acid is produced in a closed system and used as an intermediate for organic chemicals. The occupational exposure is expected through inhalation and the dermal route is assumed negligible because this chemical is solid. As the atmospheric concentration in plant was not measured, the maximum exposure level is estimated according to working schedules as follows. If a single worker (body weight; 70 kg, respiratory volume; 1.25 m^3/hr) is assigned to implement this operation without protection, the highest daily intake (EHE) is calculated as 0.23 mg/kg/day as the worst case. Practically, workers always wear protective gloves and respiratory protective equipment (mask) during the operation.

	Frequency Times/day	Duration hr	Working hr/day	Maximum Concentration mg/m^3	Maximum EHE mg/kg/day
Bag Filling	80	0.08	6.5	2	0.23

EHE: Estimated Human Exposure

4.1.2 Consumer exposure

Chloroisocyanurates such as sodium dichloroisocyanurate, potassium dichloroisocyanurate, sodium dichloroisocyanurate hydrate, potassium dichloroisocyanurate hydrate and trichloroisocyanuric acid have been used in sterilizing water tank, swimming pool, bathing water, and kitchen. In water, chloroisocyanurates are hydrolized to isocyanuric acid and hypochloric acid, that is the active agent (Golaszewski & Seux: 1994). The antimicrobial activity of sodium dichloroisocyanurate was evaluated against Gram negative bacteria such as *E. coli* or *Salmonella typhimurium* and against some fungi (D'Auria, *et al.*: 1989).

It is considered that the potential for exposure to pool chemicals through swallowing water and/or dermal absorption is quite high. Allen et al. (1982) reported cumulative recovery of isocyanuric acid in the urine of swimmers, 20 hr after swimming, averaging 9.8 mg. As the worst case, high performance athletes in training are known to spend up to 4 hr/day in the pool for 300 day/year and are estimated to swallow up to 60 ml/hr of pool water (Datta: 1979). In Japan, trichloroisocyanurate is mainly used in swimming pool and the average concentration of isocyanuric acid is estimated as 50 to 100 μg/ml. Based on this information, oral daily intake of isocyanuric acid for 60 kg b.w.

person is calculated as 0.17 to 0.33 mg/kg/day. Continuous-dose automated *in vitro* dermal absorption studies conducted with isocyanuric acid demonstrated minimal absorption through rat, hairless guinea pig, human, and Test skin (Moody: 1993). Total cumulative absorption of isocyanuric acid by 24 h in Test skin and human skin was 0.02 μg/cm^2 in both cases. As 1.5 m^2 of body surface is estimated for 60 kg b.w. person, the daily intake through skin is calculated as 5 μg/kg/day as the maximum value.

4.1.3 Indirect exposure via the environment

As isocyanuric acid is persistent in water and low bioaccumulative, the exposure to the general population via the environment would be possible through drinking water processed from surface water and through fish which may accumulate this chemical.

The concentration in drinking water should be estimated to be equal to PEC calculated in Section 3.1, i.e. 0.186 mg/l. The daily intake through drinking water is calculated as 6.20 x 10^{-3} mg/kg/day (2 l/day, 60 kg b.w.).

Using the maximum bioconcentration factor of 0.5 obtained by tests, the concentration of this chemical in fish can be calculated as follows:

$$PEC_{fish} = 0.186 \text{ mg/l} \times 0.5 = 9.03 \times 10^{-5} \text{ mg/g-wet}$$

As a daily intake of fish in Japan is estimated to be 90 g for 60 kg body weight person, a daily intake of this chemical will be 1.40 x 10^{-4} mg/kg/day.

4.2 Effects on Human Health

a) Acute toxicity

[SIDS data] Oral LD_{50} for isocyanuric acid was 7,700 mg/kg b.w. for rats. In inhalation study, the minimum toxic concentration was reported to be 612 mg/m^3 in rats. (Babayan and Aleksandryan: 1985) Dermal LD_{50} for isocyanuric acid was higher than 7940 mg/kg b.w. for rabbits (Toxikologische Bewertung: 1993).

Other acute toxicity information including sodium isocyanurate are given in Table. In addition, it is also reported that a single oral dosage of isocyanuric acid up to 10 g/kg was tolerated by rats and daily dosage of 20 g/kg was tolerated by rabbits for periods up to 4 days (Hodge et al.: 1965). Based on these data, isocyanuric acid is considered to be low toxic when administered as a single dose.

Routes	Strain	Type	Values	
Isocyanic acid				
Oral	Rats	LD_{50}	7,700 mg/kg	SIDS data, Ref.1
	Mice	LD_{50}	3,400 mg/kg	Ref.1
	Rabbits	LDL_0	> 10 g/kg	Ref.2
Inhalation	Rats	Other*	612 mg/m^3	SIDS data, Ref.1
Dermal	Rabbits	LD_{50}	> 7,940 mg/kg	SIDS data, Ref.3

Intravenous	Rats	LD_{50}	> 100 mg/kg	Ref.4
	Mice	LD_{50}	> 500 mg/kg	Ref.4
Sodium isocyanurate				
Oral	Rats	LD_{50}	> 7,500 mg/kg	Ref.4
Intravenous	Cats	LD_{50}	2,144 mg/kg	Ref.5

Ref.1: Babayan & Aleksandryan: 1985, Ref.2: Toxicity Information: 1972, Ref.3: Toxikologische Bewertung: 1993, Ref.4: *Gigiena i Sanitariya*: 1962, Ref.5: *J Pharmacol Exp Ther*: 1951, *: Minimum toxic concentration

b) Irritation

Federal Hazardous Substances Act (FHSA) tests of isocyanuric acid were performed in rabbits. As a result, isocyanuric acid slightly irritated to eyes but not to the skin (Hammond *et al.*: 1986). As for eye irritation, there are two other data. Moderate eye irritation followed administration into the rabbit eyes for 24 hr at 20 or 500 mg (Toxicity Information: 1972, Marhold: 1972). This chemical is not listed in IUCLID labelling and classification.

Based on these data, this chemical is considered as a slightly irritant to eyes, but not to the skin.

c) Sensitisation

There is no available data.

d) Repeated toxicity

[SIDS data] Oral toxicity study was performed in SD (Crj: CD) rats by an OECD combined repeat dose and reproductive/developmental toxicity screening test. Isocyanuric acid was administered by gavage at doses of 10, 40, 150 and 600 mg/kg/day for 45 days in males and from 14 days before mating to day 3 of lactation in females. (MHW, Japan: 1997)

Isocyanuric acid induced toxic effects at 600 mg/kg in both sexes. Excretion of reddish urine was evident. In addition, depression of body weight gain was observed in males. Urinalyses of males revealed appearance of crystals, which is considered this chemical precipitated from urine, and increases of erythrocytes and leukocytes. In hematological examination of males, significant decreases in erythrocyte counts, hemoglobin concentrations and hematocrit values were observed. In blood chemical examination of males, increases in urea nitrogen and creatinine, and a decrease of sodium were revealed. In histopathological examination, dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis in the kidney, hyperplasia of the mucosal epithelium in the urinary bladder and vacuolization of the zona fasciculata in the adrenals were observed in both sexes. In addition, the incidence of atrophic thymus also showed a tendency for increase in females. Absolute and relative kidney weights and relative adrenal weights were increased in both sexes. As no toxic sign was observed at doses of 150 mg/kg and the less, NOAEL was considered to be 150 mg/kg/day in both sexes.

Oral toxicity study of sodium isocyanurate for 90 days was performed in B6C3F1 mice at doses of 896, 1,792 and 5,375 ppm in drinking water. Sodium hippurate was used as a second control in order

to have the sodium burden as the top concentration. Although an increase in water consumption in both sexes and absolute and relative weights of ovaries in females were observed, these changes were considered due to the high sodium intake. Therefore, NOAEL was considered to be 5,375 ppm (male: 1,994 mg/kg/day, female: 2,200mg/kg/day). (Hazleton: 1982)

Hodge *et al.* (1965) conducted oral toxicity study in rats and beagle dogs, and skin and eye application study in rabbits.

In first study, rats of the Rochester strain were maintained for 20 weeks on diets containing 0.8 %, and 8 % sodium isocyanurate. As a result, 14/20 males and 4/20 females died at 8 %, but no died at 0.8 %. Considerable decrease in body weight gain was observed at 8 %. Urine samples taken prior to the start of feeding and again near termination of the study showed normal concentrations of protein and sugar. In hematological examination no change was observed. There were no changes in organ weights (thyroid, liver, brain, lungs, heart, etc.), except kidney weight, which increased at 8 % in females. In histologic study, dilatation of distal collecting tubules and ducts of Bellini, with focal areas of epithelial proliferation were observed at 8 % in both sexes. Therefore, NOAEL was considered to be 0.8 % (56 mg/kg/day).

In second study, groups of 3 dogs were maintained in diets of 0.8 % sodium isocyanurate for 6 months and 8 % for 2 years. In 0.8 % dogs, there were no changes in body weight gain, organ weight, and sugar and protein in urine. In addition, hematological and histological changes were not observed. In 8 % group, 2 dogs died after 16 and 21 months on the regimen. No change or slight increase in body weights was observed. Periodic urinalyses gave normal trace values for sugar and protein. In hematologic study, only a survival dog showed changes, which are low red blood cell counts, hemoglobin values, and hematocrits. There was no change in organ weights (thyroid, liver, brain, lungs, heart, etc.), except decrease in kidney weight of 2 dogs surviving more than 20 months. In these dogs, there was gross evidence of kidney fibrosis. Sections revealed numerous linear streaks of gray fibrous tissue extending from the papillary tip to the cortical surface. Microscopically, similar changes were observed in the kidneys of all three dogs. The collecting tubules were more uniformly and severely involved, but all portions of the nephron were compressed by fibrosis. There were slight focal dilatation and epithelial proliferation in the ducts of Bellini. In survival dog, focal areas of thyroid atrophy were found with lymphocytic infiltration, but without evidence of hyperplasia. Therefore, NOAEL for 6 months study was considered to be 0.8 % (291 mg/kg/day) and LOAEL for 2 years study to be 8 % (2,912 mg/kg/day).

In skin application study, 5 ml of 0.8 % or 8 % aqueous suspension were administered to the skin of albino rabbits 5 days/week for about 3 months, respectively. Urinalyses (sugar and protein) and hematological study showed no changes. There were no irritation or other adverse effects on the skin. In histological findings of liver and skin from treated and untreated area, no change was observed at the termination of the study. In the kidneys of the rabbits treated with the 8 % sodium isocyanurate suspension, slight dilation of the ducts of Bellini and mild tubular changes were found. Therefore, NOAEL was considered to be 0.8 %.

In eye application studies, 0.1 ml of 0.8 % or 8 % aqueous suspension were administered to eye of albino rabbits 5 days/week for about 3 months, respectively. Increase in body weight was observed during the period of the study in all treated groups. No eye injury and irritation was caused. Therefore, NOAEL was considered to be 8 %.

e) Reproductive/developmental toxicity

Reproductive toxicity

[SIDS data] Oral toxicity study was performed in SD (Crj: CD) rats by an OECD combined repeated dose and reproductive/developmental toxicity screening test. Isocyanuric acid was administered by gavage at doses of 10, 40, 150 and 600 mg/kg/day for 45 days in males and from 14 days before mating to day 3 of lactation in females. (MHW, Japan: 1997)

The parental animals exhibited no alteration in reproductive parameters including the copulation index, fertility index, gestation length, numbers of corpora lutea or implantation, implantation index, gestation index, delivery index, and behavior at delivery and lactation. There were no significant differences in offspring parameters including number of offspring or live offspring, the sex ratio, live birth index, viability index and body weight. No external or visceral abnormalities related to the test substance were detected in any of the offspring. Therefore, NOAEL for parents and offsprings was considered to be 600 mg/kg/day.

Three-generation study was conducted. Sodium isocyanurate was given by drinking water at concentrations of 400, 1,200 and 5,375 ppm to CD rats. Treatment was initiated at 36 days of age and continued for a minimum of 100 days before mating. Weanlings from the F1 and F2 litters were randomly selected as the next parents and continued on treatment for the additional 120 days. Selected litters and F3 offsprings were sacrificed 4 weeks after weaning, and organ weight measurements and microscopic examination of tissues were carried out. (Wheeler *et al.*: 1985)

No compound-related changes were observed in mortality, body weights, food consumption, gestation length, litter size, pup survival to weaning, sex ratio, and pup weight. In pathological and histological findings, epithelial hyperplasia with chronic cystitis was observed only in a few of high-dose treated males in F2 offsprings, which were attributed to chronic irritation by the calculi in the urinary bladder. However, this change is considered not to be due to reproductive toxicity of this chemical. In other treated groups, there were no changes. Therefore, NOAEL for reproductive toxicity was considered to be 5,375 ppm (approx. 370 mg/kg/day for male and 630 mg/kg/day for female).

Male CD-1 mice were treated intraperitoneally at doses of sodium isocyanurate (125 and 250 mg/kg/day). As positive control, methyl methane sulfonate was used at dose of 50 mg/kg/day. Males were mated with non-treated females. Although early resorptions were observed in females mated with males treated with methyl methane sulfonate, any chemical-related effects were not observed in females, mated with sodium isocyanurate treated males. Therefore, NOAEL was considered to be 250 mg/kg/day. (FMC Corporation: 1972)

Developmental toxicity

[SIDS data] Pregnant Dutch belted rabbits were given sodium isocyanurate at doses of 50, 200 and 500 mg/kg/day by gavage during days 6-18 of gestation. (FMC Corporation, unpublished observations)

Although slight decrease in body weight was observed in mid- and high-dose dams during the treatment period, compensatory weight gains occurred after termination of treatment on day 18. There were no compound related mortality or other adverse reactions in all treated dams. The mean number of live fetus/dam and sex ratio was essentially comparable for all groups. Fetal body weights and crown/rump lengths were reduced slightly in high-dose groups, compared to control. These changes may have resulted from the slight manifestations of maternal toxicity that occurred during treatment. There was no evidence of external or internal malformations or skeletal anomalies. Therefore, NOAEL for developmental toxicity was considered to be 200 mg/kg/day.

Sodium isocyanurate was administered at doses of 200, 1,000, and 5,000 mg/kg/day by oral gavage to pregnant CD rats during days 6-15 of gestation. Sodium control groups received sodium hippurate at dose of 1,118 and 5,590 mg/kg/day. (Industry ad hoc Committee for Isocyanurates: 1982)

There was no mortality in all treated groups. Although decrease in body weight and crown/rum length, increase in post-implantation loss, incidence incomplete ossification were observed in sodium control group, no treatment related effect on maternal appearance, behaviour and body weight gain, and no teratogenic effect were observed in all groups treated with sodium isocyanurate. Therefore, NOAEL for developmental toxicity was considered to be 5,000 mg/kg/day.

f) Genetic toxicity

Bacterial test
[SIDS data] Isocyanuric acid was not mutagenic to *S. typhimurium* TA1535, TA1537, TA98, TA100 with or without metabolic activation (Hayworth *et al.*: 1983).

Isocyanuric acid did not induce the bacteriophage Lambda in *Escherichia coli* K12 en VA UVRB (NORSOLOR/APC: 1977).

Non-bacterial test *in vitro*
[SIDS data] In chromosomal aberration test *in vitro*, clastogenicity or polyploidy in CHL/IU cells was not induced in the absence or presence of an exogenous metabolic activation system (MHW, Japan: 1997).

In lymphoma assay, this chemical also showed negative result at up to a concentration of 2000 μg/ml in the TK locus of L5178Y mouse lymphoma cells (Industry ad hoc Committee for Isocyanurates: 1981a). This chemical did not induce sister chromatid exchange in CHO cells (Industry ad hoc committee for Isocyanurates: 1981b), and this negative result was confirmed on human lymphoid cell line (LAZ-007) by Sobti *et al.* (1981), although the concentration was very low (2μg/ml).

in vivo Test
[SIDS data] In chromosomal aberration test *in vivo*, rats were killed 24 and 48 hr after administration of sodium isocyanurate by gavage at single dosages up to 5000 mg/kg, and bone marrow cells were collected and examined. As a result, this chemical did not induce chromosomal aberrations in rat bone marrow cells (Hammond *et al*: 1985).

g) Carcinogenicity

CD rats were administered sodium isocyanurate in drinking water at concentrations of 400, 1,200, 2,400 or 5,375 ppm for 2 years. Estimated daily doses were indicated only for 2,400 and 5,375 ppm (male: 154 and 371 mg/kg/day, female: 266 and 634 mg/kg/day, respectively). For a second control, sodium hippurate was administered as the same amount of sodium as the highest dose. Treatment-related mortality was observed in some males of the highest dose group, which died during the first 12 months of the study. This mortality was due to the development of calculi in the urinary tract. In some males that died on test and in some that were sacrificed at 12 months, there were pathologic changes, including hyperplasia, bleeding, and inflamed ureters, and renal tubular nephrosis. Although slight tubular nephrosis was also observed in a few females of the highest dose group during the first 12 months, these animals did not exhibit bladder calculi. Inflammatory

lesions in the heart were also apparent in some of the highest dose males that died early. There was no evidence of a test article related carcinogenic effect. (Cascieri *et al.*: 1985)

B6C3F1 mice were administered sodium isocyanurate in drinking water at concentrations of 100, 400, 1,200 and 5,375 ppm for 2 years. Apparently swollen enlarged abdomen was observed at the highest dose groups, related to increase in water consumption. There were no effects on survival, clinical pathology (except for urinary sodium), organ weight, gross and histopathology. There was no evidence of a test article related carcinogenesis. (Industry Ad hoc Committee for Isocyanurates: 1986)

h) Toxicodynamics/toxicokinetics

Toxicokinetics study of sodium isocyanurate was performed in rats and dogs, using [^{14}C] sodium isocyanurate. Administration was performed at 5 mg/kg by oral or intravenous route and at 500 mg/kg by oral route. At 5 mg/kg, this chemical was completely absorbed and largely eliminated in urine, while at 500 mg/kg, this chemical was incompletely absorbed and largely eliminated in feces. The elimination half-life was 30 to 60 min in rats and 1.5 to 2 hr in dogs after oral or intravenous administration. In dogs, sodium isocyanurate distributed into an apparent volume of distribution of 0.7 L/kg, which is somewhat greater than total body water volume. Rats and dogs were also administered unlabeled sodium isocyanurate orally at 5 mg/kg/day followed by the single exposure of 5 mg/kg radiolabeled sodium isocyanurate on day 15. In rats, the remainder of radioactivity in most tissues was below the level of detection 7 days after treatment for repeated dose administration and for all sampling times for both single and repeated dose administration in dogs. As results of repeated dose study, it was shown that isocyanurate did not bioaccumulate in tissues. There was no evidence that isocyanurate was biodegraded, as only unchanged isocyanurate was found in excreta. (Barbee *et al.*: 1983)

Toxicokinetics study by dermal route was performed, in which species was not indicated. After dermal application, the ^{14}C-labelled substance is not detectable in the blood and < 0.01 % of the administered dose is found in the urine. This result showed that isocyanuric acid was absorbed only in very small quantities. (Toxikologische Bewertung: 1993)

i) Experience with human exposure

Toxicokinetics of isocyanuric acid was investigated in 5 volunteers, who soaked in a swimming pool for 120 minutes. As a result, the cumulative excretion of isocyanuric acid was 0.03-2.8 mg, equivalent to 3.0-3.6 ml of pool water and the elimination half-life is calculated as 3 hr. On the other hand, recovery of ingested isocyanuric acid was 98 % in urine. There was no correlation between toxicokinetics and gamma glutamyl transpeptidase activity. (Allen *et al.*: 1982)

4.3 Initial Assessment for Human Health

Isocyanuric acid is lowly toxic in acute toxicity studies. This chemical is considered to be slightly irritating to eyes, but not to the skin. Several subchronic oral toxicity studies demonstrated renal damages, such as dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis. These changes were probably caused by crystal of this chemical in renal tubules. The mechanism of this renal toxicity is supported by the toxicokinetics studies in animals and humans, showing that this chemical is quickly absorbed and excreted to urine within a few hours as an unchanged form. NOAEL is considered to be 150 mg/kg/day. In a developmental toxicity study, reduction of fetal body weights and crown/rump lengths was observed and NOAEL was 200 mg/kg/day, but this most

likely reflects toxicty to the dams. No reproductive toxicity was observed (NOAEL: 600 mg/kg/day). A variety of *in vitro* and *in vivo* genotoxicity studies show this chemical is not genotoxic. Two years studies of rats and mice indicate this chemical has no carcinogenic potential.

Occupational exposure

Isocyanuric acid is used in a closed system at industries and workers wear protective gloves and respiratory protective equipment during the operation. Although the occupational exposure route is expected as an inhalation in limited workers, there is no available data of the atmosphere concentration. Based on the predicted high concentration and the possibility of exposure period, the daily intake is calculated as 0.23 mg/kg/day as the worst case. Occupational risk is presumably low because the margin of safety is 652.

Consumer exposure

Isocyanuric acid is used in the form of chlorides in sterilizing water tank, swimming pool, bathing water, and kitchen. In Japan, trichloroisocyanurate is mainly used in swimming pool and the average concentration of isocyanuric acid is estimated as 50 to 100 μg/ml. The exposure of high performance athletes in training is expected through a swallow and skin absorption. The combined daily intake is calculated as 0.34 mg/kg/day as the worst case. Consumer risk is presumably low because the margin of safety is 441.

Indirect exposure via environment

As for indirect exposure via environment, PEC_{local} of 0.186 mg/l from local exposure scenario was used for the estimation. The daily intakes through drinking water and fish were calculated as 6.20×10^{-3} mg/kg/day and 1.40×10^{-4} mg/kg/day, respectively. Since the margin of safety is very large, such as 2.42×10^{4} for drinking water and 1.08×10^{6} for fish, health risk via environment is presumably low.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Isocyanuric acid is not readily biodegradable (OECD 301C: 0 % after 14-d) and stable in water. Bioaccumulation factor of this chemical is low (BCF < 0.5, Carp). PEC/PNEC ratio (0.186/0.32 = 0.58) is less than 1 based on the local exposure scenario in the Sponsor country. It is currently considered of low potential risk to environments and low priority for further work. However, relatively high PEC/PNEC value suggests necessity for assessment of this chemical to the river ecosystem contaminated with this chemical.

Isocyanuric acid is moderately toxic in a repeated dose study (i.e. kidney) but not toxic in reproductive toxicity study. In a developmental toxicity study, this chemical is toxic to dams, which resulted in slight fetal toxicity (reduction of body weights and crown/rump lengths). This chemical is neither genotoxic nor carcinogenic but slightly irritating to eyes. Occupational and consumer risks are expected to be low because the margin of safety is 652 and 441, respectively. As the margin of safety via indirect exposure is more than 10,000, it is currently considered of low potential human risk and low priority for further work.

5.2 Recommendations

Environment: Relatively high PEC (0.18 mg/l) and PEC/PNEC ratio (0.58) in the river receiving the effluents from the production site.

Human health: No recommendation

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Appendix 1

Method for Prediction of Environmental Concentration of Pollutant in Surface Water

1. Predicted environmental concentration in the local environment (PEC_{local}) with effluent release into river

When decomposition, precipitation and vaporization of pollutant can be ignored, it is used that simplified equation by complete mixing model shown with equation (1) to calculate predicted environmental concentration in the local environment (PEC_{local}) as for release effluent into river.

$$PEC_{local}\ (mg/L) = \frac{Co\ Q + Cs\ Qs}{Q + Qs} \qquad (1)$$

Where

Co: Concentration of pollutant in upper stream of release point (mg/L)
Cs: Concentration of pollutant in effluent (mg/L)
Q: Flow rate of river (m^3/day)
Qs: Flow rate of effluent released into river (m^3/ day)

At the equation (1), when Co can be considered as 0, dilution factor of pollutant in the river (R) can be shown with following equation.

$$R = Cs/C = (Q + Qs) / Qs \qquad (2)$$

As the worst case, it is used to employ a flow rate at dry season as flow rate of river (Q). When flow rate at dry season is indistinct, it is estimated using the following equation in Japan.

$$\text{Flow rate at dry season} = \text{mean flow late} / 2.5 \qquad (3)$$

2. Predicted environmental concentration in the local environment (PEC_{local}) with effluent release into sea

For prediction of concentration of pollutant in the sea water with effluent, it is employed generally Joseph-Sendnersymbol 146 ¥f "Times New Roman" ¥s 11'}s equation (4). This equation is one of analytic solution led under the following conditions from diffusion equation.

1 It is adopted large area of sea or lake.
2 The flow rate of effluent and concentration of pollutant in the effluent are constant, and distribution of concentration is able to regard as equilibrium state.
3 Effluent is distributed uniformly to vertical direction, and it spreads in a semicircle or segment to horizontal direction.
4 Diffusion coefficient of pollutant at the sea is in proportion to distance from release point of effluent.
5 There is not any effect of tidal current.
6 Decomposition of pollutant can be ignored.

$$C(x) = (C_s - C(r))\left(1 - \exp\left(-\frac{Q_s}{d\,p}\left(\frac{1}{x} - \frac{1}{r}\right)\right)\right) + C(r) \quad (4)$$

Where

C (x): Concentration of pollutant at distance x (m) from release point
Cs: Concentration of pollutant in effluent
C (r): Concentration of pollutant at distance r (m) from release point
Qs: Flow rate of effluent (m^3/day)
: Opening angle of seacoast (rad.)
d: Thickness of diffusion layer (m)
P: Diffusion velocity (m/day) (1.0 0.5 cm/sec)

When C(x) is 0 at r = and density stratification is ignored for simplification, Joseph-Sendnersymbol 146 ¥f "Times New Roman" ¥s 11'}s equation (4) is simplified to equation (5)

$$C(x) = C_s\left(1 - \exp\left(-\frac{Q_s}{d\,p\,x}\right)\right) \quad (5)$$

Because of Qs/ d p x << 1 except vicinity of release point, dilution factor in distance x from release point R(x) can be shown with equation (6).

$$R(x) = C_s/C(x) = d\,p\,x/Q_s \quad (6)$$

When it is employed following parameters in equation (6) as default, dilution factor R can be shown with equation (7).

P = 1 cm/sec (860 m/day)
= 3.14
d = 10 m
x = 1000 m

$$R = 2.7\ 10^7/Q_s \quad (7)$$

Qs: volume of effluent (m^3/day)

REVISED OECD HPV FORM 1

SIDS DOSSIER ON THE HPV PHASE 5 CHEMICAL

Isocyanuric acid

CAS No. 108-80-5

Sponsor Country: Japan

DATE: March 15, 1999

CONTENTS

B. Ph Value, Pka Value
2.7 Flash Point (Liquids)
2.8 Auto Flammability (Solid/Gases)
2.9 Flammability
2.10 Explosive Properties
2.11 Oxidising Properties
2.12 † Oxidation: Reduction Potential
2.13 Additional Remarks
A. Partition Co-Efficient Between Soil/Sediment And Water (Kd)
B. Other Remarks

3. Environmental Fate And Pathways

3.1 Stability
3.1.1 * Photodegradation
3.1.2 * Stability In Water
3.1.3 Stability In Soil
3.2 * Monitoring Data (Environment)
3.3 * Transport And Distribution Between Environmental Compartments Including Estimated Environmental Concentrations And Distribution Pathways
3.3.1 Transport
3.3.2 Theoretical Distribution (Fugacity Calculation)
3.4 Mode Of Degradation In Actual Use
3.5 * Biodegradation
3.6 Bod-5, Cod Or Ratio Bod-5/Cod
3.7 Bioaccumulation
3.8 Additional Remarks
A. Sewage Treatment
B. Other

4. Ecotoxicity

4.1 * Acute/Prolonged Toxicity To Fish
4.2 Acute Toxicity To Aquatic Invertebrates
* A. Daphnia
B. Other Aquatic Organisms
4.3 * Toxicity To Aquatic Plants E.G., Algae
4.4 Toxicity To Bacteria
4.5 Chronic Toxicity To Aquatic Organisms
4.5.1 Chronic Toxicity To Fish
4.5.2 (*) Chronic Toxicity To Aquatic Invertebrates (E.G., Daphnia Reproduction)
4.6 Toxicity To Terrestrial Organisms
4.6.1 Toxicity To Soil Dwelling Organisms
4.6.2 Toxicity To Terrestrial Plants
4.6.3 Toxicity To Other Non-Mammalian Terrestrial Species (Including Birds)
4.7 Biological Effects Monitoring (Including Biomagnification)
4.8 Biotransformation And Kinetics
4.9 Additional Remarks

5. Toxicity

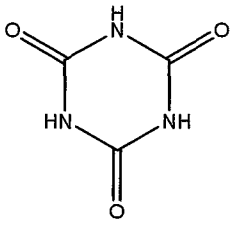
5.1 * Acute Toxicity
5.1.1 Acute Oral Toxicity
5.1.2 Acute Inhalation Toxicity
5.1.3 Acute Dermal Toxicity
5.1.4 Acute Toxicity By Other Routes Of Administration
5.2 Corrosiveness/Irritation
5.2.1 Skin Irritation/Corrosion
5.2.2 Eye Irritation/Corrosion
5.3 Skin Sensitisation
5.4 * Repeated Dose Toxicity
5.5 * Genetic Toxicity In Vitro
A. Bacterial Test
B. Non-Bacterial In Vitro Test
5.6 * Genetic Toxicity In Vivo
5.7 Carcinogenicity
5.8 * Toxicity To Reproduction
5.9 * Developmental Toxicity / Teratogenicity
5.10 Other Relevant Information
A. Specific Toxicities (Neurotoxicity, Immunotoxicity Etc.)
B. Toxicodynamics, Toxicokinetics
5.11 * Experience With Human Exposure

6. References

Appendix-1

Note: *; Data Elements In The Sids
†; Data Elements Specially Required For Inorganic Chemicals

SIDS PROFILE

1.01 A.	**CAS No.**	108-80-5
1.01 C.	**CHEMICAL NAME (OECD Name)**	Isocyanuric acid
1.01 D.	**CAS DESCRIPTOR**	
1.01 G.	**STRUCTURAL FORMULA**	
	OTHER CHEMICAL IDENTITY INFORMATION	
1.5	**QUANTITY**	20,000 tonnes/year in Japan
1.7	**USE PATTERN**	Intermediate in closed system.
1.9	**SOURCES AND LEVELS OF EXPOSURE**	407.7 tonnes/year Release into river
ISSUES FOR DISCUSSION (IDENTIFY, IF ANY)	SIDS testing required: Water solubility, Vapour pressure, Octanol/water partition coefficient, Stability in water, Biodegradation, Chronic toxicity to daphnia, Combined repeat dose and reproductive toxicity, Chromosomal aberration test in vitro	

SIDS SUMMARY

CAS NO: 108-80-5		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL-CHEMICAL DATA								
2.1	Melting Point	Y	N	N	Y	N	Y	N
2.2	Boiling Point	Y	N	N	Y	N	Y	N
2.3	Density	N						N
2.4	Vapour Pressure	N						Y
2.5	Partition Coefficient	N						Y
2.6	Water Solubility	N						Y
	pH and pKa values	N						N
2.12	Oxidation: Reduction potential	N						N
OTHER P/C STUDIES RECEIVED								
ENVIRONMENTAL FATE and PATHWAY								
3.1.1	Photodegradation	N						N
3.1.2	Stability in water	N						Y
3.2	Monitoring data	N						N
3.3	Transport and Distribution	N						N
3.5	Biodegradation	N						Y
OTHER ENV FATE STUDIES RECEIVED								
ECOTOXICITY								
4.1	Acute toxicity to Fish	Y	N	N	Y	N	N	Y
4.2	Acute toxicity to Daphnia	Y	N	N	Y	N	N	Y
4.3	Toxicity to Algae	N						Y
4.5.2	Chronic toxicity to Daphnia	N						Y
4.6.1	Toxicity to Soil dwelling organisms	N						N
4.6.2	Toxicity to Terrestrial plants	N						N
4.6.3	Toxicity to Birds	N						N
OTHER ECOTOXICITY STUDIES RECEIVED								
TOXICITY								
5.1.1	Acute Oral	Y	N	N	Y	N	Y	N
5.1.2	Acute Inhalation	Y	N	N	Y	N	Y	N
5.1.3	Acute Dermal	Y	N	N	Y	N	Y	N
5.4	Repeated Dose	Y	N	Y	Y	N	Y	Y
5.5	Genetic Toxicity *in vitro*							
	. Gene mutation	Y	N	N	Y	N	Y	N
	. Chromosomal aberration	N						Y
5.6	Genetic Toxicity *in vivo*	Y	N	N	Y	N	Y	N
5.8	Reproduction Toxicity	Y	N	Y	Y	N	Y	Y
5.9	Development / Teratogenicity	Y	N	Y	Y	N	Y	N
5.11	Human experience	Y	N	N	Y	N	Y	N
OTHER TOXICITY STUDIES RECEIVED		Y	N	N	Y	N	Y	N

1. GENERAL INFORMATION

1.01 SUBSTANCE INFORMATION

***A. CAS number** 108-80-5

B. Name *(IUPAC name)*

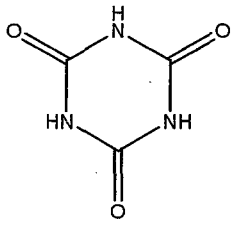
***C. Name** *(OECD name)* Isocyanuric acid

†D. CAS Descriptor

E. EINECS-Number 203-618-0

F. Molecular Formula $C_3H_3N_3O_3$

***G. Structural Formula**



H. Substance Group

I. Substance Remark

J. Molecular Weight 129.08

1.02 OECD INFORMATION

A. Sponsor Country: Japan

B. Lead Organisation:

Name of Lead Organisation: Ministry of Health and Welfare (MHW)
Ministry of International Trade and Industry (MITI)
Environmental Agency (EA)
Ministry of Labour (MOL)

Contact person: Mr. Kazuhide Ishikawa
Second International Organization Division
Economic International Bureau
Ministry of Foreign Affairs

Address:

Street: 2-2-1 Kasumigaseki, Chiyoda-ku, Tokyo 100 Japan
Tel: 81-3-3581-0018
Fax: 81-3-3503-3136

C. Name of responder

Same as above contact person

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance

element [ ]; inorganic[ ]; natural substanc [ ]; organic[**X**]; organometallic [ ]; petroleum product [ ]

B. Physical State *(at 20°C and 1.013 hPa)*

gaseous [ ]; liquid [ ]; solid [**X**]

C. Purity 99.7 %

1.2 SYNONYMS

sym-Triazine-2,4,6-triol; sym-Triazinetriol; normal Cyanuric acid; 2,4,6-Trihydroxy-1,3,5-triazine; Trihydroxycyanidine; Tricyanic acid; Pseudocyanuric acid; 1,3,5-Triazine-2,4,6(1H,3H,5H)-trione; 1,3,5-Triazine-2,4,6-triol; 1,3,5-Triazinetriol; 1,3,5-Triazinetrione; Tricarbimide; Trihydroxy-1,3,5-triazine

1.3 IMPURITIES

None

1.4 ADDITIVES

None

*1.5 QUANTITY

Remarks: 20,000 tonnes/year
Reference: MITI, Japan

1.6 LABELLING AND CLASSIFICATION

None

*1.7 USE PATTERN

A. General

Type of Use:	**Category:**
main	Intermediate
industrial	Intermediate in closed system
use	Intermediate for various chemicals

Remarks: None
Reference: MITI, Japan

1.8 OCCUPATIONAL EXPOSURE LIMIT

None

* 1.9 SOURCES OF EXPOSURE

In Japan, isocyanuric acid is produced in 2 companies.

Source:	Media of release:	River
	Quantities per media:	407.7 tonnes/year
Remarks:		
Reference:	MITI, Japan	

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	330 °C
Decomposition:	Yes [X] No [ ] Ambiguous [ ]
Sublimation:	Yes [ ] No [X] Ambiguous [ ]
Method:	
GLP:	Yes [ ] No [X] ? [ ]
Remarks:	
Reference:	Organic Chemical Dictionary

*2.2 BOILING POINT

Value:	not measurable
Pressure:	
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Method:	
GLP:	Yes [ ] No [X] ? [ ]
Remarks:	
Reference:	MITI, Japan

*2.4 VAPOUR PRESSURE

Value:	$< 5.0 \times 10^{-3}$ Pa
Temperature:	25 °C
Method:	calculated [ ]; measured [X] OECD TG 104
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: 99.9 %
Remarks:	
Reference:	MITI, Japan

*2.5 PARTITION COEFFICIENT $\log_{10} P_{ow}$

Log Pow:	< 0.3
Temperature:	25 °C

Method:	calculated [ ]; measured [X] OECD TG 107 HPLC method
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: 99.9 %
Remarks:	
Reference:	MITI, Japan

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	2.7 g/l
Temperature:	25 °C
Description:	Miscible [ ]; Of very high solubility [X]; Soluble [ ]; Slightly soluble [ ]; Of low solubility [ ]; Of very low solubility [ ]; Not soluble [ ]
Method:	OECD TG 105
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: 99.9 %
Remarks:	
Reference:	MITI, Japan

B. pH Value, pKa Value

Value:	$pK_1 = 6.88$ $pK_2 = 11.40$ $pK_3 = 13.50$
Reference:	Merck Index

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

***3.1.2 STABILITY IN WATER**

Type:	Abiotic (hydrolysis) [X]; biotic (sediment)[ ]
Half life:	Stable in pH 4, 7, 9 at 25 °C
Method:	OECD TG 111
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: 99.9 %
Remarks:	
Reference:	MITI, Japan

***3.2 MONITORING DATA (ENVIRONMENTAL)**

(a)

Type of Measurement:	Background [ ]; At contaminated site [ ]; Other [X]
Media:	Surface water (lake)
Results:	ND (Detection limits: 0.002 mg/l) in 3 areas in Japan as of 1983

Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

(b)
Type of Measurement: Background [ ]; At contaminated site [ ]; Other [X]
Media: Surface water (estuary)
Results: ND (Detection limits: 0.004 mg/l) in 1 area in Japan as of 1983
Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

(c)
Type of Measurement: Background [ ]; At contaminated site [ ]; Other [X]
Media: Surface water (sea)
Results: ND (Detection limits: 0.002 - 0.004 mg/l) in 6 areas in Japan as of 1983
Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

(d)
Type of Measurement: Background [ ]; At contaminated site [ ]; Other [X]
Media: Sediment (lake)
Results: ND (Detection limits: 0.12 - 0.24 mg/kg-dry) in 3 areas in Japan as of 1983
Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

(e)
Type of Measurement: Background [ ]; At contaminated site [ ]; Other [X]
Media: Sediment (estuary)
Results: ND (Detection limit: 0.09 mg/kg-dry) in 1 area in Japan as of 1983
Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

(f)
Type of Measurement: Background [ ]; At contaminated site [ ]; Other [X]
Media: Sediment (sea)
Results: ND (Detection limit: 0.025 - 0.15 mg/kg-dry) in 6 areas in Japan as of 1983
Remarks: ND: Not detected
Reference: Chemicals in the environment, EA, Japan (1984)

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota [ ]; Air-biota-sediment-soil-water [X]; Soil-biota [ ]; Water-air [ ]; Water-biota [ ]; Water-soil [ ]; Other [ ]

Method: Fugacity level I [ ]; Fugacity level II [ ]; Fugacity level III **[X]**; Fugacity level IV [ ]; Other (calculation) [ ]; Other (measurement)[ ]

Results:

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	0.1 %	0.0 %	0.0 %
Water	46.5 %	99.6 %	40.5 %
Soil	53.3 %	0.0 %	59.3 %
Sediment	0.2 %	0.4 %	0.2 %

Remarks: Appendix 1
Reference: MITI. Japan

*3.5 BIODEGRADATION

Type: aerobic **[X]**; anaerobic [ ]
Inoculum: adapted [ ]; non-adapted **[X]**;
Concentration of the chemical: related to COD [ ]; DOC [ ]; test substance **[X]**
Medium: water **[X]**; water-sediment [ ]; soil [ ]; sewage treatment [ ]
Degradation: 0 % by BOD after 14 days
7.8 % by TOC after 14 days
5.3 % by HPLC after 14 days
Results: readily biodeg. [ ]; inherently biodeg. [ ]; under test condition no biodegradation observed **[X]**, other [ ]
Method: OECD TG 301C
GLP: Yes **[X]** No [ ] ? [ ]
Test substance: purity: 99.9 %
Reference: MITI, Japan

3.7 BIOACCUMULATION

Species: Carp (*Cyprinus carpio*)
Exposure period: 6 weeks
Temperature: 25 °C
Concentration: (1) 10 mg/L
(2) 1 mg/L
BCF: (1) < 0.1
(2) < 0.5
Method: OECD TG 305C
Type of test: calculated [ ]; measured **[X]**
static[ ]; semi-static[ ]; flow-through**[X]**; other*(e.g. field test)*[ ]
GLP: Yes **[X]** No [ ] ? [ ]
Test substance: purity: 99.9 %
Remarks:
Reference: MITI, Japan

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

(a)

Type of test:	static [ ]; semi-static [X]; flow-through [ ]; other *(e.g. field test)* [ ] open-system [X]; closed-system [ ]
Species:	*Oryzias latipes* (Himedaka)
Exposure period:	96 h
Results:	LC_{50} (96h) > 100 mg/l
Analytical monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD TG 203 (1992)
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	As prescribed by 1.1 - 1.4, purity: 99.7 %
Remarks:	Groups of 10 Himedaka were exposed to the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/l and laboratory water control. Solubilizer was not used. Concentrations of the test substance were kept close to the nominal concentrations (99.5 to 103 %).
Reference:	Environment Agency of Japan (1996)

(b)

Type of test:	static [ ]; semi-static[ ]; flow-through [X]; other *(e.g. field test)* [ ] open-system [X]; closed-system [ ]
Species:	*Oryzias latipes* (Himedaka)
Exposure period:	14 d
Results:	LC_{50} (14d) > 100 mg/l
Analytical monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD TG 203 (1992)
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	As prescribed by 1.1 - 1.4, purity: 99.7 %
Remarks:	Groups of 10 Himedaka were exposed to the nominal concentrations of 10, 32 and 100 mg/l and laboratory water control. Solubilizer was not used. Concentrations of the test substance were kept close to the nominal concentrations throughout the 14-d test (99 to 102 %).
Reference:	Environment Agency of Japan (1996)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test:	static [X]; semi-static [ ]; flow-through [ ]; other*(e.g. field test)*[ ]; open-system [X]; closed-system [ ]
Species:	*Daphnia magna.*
Exposure period:	48 h
Results:	EC_{50} (48h) = 1000 mg/l
Analytical monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD TG 202
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	As prescribed by 1.1 - 1.4, purity: 99.7 %

Remarks:	20 daphnids (4 replicates; 5 organisms per replicate) were exposed to measured concentrations of 100, 180, 320, 580 and 1000 mg/l and laboratory water control. Solubilizer was not used. Concentrations of the test substance were kept close to the nominal concentrations throughout the 48-h test (99.2 to 103.0 %).
Reference:	Environment Agency of Japan (1996)

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Species:	*Selenastrum capricornutum* ATCC 22662
Endpoint:	Biomass [X]; Growth rate [ ]; Other [ ]
Exposure period:	72 h
Results:	Biomass $EC_{50\ (72h)}$ = 620 mg/l *(Endpoint)* NOEC = 62.5 mg/l
Analytical monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD TG 201 (1984) open-system [ ]; closed-system [X]
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	As prescribed by 1.1 - 1.4, purity: 99.7 %
Remarks:	Static test. The EC_{50} value for biomass was calculated based on the measured concentrations of the nominal concentrations 62.5, 125, 250, 500 and 1000 mg/l. No solubilizer was used. Concentrations of the test substance were kept close to the nominal concentrations throughout the 72-h test (98 to 105 %).
Reference:	Environment Agency of Japan (1996)

4.4 TOXICITY TO BACTERIA

No data

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

(*)4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Type of test:	static [ ]; semi-static [X]; flow-through [ ]; other *(e.g. field test)* [ ]; open-system [X]; closed-system [ ]
Species:	*Daphnia magna*
Endpoint:	Mortality [ ]; Reproduction rate [X]; Other [X]
Exposure period:	21 d
Results:	Reproduction rate: $EC_{50\ (21\ d)}$ = 65.9 mg/l *(Endpoint)* NOEC = 32.0 mg/l
Analytical monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD TG 202(1984)
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	As prescribed by 1.1 - 1.4, purity: 99.7 %
Remarks:	40 daphnids (4 replicate; 10 daphnids per replicate) were exposed to the nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg/l and laboratory water control (dechlorinated tap water).

Concentrations of the test substance were kept close to the nominal concentrations throughout the 21-d test (95 to 103 %). The test water was renewaled every 2 or 3 days.

Reference: Environment Agency of Japan (1996)

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

No data

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

No data

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

No data

4.8 BIOTRANSFORMATION AND KINETICS

No data

4.9 ADDITIONAL REMARKS

None

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a)	Type:	LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
	Species/strain:	Rats/albino
	Value:	7,700 mg/kg b.w.
	Method:	Other
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	
	Reference:	Babayan & Aleksandryan: 1985
(b)	Type:	LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
	Species/strain:	Rats
	Value:	> 7,500 mg/kg b.w.

Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Remarks:
Reference: *Gigiena i Sanitariya*: 1962

(c) Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain: Mice
Value: 3,400 mg/kg b.w.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: purity: unknown
Remarks:
Reference: Babayan & Aleksandryan: 1985

(d) Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [ ]; LDL_0 [X]; Other [ ]
Species/strain: Rabbits
Value: > 10 g/kg b.w.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: purity: unknown
Remarks:
Reference: Toxicity Information: 1972

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_0 [ ]; LC_{100} [ ]; LC_{50} [ ]; LCL_0 [ ]; Other [X]
Species/strain: Rats
Exposure time: not indicated
Value: 612 mg/m^3
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: As an aerosol, purity: unknown
Remarks: Minimum toxic concentration
Reference: Babayan & Aleksandryan: 1985

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain: Rabbits
Value: > 7,940 mg/kg b.w.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: purity: unknown
Remarks:
Reference: Toxikologische Bewertung: 1993

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain: Rats

Route of Administration:	i.m. [ ]; i.p. [ ]; i.v. [X]; infusion [ ]; s.c. [ ]; other [ ]
Exposure time:	
Value:	> 100 mg/kg b.w.
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	*Gigiena i Sanitariya*: 1962

Type:	LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain:	Mice
Route of Administration:	i.m. [ ]; i.p. [ ]; i.v. [X]; infusion [ ]; s.c. [ ]; other [ ]
Exposure time:	
Value:	> 500 mg/kg b.w.
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	*Gigiena i Sanitariya*: 1962

Type:	LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain:	Cats
Route of Administration:	i.m. [ ]; i.p. [ ]; i.v. [X]; infusion [ ]; s.c. [ ]; other [ ]
Exposure time:	
Value:	2,144 mg/kg b.w.
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	Sodium isocyanurate, purity: unknown
Remarks:	
Reference:	*J. Pharmacol. Exp. Ther.*: 1951

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/strain:	Rabbits
Results:	Highly corrosive [ ]; Corrosive [ ]; Highly irritating [ ]; Irritating [ ]; Moderate irritating [ ]; Slightly irritating [ ]; Not irritating [X]
Classification:	Highly corrosive (causes severe burns) [ ]; Corrosive (causes burns)[ ]; Irritating [ ]; Not irritating [ ]
Method:	Federal Hazardous Substances Act (FHSA) tests
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	Hammond *et al.*: 1986

5.2.2 EYE IRRITATION/CORROSION

(a) Species/strain: Rabbits

	Results:	Highly corrosive [ ]; Corrosive [ ]; Highly irritating [ ]; Irritating [ ]; Moderate irritating [ ]; Slightly irritating [X]; Not irritating [ ]
	Classification:	Irritating [ ]; Not irritating [ ]; Risk of serious damage to eyes [ ]
	Method:	Federal Hazardous Substances Act (FHSA) tests
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	
	Reference:	Hammond *et al.*: 1986
(b)	Species/strain:	Rabbits
	Results:	Highly corrosive [ ]; Corrosive [ ]; Highly irritating [ ]; Irritating [ ]; Moderate irritating [X]; Slightly irritating [ ]; Not irritating [ ]
	Classification:	Irritating [ ]; Not irritating [ ]; Risk of serious damage to eyes [ ]
	Method:	Rinsed with water
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	Administration into the eye at 20 mg/24 hr
	Reference:	Toxicity Information: 1972
(c)	Species/strain:	Rabbits
	Results:	Highly corrosive [ ]; Corrosive [ ]; Highly irritating [ ]; Irritating [ ]; Moderate irritating [X]; Slightly irritating [ ]; Not irritating [ ]
	Classification:	Irritating [ ]; Not irritating [ ]; Risk of serious damage to eyes [ ]
	Method:	Standard Draize test
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	Administration into the eye at 500 mg/24 hr
	Reference:	Marhold: 1972

5.3 SKIN SENSITISATION

No data

*5.4 REPEATED DOSE TOXICITY

(a)	Species/strain:	Rats/Crj: CD (SD)
	Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
	Route of Administration:	Oral (by gavage)
	Exposure period:	Male: 44 days Female: From 14 days before mating to day 3 of lactation
	Frequency of treatment:	Daily
	Post exposure observation period:	
	Dose:	0, 10, 40, 150, 600 mg/kg/day
	Control group:	Yes [X]; No [ ]; No data [ ]; Sesame oil Concurrent no treatment[ ]; Concurrent vehicle[X];Historical[ ]
	NOAEL:	150 mg/kg/day
	LOAEL:	600 mg/kg/day

	Results:	Isocyanuric acid indicated toxic effects at 600 mg/kg in both sexes. Excretion of reddish urine was evident. In addition, depression of body weight gain was observed in males. Urinalyses of males revealed appearance of crystals, which is considered this chemical precipitated from urine, and increases of erythrocytes and leukocytes. In hematological examination of males, significant decreases in erythrocyte counts, hemoglobin concentrations and hematocrit values were observed. In blood chemical examination of males, increases in urea nitrogen and creatinine, and a decrease of sodium were revealed. In histopathological examination, dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis in the kidney, hyperplasia of the mucosal epithelium in the urinary bladder and vacuolization of the zona fasciculata in the adrenals were observed in both sexes. In addition, the incidence of atrophic thymus also showed a tendency for increase in females. Absolute and relative kidney weights and relative adrenal weights were increased in both sexes.
	Method:	OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test
	GLP:	Yes [X] No [ ] ? [ ]
	Test substance:	purity: 99.8 %
	Reference:	MHW, Japan: 1997
(b)	Species/strain:	Rats/Rochester strain (Wistar-derived)
	Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
	Route of Administration:	Oral (in diet)
	Exposure period:	20 weeks
	Frequency of treatment:	Daily
	Post exposure observation period:	
	Dose:	0, 0.8, 8 % (calculated daily dose: 0, 56, 560 mg/kg)
	Control group:	Yes [X]; No [ ]; No data [ ]; Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
	NOAEL:	0.8 % (56 mg/kg/day)
	LOAEL:	8 % (560 mg/kg/day)
	Results:	14/20 males and 4/20 females died at 8 %, but no died at 0.8 %. Considerable decrease in body weight gain was observed at 8 %. Urine samples taken prior to the start of feeding and again near termination of the study showed normal concentrations of protein and sugar. In hematological examination no change was observed. There were no changes in organ weights (thyroid, liver, brain, lungs, heart, etc.), expect for kidney weight, which increased at 8 % in females. In histologic study, dilatation of distal collecting tubules and dusts of Bellini, with focal areas of epithelial proliferation were observed at 8 % in both sexes.
	Method:	Other
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	Sodium isocyanurate, purity: unknown
	Reference:	Hodge *et al.*: 1965

(c) Species/strain: Mice/B6C3F1
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral (in drinking water)
Exposure period: 90 days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 896, 1,792, 5,375 ppm
Control group: Yes [X]; No [ ]; No data [ ];
Concurrent no treatment[X];Concurrent vehicle[X];Historical[ ]
NOAEL: 5,375 ppm (male: 1,994 mg/kg/day, female: 2,200mg/kg/day)
LOAEL:
Results: Although increase in water consumption in both sexes and absolute and relative weights of ovaries in females were observed, these changes were considered due to the high sodium content. No adverse effect was observed.
Method: Other
GLP: Yes [X] No [ ] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Remarks: Sodium hippurate was used as a second control in order to have the sodium burden as the top concentration.
Reference: Hazleton U.S.: 1982

(d) Species/strain: Dogs/Beagle
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral (in diet)
Exposure period: 6 months
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0 (vehicle), 0.8 % (calculated daily dose: 291 mg/kg)
Control group: Yes [ ]; No [X]; No data [ ];
Concurrent no treatment[ ]; Concurrent vehicle[ ]; Historical[ ]
NOAEL: 0.8 % (291 mg/kg/day)
LOAEL:
Results: There were no changes in body weight gain, organ weight, and sugar and protein in urine. In addition, hematological and histological changes were not observed.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Reference: Hodge *et al.*: 1965

(e) Species/strain: Dogs/Beagle
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral (in diet)
Exposure period: 2 years
Frequency of treatment: Daily
Post exposure observation period:
Dose: 8 % (calculated daily dose: 2,912 mg/kg)
Control group: Yes [ ]; No [X]; No data [ ];

Concurrent no treatment[ ]; Concurrent vehicle[ ]; Historical[ ]

NOAEL:
LOAEL: 8 % (2912 mg/kg/day)
Results: Two of three dogs died after 16 and 21 months on the regimen, respectively. No change or slight increase in body weights was observed. Periodic urinalyses gave normal trace values for sugar and protein. In hematologic study, only a survival dog showed changes, which are low red blood cell counts, hemoglobin values, and hematocrits. There was no change in organ weights (thyroid, liver, brain, lungs, heart, etc.), expect for decrease in kidney weight of two dogs surviving more than 20 months. In these dogs, there was gross evidence of kidney fibrosis. Sections revealed numerous linear streaks of gray fibrous tissue extending from the papillary tip to the cortical surface. Microscopically, similar changes were observed in the kidneys of all three dogs. The collecting tubules were more uniformly and severely involved, but all portions of the nephron were compressed by fibrosis. There were slight focal dilatation and epithelial proliferation in the ducts of Bellini. In survival dog, focal areas of thyroid atrophy were found with lymphocytic infiltration, but without evidence of hyperplasia.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Reference: Hodge *et al.*: 1965

(f) Species/strain: Rabbits/Albino
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Dermal
Exposure period: Approx. 3 months
Frequency of treatment: 5 days/week
Post exposure observation period:
Dose: 5 ml of 0.8 % or 8 % aqueous suspension
Control group: Yes [ ]; No [X]; No data [ ];
Concurrent no treatment[ ]; Concurrent vehicle[ ]; Historical[ ]
NOAEL: 0.8 %
LOAEL: 8 %
Results: Urinalyses (sugar and protein) and hematological study showed no change. There were no irritation or other adverse effects on the skin. In histological findings of liver and skin from treated and untreated area, no change was observed at the termination of the study. In the kidneys of the rabbits treated with the 8 % isocyanurate suspension, slight dilatation of the ducts of Bellini and mild tubular changes were found.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Reference: Hodge *et al.*: 1965

(g) Species/strain: Rabbits/Albino
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]

Route of Administration:	Eye application
Exposure period:	Approx. 3 months
Frequency of treatment:	5 days/week
Post exposure observation period:	
Dose:	0.1 ml of 0.8 % or 8 % aqueous suspension
Control group:	Yes [X]; No [ ]; No data [ ]; Concurrent no treatment[X]; Concurrent vehicle[ ];Historical[ ]
NOAEL:	0.8 %
LOAEL:	8 %
Results:	Increase in body weight was observed during the period of the study in all treated groups. No eye injury was caused and no eye irritation was observed in rabbits treated with an 8 % aqueous suspension of the sodium salt.
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	Sodium isocyanurate, purity: unknown
Reference:	Hodge *et al.*: 1965

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type:	Ames test
System of testing:	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100
Concentration:	100 to 1000 µg/plate
Metabolic activation:	With [ ]; Without[ ]; With and Without [X]; No data [ ]
S9:	Hamster liver - Arochlor 1254
Results:	

		+	?	-
Cytotoxicity conc:	With metabolic activation:			
	Without metabolic activation:			
Precipitation conc:				
Genotoxic effects:		+	?	-
With metabolic activation:		[ ]	[ ]	[X]
Without metabolic activation:		[ ]	[ ]	[X]

Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	Hayworth *et al.*: 1983

Type:	Other: Inductest Pasteur
System of testing:	Induction of bacteriophage Lambda in *Escherichia Coli* K12 en VA UVRB
Concentration:	0.2 to 2000 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	

		+	?	-
Cytotoxicity conc:	With metabolic activation:			
	Without metabolic activation:			
Precipitation conc:				
Genotoxic effects:		+	?	-
With metabolic activation:		[ ]	[ ]	[X]

	Without metabolic activation: [ ] [ ] [X]
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	NORSOLOR/APC: 1977

B. NON-BACTERIAL IN VITRO TEST

Type:	Chromosomal aberration test
System of testing:	Chinese hamster lung (CHL/IU) cells
Concentration:	+S9 (short-term treatment): 0, 0.33, 0.65, 1.3 mg/ml -S9 (continuous treatment): 0, 0.33, 0.65, 1.3 mg/ml -S9 (short-term treatment): 0, 0.33, 0.65, 1.3 mg/ml
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
S9:	Rat liver, induced with phenobarbital and 5,6-benzoflavone
Results:	
	Cytotoxicity conc: Not observed Precipitation conc: Genotoxic effects: clastogenicity (+ ? -) polyploidy (+ ? -) With metabolic activation: [ ] [ ] [X] [ ] [ ] [X] Without metabolic activation: [ ] [ ] [X] [ ] [ ] [X]
Method:	Guidelines for Screening Mutagenicity Testing of Chemicals (Japan), and OECD TG (473).
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: 99.5 %
Remarks:	Exposure period: short-term treatment: 6 hr continuous treatment: 24, or 48 hr Positive control: -S9: Mitomycin, +S9: Cyclophosphamide
Reference:	MHW, Japan: 1997

Type:	Mouse lymphoma assay
System of testing:	L 5178 TK +/-
Concentration:	50 to 2000 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	
	Cytotoxicity conc: With metabolic activation: Without metabolic activation: Precipitation conc: Genotoxic effects: + ? - With metabolic activation: [ ] [ ] [X] Without metabolic activation: [ ] [ ] [X]
Method:	Other
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	Industry ad hoc Committee for Isocyanurates: 1981a

Type:	Sister chromatid exchange assay
System of testing:	CHO cells

Concentration:	93 to 1500 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	
	Cytotoxicity conc: With metabolic activation:
	Without metabolic activation:
	Precipitation conc:
	Genotoxic effects: + ? -
	With metabolic activation: [ ] [ ] [X]
	Without metabolic activation: [ ] [ ] [X]
Method:	Other
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	purity: unknown
Remarks:	
Reference:	Industry ad hoc committee for Isocyanurates: 1981b

* 5.6 GENETIC TOXICITY IN VIVO

Type:	Chromosomal aberration test
Species/strain:	Rats
Sex:	Female [ ]; Male [ ]; Male/Female [ ]; No data [X]
Route of Administration:	Oral (single gavage administration)
Exposure period:	
Doses:	Up to 5000 mg/kg
Results:	
	Effect on mitotic index or P/N ratio:
	Genotoxic effects: + ? -
	[ ] [ ] [X]
Method:	Other
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	Sodium isocyanurate, purity: unknown
Remarks:	Rats were killed 24 and 48 hr after dosing, and bone marrow cells were collected and examined for chromosomal aberrations.
Reference:	Hammond *et al.*: 1985

5.7 CARCINOGENICITY

(a)	Species/strain:	Rats/CD
	Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
	Route of Administration:	Oral (in drinking water)
	Exposure period:	2 years
	Frequency of treatment:	Daily
	Postexposure observation period:	
	Doses:	0 (vehicle), 400, 1,200, 2,400, 5,375 ppm (Estimated daily doses were indicated only for 2,400 and 5,375 ppm (male: 154 and 371 mg/kg/day, female: 266 and 634 mg/kg/day))
	Control group:	Yes [X]; No [ ]; No data [ ]; tap water Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
	Results:	No test article related carcinogenesis.

	Method:	Other
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	Sodium isocyanurate, purity: unknown
	Remarks:	Sodium hippurate was administered at the equivalent amount of sodium to the highest dose group as a second control. Treatment-related mortality was observed in some males of highest dose group, which died during the first 12 months of the study. This mortality was due to the development of calculi in the urinary tract. In some males that died on test and in some that were sacrificed at 12 months, there were pathologic changes, including hyperplasia, bleeding, and inflamed ureters, and renal tubular nephrosis. Although slight tubular nephrosis was also observed in a few females of highest dose group during the first 12 months, these animals did not exhibit bladder calculi. Inflammatory lesions in the heart were also apparent in some of the highest dose males that died early.
	Reference:	Cascieri *et al.*: 1985
(b)	Species/strain:	Mice/B6C3F1
	Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
	Route of Administration:	Oral (in drinking water)
	Exposure period:	2 years
	Frequency of treatment:	Daily
	Postexposure observation period:	
	Doses:	0 (vehicle), 100, 400, 1,200, 5,375 ppm
	Control group:	Yes [X]; No [ ]; No data [ ]; Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
	Results:	There was no evidence of test article related carcinogenesis.
	Method:	Other
	GLP:	Yes [X] No [ ] ? [ ]
	Test substance:	Sodium isocyanurate, purity: unknown
	Remarks:	Sodium hippurate was administered at the equivalent amount of sodium to the highest dose group as a second control. Apparent swollen enlarged abdomen was observed at the highest dose groups (both isocyanurate and hippurate). There were no effects on survival, clinical pathology (except for urinary sodium), organ weight, gross and histopathology.
	Reference:	Industry Ad hoc Committee for Isocyanurates, Hazleton laboratories, Report 2169-100 (1986)
(c)	Species/strain:	Rats
	Sex:	Female [ ]; Male [ ]; Male/Female [ ]; No data [X]
	Route of Administration:	Subcutaneous
	Exposure period:	2 years
	Frequency of treatment:	Once a week
	Postexposure observation period:	
	Doses:	Total dose: 6.06 g (approx. daily dose: 8.3 mg/day)
	Control group:	Yes [ ]; No [ ]; No data [X]; Concurrent no treatment[ ]; Concurrent vehicle[ ]; Historical[ ]

	Results:	A lymphosarcoma in lungs has been observed in 1 of the 5 surviving rats after 28 months, and a subdermal lipoma in 1 of the other rats after 30.5 months.
	Method:	Other
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	
	Reference:	Toxikologische Bewertung.: 1993
(d)	Species/strain:	Mice
	Sex:	Female [ ]; Male [ ]; Male/Female [ ]; No data [X]
	Route of Administration:	Subcutaneous
	Exposure period:	2 years
	Frequency of treatment:	Once a week
	Postexposure observation period:	
	Doses:	Total dose: 0.6 g (estimated daily dose: 0.82 mg/day)
	Control group:	Yes [ ]; No [ ]; No data [X]; Concurrent no treatment[ ];Concurrent vehicle[ ]; Historical [ ]
	Results:	No tumours were observed.
	Method:	Other
	GLP:	Yes [ ] No [X] ? [ ]
	Test substance:	purity: unknown
	Remarks:	
	Reference:	Toxikologische Bewertung.: 1993

*5.8 TOXICITY TO REPRODUCTION

(a)	Type:	Fertility [ ]; One-generation study [ ]; Two-generation study [ ]; Other [X]
	Species/strain:	Rats/Crj: CD (SD)
	Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
	Route of Administration:	Oral (by gavage)
	Exposure period:	Male: 14 days before mating Female: 14 days before mating to day 3 of lactation
	Frequency of treatment:	Daily
	Post exposure observation period:	
	Premating exposure period:	14 days
	Duration of the test:	
	Dose:	0, 10, 40, 150, 600 mg/kg/day
	Control group:	Yes [X]; No [ ]; No data [ ]; Sesame oil Concurrent no treatment[·];Concurrent vehicle[X]; Historical[ ]
	NOEL Parental:	Male: 600 mg/kg/day, Female: 600 mg/kg/day
	NOEL F1 Offspring:	600 mg/kg/day
	NOEL F2 Offspring:	
	Results:	General parental toxicity: Isocyanuric acid indicated no alteration in reproductive parameters including the copulation index, fertility index, gestation length, numbers of corpora lutea or implantations, implantation index, gestation index, delivery index, and behavior at delivery and lactation.

Toxicity to offspring:
There were no significant differences in offspring parameters including number of offspring or live offspring, the sex ratio, live birth and viability indices, and body weight. No external or visceral abnormalities related to the test substance were detected in any of the offspring.

Method: OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test
GLP: Yes [X] No [ ] ? [ ]
Test substance: purity: 99.8 %
Remarks:
Reference: MHW, Japan: 1997

(b) Type: Fertility [ ]; One-generation study [ ]; Two-generation study [ ]; Other [X] *Three generation study
Species/strain: Rats/CD
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral (in drinking water)
Exposure period: P0: A minimum of 100 days from 36 days of age to mating
F1 and F2: 120 days after weaning
F3: 4 weeks
Frequency of treatment: Daily
Post exposure observation period:
Premating exposure period: A minimum of 100 days
Duration of the test:
Dose: 0 (vehicle), 400, 1,200, 5,375 ppm
Control group: Yes [X]; No [ ]; No data [ ]; tap water
Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
NOAEL Parental: 5,375 ppm (Approx. 370 mg/kg/day for male, 634 mg/kg/day for female)
NOAEL F1 Offspring: 5,375 ppm
NOAEL F2 Offspring: 5,375 ppm
NOAEL F3 Offspring: 5,375 ppm
Results:

General parental toxicity:
No compound related changes were observed in mortality, body weight, food consumption, and gestation length. In pathological and histological findings, there were also no changes.

Toxicity to offspring:
No compound-related changes were observed in mortality, body weights, food consumption litter size, pup survival to weaning, sex ratio, and pup weight. In pathological and histological findings, epithelial hyperplasia with chronic cystitis was observed in a few of high-dose treated males in F2 offsprings, which were attributed to chronic irritation by the calculi in the urinary bladder. In other treated groups, there were no changes.

Method: Other
GLP: Yes [X] No [ ] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown

Remarks: Sodium hippurate was provided an equivalent amount of sodium administered to high-dose sodium isocyanurate animals as second control.
Weanlings from the F1 and F2 litters were randomly selected as parents for the next generation and continued on treatment. Related litters and F3 offsprings were sacrificed 4 weeks after weaning and organ weight measurements and microscopic examination of tissues were carried out.
Reference: Wheeler *et al.*: 1985

(c) Type: Fertility [ ]; One-generation study [ ]; Two-generation study [ ]; Other [X]
Species/strain: Mice/CD-1
Sex: Female [ ]; Male [X]; Male/Female [ ]; No data [ ]
Route of Administration: i.p.
Exposure period: 6 weeks
Frequency of treatment:
Post exposure observation period:
Premating exposure period:
Duration of the test: 6 weeks
Doses: 0 (vehicle), 125 and 250 mg/kg/day
Control group: Yes [X]; No [ ]; No data [ ];
Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
NOAEL Parental: 250 mg/kg/day
NOAEL Foetal: 250 mg/kg/day
Results:
General parental toxicity:
Any treatment related effects were not observed in females, mated with sodium isocyanurate treated males.
Toxicity to fetus:
Any toxicity was not observed.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Remarks: As positive control, methyl methane sulfonate was used at dose of 50 mg/kg/day.
Non-treated females are mated with the treated males every week.
As a result, early resorptions were observed in females mated with males treated with methyl methane sulfonate.
Reference: FMC Corporation: 1972

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Species/strain: Rabbits/Dutch belted
Sex: Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration: Oral (by gavage)
Duration of the test: 22 days
Exposure period: Days 6-18 of gestation
Frequency of treatment: Daily
Doses: 0 (vehicle), 50, 200, 500 mg/kg/day

Control group: Yes [X]; No [ ]; No data [ ]; 20 mL/kg water
Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
NOAEL Maternal Toxicity: 50 mg/kg/day
NOAEL teratogenicity: 200 mg/kg/day
Results:
Maternal general toxicity:
Although slight decrease in body weight were observed in mid- and high-dose groups during the treatment period, compensatory weight gains occurred after termination of treatment on day 18. There were no compound related mortality or other adverse reactions.
Pregnancy/litter data:
Foetal data:
The mean number of live fetus/dam and the sex ratio were essentially comparable for all groups. Body weights and crown/rump lengths were reduced slightly in high-dose groups, compared to control. There was no evidence of external or internal malformations or skeletal anomalies.
Method: Other
GLP: Yes [ ] No [X] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Remarks:
Reference: FMC Corporation, unpublished observations

Species/strain: Rats/Sprague-Dawley
Sex: Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration: Oral (by gavage)
Duration of the test: 20 days
Exposure period: Days 6-15 of gestation
Frequency of treatment: Daily
Doses: 0 (vehicle), 200, 1,000, 5,000 mg/kg/day
Control group: Yes [X]; No [ ]; No data [ ];
Concurrent no treatment[ ];Concurrent vehicle[X]; Historical[ ]
NOAEL Maternal Toxicity: 5,000 mg/kg/day
NOAEL teratogenicity: 5,000 mg/kg/day
Results:
Maternal general toxicity:
There were no treatment-related effects on maternal appearance, behavior and body weight gain in all groups treated with sodium isocyanurate.
Pregnancy/litter data:
Foetal data:
No teratogenic effects were observed in all groups treated with sodium isocyanurate.
Method: Other
GLP: Yes [X] No [ ] ? [ ]
Test substance: Sodium isocyanurate, purity: unknown
Remarks: Sodium control groups received sodium hippurate at doses of 1,118 and 5,590 mg/kg/day.

In sodium control group, decrease in body weight and crown/rum length, and increase in post-implantation loss and incidence of incomplete ossification were observed.

Reference: Industry ad hoc Committee for Isocyanurates: 1982

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

There is no available data.

B. Toxicodynamics, toxicokinetics

Type: Toxicokinetics

Results: Toxicokinetics study of sodium isocyanurate was performed in rats, using [^{14}C] sodium isocyanurate. The elimination half-life was 30 to 60 min after oral or intravenous administration at 5 mg/kg and 2.5 hr after oral administration at 500 mg/kg. At 5 mg/kg, this chemical was completely absorbed and largely eliminated in urine, while at 500 mg/kg, this chemical was incompletely absorbed and largely eliminated in feces. The remainder of radioactivity in most tissues was below the level of detection (0.1-1.0 μg/g) 7 days after treatment. In second study, rats were administered unlabeled sodium isocyanurate orally at 5 mg/kg/day for 14 days followed by the single exposure on day 15. As results of second study, no bioaccumulation and no significant changes in disposition or metabolism were observed, compared to the single exposure. In excreta, only unchanged isocyanurate was found.

Remarks:

References: Barbee *et al.*: 1983

Type: Toxicokinetics

Results: Toxicokinetics study of sodium isocyanurate was conducted in dogs, using [^{14}C] sodium isocyanurate. Administration was performed at 5 mg/kg by oral or intravenous route and at 500 mg/kg by oral route. At 5 mg/kg, this chemical was completely absorbed and largely eliminated in urine, while at 500 mg/kg, this chemical was only partially absorbed and largely eliminated in feces. Sodium isocyanurate distributed into an apparent volume of distribution of 0.7 L/kg, which is somewhat greater than total body water volume. The elimination half-life was from 1.5 to 2 hr after administration. Dogs were also administered unlabeled sodium isocyanurate orally at 5 mg/kg/day followed by the single exposure of 5 mg/kg radiolabeled sodium isocyanurate on day 15. The remainder of radioactivity in most tissues was below the level of detection (0.1-3.3 μg/g) for all sampling times for both single and repeated dose administration. In excreta, only unchanged isocyanurate was found.

Remarks:

References: Barbee *et al.*: 1984

Type: Toxicokinetics
Results: Toxicokinetics study by dermal route was performed, in which species was not indicated. After dermal application, the ^{14}C-labelled substance is not detectable in the blood and < 0.01% of the administered dose is found in the urine.
Remarks:
References: Toxikologische Bewertung: 1993

* 5.11 EXPERIENCE WITH HUMAN EXPOSURE

Results: Toxicokinetics of isocyanuric acid was investigated in 5 volunteers, who soaked in a swimming pool for 120 minutes. As a result, the cumulative excretion of isocyanuric acid was 0.03-2.8 mg, equivalent to 3.0-3.6 ml of pool water and the elimination half-life is caluculated as 3 hr. On the other hand, recovery of ingested isocyanuric acid is 98 % in urine. No correlation observed between toxicokinetics and gamma glutamyl transpeptidase activity.
Distribution 1 compartment open model.
Remarks:
Reference: Allen *et al.*: 1982

6. REFERENCES

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Appendix 1

scenario 1

	emission rate	conc.	amount	percent	transformation rate [kg/h]	
	[kg/h]	[g/m^3]	[kg]	[%]	reaction	advection
air	1,000	9.5.E-08	9.5.E+02	0.1	2.4E+00	9.5.E+00
water	0	4.2.E-02	8.4.E+05	46.5	6.8E+01	8.4.E+02
soil	0	6.0.E-01	9.7.E+05	53.3	7.7E+01	
sediment		3.3.E-02	3.3.E+03	0.2	2.7E-01	6.7.E-02
		total amount	1.8.E+06			

scenario 2

	emission rate	conc.	amount	percent	transformation rate [kg/h]	
	[kg/h]	[g/m^3]	[kg]	[%]	reaction	advection
air	0	4.3.E-12	4.3.E+02	0.0	1.1.E-04	4.3.E-04
water	1000	4.6.E-02	9.3.E+05	99.6	7.4.E+01	9.3.E+02
soil	0	2.7.E-05	4.3.E+01	0.0	3.5.E-03	
sediment		3.7.E-02	3.7.E+03	0.4	2.9.E-01	7.3.E-02
		total amount	9.3.E+05			

scenario 3

	emission rate	conc.	amount	percent	transformation rate [kg/h]	
	[kg/h]	[g/m^3]	[kg]	[%]	reaction	advection
air	0	7.9.E-10	7.9.E+00	0.0	2.0.E-02	7.9.E-02
water	0	4.2.E-02	8.3.E+05	40.5	6.7.E+01	8.3.E+02
soil	1000	7.6.E-01	1.2.E+06	59.3	9.8.E+01	
sediment		3.3.E-02	3.3.E+03	0.2	2.6.E-01	6.6.E-02
		total amount	2.1.E+06			

scenario 4

	emission rate	conc.	amount	percent	transformation rate [kg/h]	
	[kg/h]	[g/m^3]	[kg]	[%]	reaction	advection
air	600	5.7.E-08	5.7.E+02	0.0	1.5.E+00	5.7.E+00
water	300	4.3.E-02	8.7.E+05	55.1	7.0.E+01	8.7.E+02
soil	100	4.4.E-01	7.0.E+05	44.6	5.6.E+01	
sediment		3.4.E-02	3.4.E+03	0.2	2.7.E-01	6.9.E-02
		total amount	1.6.E+06			

Physico-chemical parameter

molecular weight		129.08	Measured
melting point		330	Measured
vapor pressure [Pa]		5.00E-03	Measured
water solubility [g/m^3]		2700	Measured
log Kow		0.3	Measured
half life [h]	in air	272	Estimated
	in water	8640	Estimated
	in soil	8640	Estimated
	in sediment	8640	Estimated

Temp. []	25

Environmental parameter

		volume	depth	area	organic	lipid content	density	residence
		[m^3]	[m]	[m^2]	carbon []	[]	[kg/m^3]	time [h]
bulk air	air	1.0E+13					1.2	100
	particles	2.0E+03						
	total	1.0E+13	1000	1E+10				
bulk water	water	2.0E+10					1000	1000
	particles	1.0E+06			0.04		1500	
	fish	2.0E+05				0.05	1000	
	total	2.0E+10	10	2E+09				
bulk soil	air	3.2E+08					1.2	
	water	4.8E+08					1000	
	solid	8.0E+08			0.04		2400	
	total	1.6E+09	0.2	8E+09				
bulk sediment	water	8.0E+07					1000	
	solid	2.0E+07			0.06		2400	50000
	total	1.0E+08	0.05	2E+09				

Intermedia Transport Parameters m/h

air side air-water MTC	5	soil air boundary layer MTC	5
water side air water MTC	0.05	sediment-water MTC	1E-04
rain rate	1E-04	sediment deposition	5E-07
aerosol deposition	6E-10	sediment resuspension	2E-07
soil air phase diffusion MTC	0.02	soil water runoff	5E-05
soil water phase diffusion MTC	1E-05	soil solid runoff	1E-08

EXTRACT FROM IRPTC LEGAL FILES

File: 17.01 LEGAL rn : 303375

systematic name:1,3,5-Triazine-2,4,6(1H,3H,5H)-trione
common name :cyanuric acid
reported name :ISOCYANURIC ACID
cas no :108-80-5
area : CAN type : REG

subject	specification	descriptor
USE	OCC	RQR
STORE		
LABEL		

INGREDIENT DISCLOSURE LIST CONCENTRATION 1% WEIGHT/WEIGHT. THE WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM (WHMIS) IS A NATIONAL SYSTEM TO PROVIDE INFORMATION ON HAZARDOUS MATERIALS USED IN THE WORKPLACE. WHMIS IS IMPLEMENTED BY THE HAZARDOUS PRODUCTS ACT AND THE CONTROLLED PRODUCTS REGULATIONS (ADMINISTERED BY THE DEPARTMENT OF CONSUMER AND CORPORATE AFFAIRS). THE REGULATIONS IMPOSE STANDARDS ON EMPLOYERS FORTHE USE, STORAGE AND HANDLING OF CONTROLLED PRODUCTS AND ADDRESS LABELLING AND IDENTIFICATION, EMPLOYEE INSTRUCTION AND TRAINING, AS WELL AS THE UPKEEP OF A MATERIALS SAFETY DATA SHEET (MSDS). THE PRESENCE IN A CONTROLLED PRODUCT OF AN INGREDIENT IN A CONCENTRATION EQUAL TO OR GREATER THAN SPECIFIED IN THE INGREDIENT DISCLOSURE LIST MUST BE DISCLOSED IN THE SAFETY DATA SHEET.
entry date: APR 1991 effective date: 31DEC1987

amendment: CAGAAK, Canada Gazette Part II, 122 , 2 , 551 ,

****

File: 17.01 LEGAL rn : 1122611

systematic name:1,3,5-Triazine-2,4,6(1H,3H,5H)-trione
common name :cyanuric acid
reported name :cyanuric acid
cas no :108-80-5
area : RUS type : REG

subject	specification	descriptor
AIR	OCC	MAC CLASS

CLV : 0.5 MG/M3 (AEROSOL) HAZARD CLASS: II
entry date: MAY 1990 effective date: 01JAN1989

amendment: GOSTS*, GOSUDARSTVENNYI STANDART SSSR(STATE STANDARD OF USSR), 12.1.005 , , , 1988

****

File: 17.01 LEGAL rn : 1123035

systematic name:1,3,5-Triazine-2,4,6(1H,3H,5H)-trione
common name :cyanuric acid
reported name :cyanuric acid
cas no :108-80-5
area : RUS type : REG

subject	specification	descriptor
AQ	SURF	MAC CLASS

6.0 MG/L HAZARD CLASS: III
entry date: JUL 1990 effective date: 1JAN1989

amendment: SPNPV*, SANITARNYE PRAVILA I NORMY OKHRANY POVERKHNOSTNYKH VOD OT ZAGRIAZNENIA (HEALTH REGULATION AND STANDARDS OF SURFACE WATER PROTECTION FROM CONTAMINATION), 4630-88 , , , 1988

****

File: 17.01 LEGAL rn : 1320069
systematic name:1,3,5-Triazine-2,4,6(1H,3H,5H)-trione
common name :cyanuric acid
reported name :cyanuric acid
cas no :108-80-5
area : USA type : REG

subject	specification	descriptor
CLASS		RQR
MANUF		PRMT

REGISTRATION STANDARD, CHLORINATED ISOCYANURATES, 1987.; Summary - THIS SUBSTANCE IS INCLUDED ON A LIST OF ACTIVE INGREDIENTS FOR WHICH REGISTRATION STANDARDS HAVE BEEN ISSUED AS OF DECEMBER 24, 1988. A REGISTRATION STANDARD IS A DOCUMENT DESCRIBING THE AGENCY'S SCIENTIFIC CONCLUSIONS AND REGULATORY FINDINGS ABOUT CHEMI CALS THAT ARE INGREDIENTS IN PESTICIDE PRODUCTS. REGISTRANTS OF THESE PESTICIDES MUST SUBMIT DATA ON THOSE SUBSTANCES FOR WHICH THEY ARE RESPONSIBLE. INFORMATION WILL BE INCLUDED INTO A DATABASE WHICH WILL ALLOW EPA TO EVALUATE HEALTH AND ENVIRONMENTAL E FFECTS AND DETERMINE APPROPRIATE REREGISTRATION STANDARDS. THIS LIST STATES THE REGISTRATION STANDARD TITLE AND THE YEAR OF THE ISSUANCE OF THE REGISTRATION STANDARD.
entry date: JAN 1992 effective date: 1988

title: FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT: PESTICIDES FOR WHICH REGISTRATION STANDARDS HAVE BEEN ISSUED. LIST A.
original : FEREAC, Federal Register, 54 , 34 , 7740 , 1989
amendment: FEREAC, Federal Register, 54 , 34 , 7740 , 1989

FOREWORD INTRODUCTION

1-Chloro-2-nitrobenzene

CAS: 88-73-3

SIDS Initial Assessment Report

For

SIAM 13

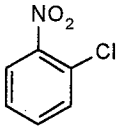
(Bern, Switzerland, 6-9 November 2001)

1. Chemical Name:	1-Chloro-2-nitrobenzene
2. CAS Number:	88-73-3
3. Sponsor Country:	Germany Name of lead organization: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Address: Postfach 12 06 29, D- 53048 Bonn- Bad Godesberg
4. Shared Partnership with:	
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	
7. Review Process Prior to the SIAM:	
8. Quality check process:	
9. Date of Submission:	14. September 2001
10. Date of last Update:	Last literature search (up date): 16 August 2001 (Human Health): databases medline, toxline; searchprofile CAS-No. and special search terms 24 July 2001 (Ecotoxicology): databases CA, biosis; searchprofile CAS-No. and special search terms
11. Comments:	OECD/ICCA - The BUA Peer Review Process Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA

guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4) not assignable)
- Review o f validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	88-73-3
Chemical Name	1-Chloro-2-nitrobenzene
Structural Formula	

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

After single oral application 1-chloro-2-nitrobenzene is toxic to moderate toxic (LD_{50}, oral: rat, male: 144, 251or 560 mg/kg bw, rat, female: 263 mg/kg bw); the acute inhalative and dermal toxicity is moderate (LC_{50}, rat: 3200 mg/m^3 (= 495 ppm, vapor/aerosol mixture); LD_{50}, dermal, rat: female: 1320 mg/kg bw, male: 655 mg/kg bw; LD_{50}, dermal, rabbit: 400 mg/kg bw (male: 455 mg/kg bw, female: 355 mg/kg bw): Cyanotic appearance was the predominant symptom for all routes of application.

The documentation of the available studies on skin irritation is incomplete in one case and in two other cases the test substance was applied undissolved or respectively diluted. However, the studies gave no evidence of a skin irritating potential. 1-Chloro-2-nitrobenzene caused slight irritation effects to the eyes of rabbits, which were reversible within 24 hours. Due to the limited and poor quality information available regarding skin sensitization, it cannot be concluded whether or not the chemical has a sensitizing activity.

Target organs of repeated dose toxicity in rats and mice are blood, liver, kidney and spleen with methemogobinaemia as the most sensitive parameter. The repeated dose toxicity was examined in rats and in mice for a period of 13 weeks via whole body inhalation. The NOAEL in rats was not achieved, the LOAEL is 1.1 ppm (7 mg/m^3). In mice, increased liver and kidney weights were observed even at 1.1 ppm and respectively 2.3 ppm. The NOAEL for histopathological injury in mice is 4.5 ppm (28.8 mg/m^3). In a subacute feeding study with mice the NOAEL was 50 ppm (males: 16 mg/kg bw/day; females: 24 mg/kg bw/day).

1-Chloro-2-nitrobenzene showed weak mutagenic activity in bacterial test systems but not in mammalian cell test systems *in vitro*. It was not mutagenic in *Drosophila melanogaster*. In mammalian cells *in vitro*, it showed weak clastogenic activity. The substance induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear. Intraperitoneal injection into mice resulted in DNA damage in the liver and kidney. The inconsistent results of the available genotoxic studies are typical for nitroaromatics. As a whole 1-chloro-2-nitrobenzene is suspected of being genotoxic, at least a weak clastogen.

1-Chloro-2-nitrobenzene induced tumours in different organs of rats and in the liver of mice. Based on the available studies, which have methodological deficiencies, there is a concern for a carcinogenic potential of 1-chloro-2-nitrobenzene. Following inhalative exposure of F344/N rats and B6C3F1 mice for 13 weeks, only in males 1-chloro-2-nitrobenzene affects the reproductive organs. Performance of a specific study on toxicity to reproduction (NTP continuous breeding protocol) reveals that 1-chloro-2-nitrobenzene was without reproductive toxicity in a different mice strain following oral treatment by gavage despite of significant changes in liver and spleen weight and despite of elevated methaemoglobin levels. Thus, the $NOAEL_{fertility}$ in Swiss CD-1 mice after oral application is 160 mg/kg bw/day whereas the dams showed general toxicity effects at this concentration. Because 1-choro-2-nitrobenzene affected the reproductive organs in systemic toxic doses in male rats and in males of one strain of mice

after subchronic inhalation there is a concern for a reproductive toxicity potential, even if an impairment of reproduction after oral administration in males of a second strain of mice could not be detected.

Developmental toxicity was examined by two studies with Sprague-Dawley rats which have methodology deficiencies. In one study, due to high mortality rate at the highest dose level, only two doses could be evaluated. NOAEL $_{\text{maternal toxicity}}$ is 25 mg/kg bw/day, a NOAEL $_{\text{developmental toxicity}}$ could not be conclusively derived since there was an increase in the number of litters exhibiting specific skeletal variations. In the second study only one dose was applied: NOAEL $_{\text{developmental toxicity}}$ is 100 mg/kg bw/day, a NOAEL maternal toxicity could not be derived. Based on the available studies the overall conclusion is, that there is no indication of developmental toxicity, although there are some limitations within the studies.

Environment

1-Chloro-2-nitrobenzene has a melting point of 32 °C, a solubility in water of 441 mg/l at 20 °C, and a vapour pressure of 4.0 Pa at 20°C. The log Kow was measured to 2.24.

According to Mackay fugacity model level I the main target compartments for 1-chloro-2-nitrobenzene are water (65.4 %) followed by air (32.9 %). 1-Chloro-2-nitrobenzene shows no ready biodegradation in aquatic compartments (OECD 301 C: 8.2% after 14d) but under the conditions of industrial waste water treatment plants removal to > 95 % was observed at one production/processing site. However, this elimination cannot be transferred to other sewage treatment plants. Special tests showed adapted cultures to be able to degrade 1-chloro-2-nitrobenzene in a cometabolic pathway. Bioconcentration factors determined for fish were in the range of 7.0 – 22.3 and thus indicate no significant bioaccumulation potential of 1-chloro-2-nitrobenzene. A calculated Koc suggests the substance to have a medium geoaccumulation potential. In the atmosphere the substance is photodegradable indirectly with a calculated half-life of 187 d.

The acute toxicity has been determined for: fish (*Cyprinus carpio*) with a 96 h-LC_{50} of 25.5 mg/l; daphnia (*Daphnia magna*) with a 24 h-$EC_{50}$of 12 mg/l and a 48 h-EC_{50} of 23.9 mg/l, and *Daphnia carinata* with a 48 h-EC_{50} of 21.3 mg/l; algae (*Chlorella pyrenoidosa*) with a 96 h-EbC_{50} of 6.9 mg/l. With another alga species (*Secendesmus subspicatus*) a 48h-ErC50 of 75 mg/l and a 48h-ErC10 of 19 mg/l was found.

Chronic toxicity has been tested for *Daphnia magna* with a 21 d-NOEC of 3 mg/l on reproduction (measured concentration) and for fish (*Pimephales promelas*) in an Early Life Stage Test with a 33 d-NOEC of 0.264 mg/l concerning the endpoint normal larvae (measured concentration). A PNECaqua of 0.026 mg/l is derived using an assessment factor of 10.

In a test with terrestrial plants a 14 d-EC50 in the range of 3.2 - 10 mg/kg soil dry weight was determined for Lactuca sativa regarding the endpoint of growth. APNECsoil of 3.2 µg/kg bw was derived from this value using an assessment factor of 1000.

Exposure

About 111,800 t/a 1-chloro-2-nitrobenzene are produced by about 30 producers worldwide. 1-Chloro-2-nitrobenzene is a basic chemical which is processed chemically to other intermediates in different fields of application. There is currently no information that there is consumer use.

NATURE OF FURTHER WORK RECOMMENDED

Human Health: The substance is a candidate for further work. Due to possible hazards (haemotoxicity, reproductive toxicity, genotoxicty, and carcinogenicity) the exposure situation in occupational settings and consumer settings should be clarified and, if then indicated, a risk assessment should be performed.

Environment: The substance is a candidate for further work. Environmental exposure at the sponsor company is adequately controlled. However, as there are no information on environmental releases from other production / processing sites, exposure assessment should be conducted and, if then indicated, a risk assessment may need to be considered. This is justified because the substance is not readily biodegradable and has a PNECaqua of 26 µg/l.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 88-73-3
IUPAC Name: 1-Chloro-2-nitrobenzene
Molecular Formula: $C_6H_4ClNO_2$

1.2 Purity/Impurities/Additives

The purity of the substance is given with > 99 % w/w.

1.3 Physico-Chemical properties

1-Chloro-2-nitrobenzene is a yellowish substance with a melting point of about 32 °C (Bayer AG 1989). With a density of 1.37 g/cm³ at 22 °C . 1-chloro-2-nitrobenzene is heavier than water (Ullmann 1991). The substance is soluble in water with 441 mg/l at 20 °C (Eckert 1962). The vapour pressure has been tested to 4.0 Pa at 20 °C (Bayer AG 2001a). Log K_{ow} is measured with 2.24 (Leo et al. 1971).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The world wide (excluding East Europe) production of 1-chloro-2-nitrobenzene amounted to 111,800 tons in 1995 (about 27,000 in West Europe, 19,000 t in USA, 9,000 t in Japan, 39,000 t in China, 15,500 t in India, and 2,300 t in South Korea) by approximately 30 producers. There is no information about production in East European countries (Bayer AG 2001).

1-Chloro-2-nitrobenzene is a basic chemical, used industrially for manufacturing of further intermediates by chlorination, nitration, sulfonation, reduction, and substitution. In the following an overview of further processing products and their percentage is given:

- 2-nitroaniline (31 %), an intermediate mainly for pesticides

- dichlorobenzidine (26 %), 2-nitroanisole (23 %), and 2-chloroaniline (8 %), processed mainly to dyestuffs and pigments

- others (12 %), including the manufacturing of nitrochlorobenzenesulphonic acid, dinitrodiphenyldisulphide, and nitrophenetole which are processed mainly to dyestuffs and pigments, of o-fluoronitrobenzene which is processed mainly to pharmaceuticals, and of nitrophenol an intermediate mainly for pesticides.

These data relate to the above cited world wide production demand in 1995 (Bayer AG 2001).

A direct use of 1-chloro-2-nitrobenzene is not known (Bayer AG 2001).

Production of 1-chloro-2-nitrobenzene takes place by mono-nitration of chlorobenzene in a continuously working closed system. Initially a mixture of chloronitrobenzenes is gained. This mixture is separated by distillation- and crystallisation procedures yielding 1-chloro-2-nitrobenzene with a purity above 99 % (Bayer AG 2001).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases into the environment may occur during production and processing.

Readily available information on exposure from production and processing to the chemical in the Sponsor country at Bayer AG is available.

The exhausts from production and processing of 1-chloro-2-nitrobenzene are connected to air washing units and thermal exhaust purification plants. Thus during normal operation no 1-chloro-2-nitrobenzene is emitted. Following the Official German Emission Declaration in year 2000, less than 25 kg/a 1-chloro-2-nitrobenzene were emitted into the atmosphere (Bayer AG 2001).

Waste water leaving the production and processing facilities are pretreated before reaching the industrial waste water treatment plant. 1-Chloro-2-nitrobenzene is monitored daily at the influent and the effluent of the waste water treatment plant.

Weekly, at changing days, the effluent is monitored on a fine analysis scale. All values of the fine analysis scale from January 2000 to May 2001 showed the substance to be eliminated to less than 5 µg/l. As worst case for the receiving water a PEC of <0.007 µg/l is calculated from this effluent concentration taking the 10 perzentil of the river flow into account (Bayer AG 2001).

There is no information on releases into the environment from other production and processing sites.

Significant environmental releases from biological reformation of 1-chloro-2-nitrobenzene from end-products are not likely to occur. This is supported by monitoring data from German surface waters for the years 1991 – 2000. These data show that the environmental concentration of 1-chloro-2-nitrobenzene (90%ile) is in the range of < 0.005 µg/l to 0.58 µg/l.

A significant exposure to the terrestrial compartment could not be identified.

2.2.2 Other Information on Environmental Fate

With regard to its chemical structure 1-chloro-2-nitrobenzene is not expected to hydrolyze under environmental conditions. According to the Mackay Fugacity Model Level I (1991), the main target compartments for 1-chloro-2-nitrobenzene are the hydrosphere with 65.4 %, followed by air with 32.9 %. The Henry constant is calculated to be 1.43 Pa m^3 mol^{-1}.

Based on the available experimental data 1-chloro-2-nitrobenzene is not readily biodegradable. In a modified MITI I test according to OECD guideline 301 C a non adapted mixed microbial inoculum mineralized 8.2 % of the initial test substance concentration within 14 days (MITI 1992).

Using the model Simpletreat 3.0 the following distribution/elimination in sewage treatment plants can be estimated using a degradation rate constant of 0 h^{-1} (not readily biodegradable), a Henry constant of 1.43 Pa m^3 mol^{-1} and a log Kow of 2.24:

% to air	2.7
% to water	95.2
% to sludge	2.1
% degraded	0
% removal	4.8

The comparison of influent and effluent concentrations of an industrial sewage treatment plant showed the substance to be removed to > 95 % [Bayer AG 2001]. However, this elimination cannot be transferred to other sewage treatment plants due to possible different waste water composition and adaptation processes. .

Examination of the degradation pathway of chloronitrobenzenes, showed these substances only to be biodegraded by isolated bacteria and adapted mixed sludge as long as the chloronitrobenzenes are not the only sole source for carbon and nitrogen (Kuhlmann 1999).

The indirect photochemical degradation in air by hydroxyl radicals is calculated with a half-life of 187.2 days.

Measured bioconcentration factors (BCF) determined for fish (Cyprinus carpio) according to OECD guideline 305 C, were in the range of 7.0 – 22.3. 1-Chloro-2-nitrobenzene concentrations of 0.25 and 0.025 mg/l had been tested. Thus no significant potential for bioaccumulation of 1-chloro-2-nitrobenzene in aquatic organisms is indicated (MITI 1992).

There is no test on geoaccumulation available. Binding to soil organic matter has been calculated with Koc = 315.5 [SRC-PcKocWIN v1.66, 2000]. According to Blume [1990] 1-chloro-2-nitrobenzene can be regarded as a substance with medium geoaccumulation properties.

2.3 Human Exposure

Note: In Germany/Europe no workplace limit concentration is laid down for 1-chloro-2-nitrobenzene as the substance is classified in Germany in Cancerogenicity Category 3 and Fertility Category 3. A technical limit concentration (TRK-Wert) is planned by German authorities according to "Bundesministerium für Arbeit und Sozialordnung: Übernahme von Luftgrenzwerten in die TRGS 900 Bundesarbeitsblatt 7-8/1998; S. 70-71".

2.3.1 Occupational Exposure

From information from the Swiss (July 2001) and Swedish product register (September 2001) there is no other use pattern of 1-chloro-2-nitrobenzene than intermediate confirmed. To protect workers from exposure to 1-chloro-2-nitrobenzene at workplace, several different precautionary and protective measures are undertaken.

Workplace monitoring is carried out periodically and appropriate personal protection equipment is prescribed in detail for different work situations.

During the past five years (1997 - 2001) 31 8-hour shift samples were taken. Thereof 25 measurements were < 0.05 mg/m³. One measurement was < 0.32 mg/m³, the higher determination limit was due to a smaller air volume taken. Four measurements, taken during filling operations showed values between 0.032 and < 0.6 mg/m³. Here masks were worn to protect the workers from inhalation of 1-chloro-2-nitrobenzene. One value of 0.11 mg/m³ was caused by not appropriate sampling within the production process. This source of exposure has been put right immediately [Bayer AG 2001].

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

1-Chloro-2-nitrobenzene, under appropriate conditions of exposure, is absorbed by the body both via the skin and the gastrointestinal tract as well as via the respiratory tract. Rat studies with labelled chemical show that 1-chloro-2-nitrobenzene absorption is 80 % following oral administration and at least 40 % after open dermal application. On 11 consecutive days, 65 mg 1-chloro-2-nitrobenzene/kg bw was administered by gavage to adult and to old rats. On d 1, 5, and 9 applied substance was labelled and urine and faeces were collected in the following 96 hours. The adult rats excreted 71-74 % of the dose in the urine and 20-27 % of the dose in the faeces. Excretion rate increased with the duration of treatment. Urinary excretion rate in the old rats consisted 71-85 % of the dose and did not increase with the duration of treatment. The radioactivity level in the tissues were determined 72 hours after d9-treatment and shown to be found 5 % of the dose in adult rats and 8 % in the old rats. At very high doses, e.g. 200 mg/kg bw given orally, urinary excretion is delayed and faecal excretion is markedly suppressed. There is evidence to suggest involvement of the enterohepatic cycle, but there are no signs of accumulation of 1-chloro-2-nitrobenzene or one of its metabolites (BG-Chemie 2000, Nomeir et al. 1992).

After oral administration of 100 mg 1-chloro-2-nitrobenzene/kg bw to rabbits 42 % of the dose was excreted in the urine as glucuronides, 24 % as sulfates, 7 % as mercapturic acids and 9 % as free 2-chloroaniline. Only 2-Chloroaniline (0.3%) could be detected in the faeces. 48 hours after administration elimination was complete (Bray et al. 1956).

In tissue, only a very small fraction of the administered radioactivity is recovered (BG-Chemie 2000).

The main metabolic routes for 1-chloro-2-nitrobenzene in the body consist in reduction of the nitro group to an amino group and hydroxylation of the benzene ring. Apart from 2-Chloroaniline, the corresponding nitrophenols and aminophenols are formed, which are excreted as conjugates of glucuronic acid and sulfuric acid. 2-Chloroaniline also appears in the urine and faeces in the unconjugated form (BG-Chemie 2000, Bray et al. 1956, Sabbioni 1994, Rickert and Held 1990).

During reduction of the nitro group to the amino group, the hydroxylamine compound is formed as a highly reactive intermediate which has been detected both in vivo in rats, and in vitro (BG-Chemie 2000, Sabbioni 1994)

3.1.2 Acute Toxicity

Inhalation

There are no studies according to the current OECD guideline but there are study reports with rats which give sufficient information to evaluate this endpoint: (Haskell Laboratory, 1992) LC_{50} ca. 3200 mg/m^3 for 4 hours (= 495 ppm, vapor/aerosol mixture) . Signs of intoxication during exposure were lethargy, slight to moderate cyanosis, slight to moderate corneal opacity, semi-prostration or prostration, reddish brown nasal discharge and tachypnoe. Signs of intoxication post exposure were pallor, reddish brown nasal discharge, semi-prostration and lethargy, corneal opacity.

Death occurred within 7 days but not dose-dependently. Thus LC_{50} value was calculated from statistically not significant regression.

Conclusion

The acute inhalative toxicity is moderate: LC_{50} (rat) ca. 3200 mg/m³ (= 495 ppm, vapor/aerosol mixture) for 4 hours. Cyanotic appearance was the predominant symptom.

Dermal

There are no studies according to the current OECD guideline but there are study reports with rats and rabbits which give sufficient information to evaluate this endpoint: (Bayer 1976): The dermal LD_{50} following a 24-hour occlusive application of the test material to the skin of rats is determined to be 1320 mg/kg bw in females and 655 mg/kg bw in males. The test material was applied as emulsion with the vehicle polyethylene glycole 400. Reduced general condition, difficulties in breathing and cyanotic appearance were the signs of intoxication starting 18 hours post application. Skin irritation was not reported. Deaths occurred within 4 days (males), and 7 days (females), respectively. A section was not performed. In rabbits (2/sex/dose, undissolved substance but warmed to make suitable for dosing, no further information on application procedure, 5 doses, exposure time: 24 hours, observation time: 14 d; Younger Labs. Inc. 1992) the LD_{50} was 400 mg/kg bw (male: 445 mg/kg bw; female: 355 mg/kg bw). Lethargy for up to three days, increasing weakness, collapse and deaths were reported. At gross autopsy, decedents showed haemorrhagic areas in the lungs, liver-, kidneys- and spleen-discoloration, gastrointestinal inflammation and enlarged gall bladder whereas in survivors the viscera appeared normal.

A further investigation on acute dermal toxicity with rabbits yielded a similar result (LD_{50} = 450 mg/kg bw, 5/dose). The sex of the animals used was not mentioned and a section was not performed (United States Testing Company 1976).

Conclusion

The acute dermal toxicity is moderate (LD_{50} (rat, male) = 655 mg/kg bw, LD_{50} (rat, female) = 1320 mg/kg bw; LD_{50}(rabbit) = 400 mg/kg bw (male: 445 mg/kg bw, female: 355 mg/kg bw)). Cyanotic appearance was the predominant symptom.

Oral

There are no studies according to the current OECD guideline but there are study reports with rats which give sufficient information to evaluate this endpoint: (Bayer, 1982 a; b) LD_{50} (Wistar, male) 251 mg/kg bw; LD_{50} (Wistar, female) 263 mg/kg bw. As signs of intoxication rats displayed reduced general condition, cyanotic appearance, rough fur, sedation, narcosis and females showed paralysis of the hind limb. Death occurred within 3 days. No macroscopic findings were recorded from decedents and from survivors 14 days post application. In another study the LD_{50} of male and female Sprague-Dawley rats was determined to be 560 mg/kg bw (Younger Labs 1991). As signs of intoxication reduced appetite and reduced activity (in survivors for at least 2-3 days), increasing weakness, ocular discharge, collapse and death were noted. Death occurred within one to four days post application of 1-chloro-2-nitrobenzene, with most death within 2 days. Hemorrhagic lungs, jaundiced liver, darkened kidneys and spleen and gastrointestinal inflammation were seen at gross autopsy of decedents. From survivors 7 days post application, lung congestion and darkened kidneys and spleen were reported.

An older study on male Wistar rats (Hoechst 1975) yielded an LD_{50} of 144 mg/kg bw. As signs of intoxication rats showed imbalance, tremor, rough fur and diarrhea. Section of the rats, that had died, could not be performed because of ongoing autolytic changes.

Conclusion

After single oral application 1-chloro-2-nitrobenzene is toxic to moderate toxic (LD_{50}, oral: rat, male: 144, 251 or 560 mg/kg bw; rat, female: 263 or 560 mg/kg bw). Cyanotic appearance was the predominant symptom.

3.1.3 Irritation

Skin Irritation

There are no studies according to the current OECD guideline but there are study reports with rabbits which give sufficient information to evaluate this endpoint:

In an older study, 0.5 ml of a 10 % sesame oil solution of 1-chloro-2-nitrobenzene was applied to the shaved (intact and abraded) skin of six rabbits for 24 hours covered by semi-occlusive dressing. When the dressing was removed (24 hour-reading) only mild erythema (score 1/0-4) was noted in both, intact and abraded skin of 4/6 rabbits. Erythema were not observed at 48 hour- and at 72 hour-reading. According to Fed. Reg. 38, No 187, p. 27109, §1500.41, 1973, the compound was evaluated as no irritant (Hoechst 1975).

In another study, 500 mg 1-chloro-2-nitrobenzene was applied undissolved to the inner surface of one ear of each of two rabbits for 24 hours covered by cellulose pads and plaster. To fix the plaster tightly a rolled gauze pad was put on it. Ear, substance, pad, plaster and rolled pad were then bandaged. No signs of irritation (sore 0/4) were observed neither when the pad, plaster, rolled pad were removed nor during the 7 day post exposure observation period (Bayer 1976). In addition, in the same report, the results of acute dermal testing in rats with the substance formulated in polyethylene glycole 400 are mentioned. Signs of irritation were not reported.

0.5 ml of warmed, undiluted 1-chloro-2-nitrobenzene was applied to the skin of six rabbits for 24 hours. No erythema or edema was observed till 168 hours after application (no information about the type of application and pretreatment of the skin) (Younger Labs. 1991).

No skin irritation was reported in an acute dermal toxicity study (see chapter 3.2.3; Bayer 1976).

Conclusion

The study documentation of the available studies is incomplete in one case and in the two other cases the test substance was applied undissolved or respectively diluted. However, the studies gave no evidence of a skin irritating potential of 1-chloro-2-nitrobenzene.

Eye Irritation

There are no studies according to the current OECD guideline but there are study reports with rabbits which give sufficient information to evaluate this endpoint:

In an older study, performed as described in Fed. Reg, Vol. 38, No.187, §1500.42, 1973, 100 mg of 1-chloro-2-nitrobenzene was applied undissolved into one eye of each of 6 rabbits (the other eye served as control). One hour post application slight conjunctival injections (score 1-2/0-3) were noted in the eyes of 6/6 rabbits, 7 hours post application in the eyes of only 2/6 rabbits (score 1/0-3) and 24 hours post application no irritational effects were observed. The compound was evaluated to be a mild irritant (Hoechst 1975).

In another study in the same report, a 10 % solution was applied into one eye of each of 6 rabbits which leads to slight irritational effects (score 1/0-3) in the eyes of 3/6 rabbits one hour post application. These effects had disappeared after 7 hours. The compound was evaluated as slightly irritating (Hoechst 1975).

In another study 50 mg 1-chloro-2-nitrobenzene was applied into the right eye of each of two rabbits. Slight redness (score 1/3) was observed in the eye of one rabbit, which disappeared within 24 hours. No signs of irritation were observed in cornea neither on the application day nor during the 7 day post exposure observation period (Bayer 1976).

Conclusion

1-Chloro-2-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 24 hours.

3.1.4 Sensitisation

Skin

Skin sensitization potency was examined in tests with 10 guinea pigs using test methods which are no longer in use and which are incompletely documented (Rusakov 1973): In a modified Draize test induction was performed with an 1 % aceton-solution of the compound on the shaved back for 5 consecutive days. At day 7 challenge was performed with the same solution. As there was no skin reaction observed, a modified Freunds complete adjuvant test was performed: the same guinea pigs were treated with a 10 % solution of 1-chloro-2-nitrobenzene at day 22: 0.2 ml Freunds Adjuvans together with 0.5 mg 1-chloro-2-nitrobenzene/kg bw was injected into the hind paw. 6 days later one drop of a 10 % solution of 1-chloro-2-nitrobenzene was applied on the shaved untreated skin as challenge. The author reported that 50 % of the treated guinea pigs showed a positive reaction. Rats exposed via inhalation to 0.008 mg/m^3 for 5 months showed also positive reactions (see above; Rusakov et al. 1973).

Conclusion

Due to the limited and poor quality information available regarding skin sensitization it cannot be concluded whether or not the chemical has a sensitizing activity.

3.1.5 Repeated Dose Toxicity

Inhalation

The repeated dose toxicity was examined in male and female Fischer 344/N rats and in male and female B6C3F1 mice for a period of 13 weeks via whole body inhalation of vapor (NTP 1993).

During exposure rats and mice were observed twice daily and were weighed at the start of the study, weekly thereafter and at necropsy. Clinical observations were recorded weekly. After cessation of exposure, complete necropsies were performed on all animals. Histopathologic evaluations, especially on target organs identified (kidney, liver, nasal cavity, and spleen (rats); liver and spleen (mice)) and on reproductive organs (see also chapter 3.2.10) were performed on all animals in the control and the highest exposure groups and on all animals that died early. Target organs identified were also examined in all lower exposure groups.

Groups of 10 male and 10 female rats were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (approx. 0, 7, 14.7, 28.8, 57.6, or 115.2 mg/m^3), 6 hours per day, 5 days per week over a period of 13 weeks. Additional 10 male and 10 female rats per group were exposed for clinical pathology studies at d 1 (only methaemoglobin - data not shown), d 4, and d 23 consisting of hematology and clinical chemistry evaluations. Animals in the base study were evaluated at the end of the study. There were no clear clinical signs of toxicity. All rats survived till the end of the study. Body weight gain was similar to the respective controls. At necropsy, males of the 18 ppm group had significant increased spleen (absol. and rel.) and from 9 ppm increased right kidney (rel.) weights. Absolute liver weights were increased from 1.1 ppm and the relative liver weight from 2.3 ppm. In males exposed to 18 ppm, abs. and rel. lung weights were significant decreased. 2/10 males in the 18 ppm group showed

darkened spleen. Histopathologic evaluation of the kidney showed tubule pigments from 4.5 ppm and tubule regeneration from 1.1 ppm. In the liver, cytoplasmic basophilia was noted from 9 ppm. Spleenic congestion was observed in all exposed and in the control male rats with dose-dependent slight increase in severity. Females, at necropsy, had increased right kidney (absol. and rel.) in the 18 ppm-group and increased absolute liver weights from 2.3 ppm and increased relative liver weights from 4.5 ppm. Significant increased spleen weights (absol. and rel.) were noted from 4.5 ppm. 1/10 females in the 18 ppm group showed darkened spleen. Histopathologic evaluation yielded in the kidney tubule pigment and cytoplasmic basophilia of the liver from 9 ppm. Spleenic congestion was noted in exposed and in the control females with dose-dependent slightly increased incidences. Hyperplasia of the nasal cavity respiratory epithelium in all exposed male and female rats was considered as a toxic effect due to 1-chloro-2-nitrobenzene exposure.

Concentration-related increase in methaemoglobinaemia (males: significant from 1.1 ppm at d 23 and from 2.3 ppm at all time points with max. of 1.14 g/dl at 18 ppm; females: significant from 1.1 ppm at week 13 and from 2.3 ppm at all time points with max. of 1.04 g/dl at 18 ppm; data from d1 not shown) and oxidative damage to red blood cells occurred from the first days of exposure (males: significant at 1.1 ppm (d23), at 4.5 ppm (week 13), at 9 ppm (d4, week13), at 18 ppm (at all time points) when compared to the control values at the respective time point; females: significant in every exposure group at week 13 when compared to the control values at the respective time point). Decrease in haematokrit, haemoglobin and increase in leukocytes predominantly in the highest dose groups of male and female rats was recorded. The beginning regeneration could be recognized in the increase in reticulocyte count at all dose groups of male and female rats at week 13. Serum activities of alanine aminotransferase and sorbitol dehydrogenase were mildly increased in different male and female exposure groups at various time points. A NOAEL was not achieved, the LOAEL is 1.1 ppm (7 mg/m³).

Male and female mice were exposed to 0, 1.1, 2.3 , 9, 4.5, 18 ppm, 6 hours per day, 5 days per week over a period of 13 weeks. There were no clinical signs of toxicity. 2/10 male mice exposed to 18 ppm died. In females from 1.1 ppm body weight gain was greater than in the concurrent control females; in males, body weight gain was similar to the respective control. Exposed mice had treatment-related increased liver and kidney weights (males: abs. and rel. right kidney weights, rel. liver weights sign. increased from 2.3 ppm, abs. liver weights from 9 ppm; females: abs. right kidney weight from 2.3 ppm, abs. liver weights in all exposed groups, rel. liver weight from 9ppm) . Pale discoloration in the liver was noted in 6/10 males and 1/10 females in the 18 ppm group. The spleen was grossly enlarged in 3 females in the 9 ppm group and 4 females in the 18 ppm group. Hepatocellular necrosis, cytomegaly, mineralization and chronic inflammation were seen in the liver, primarily in mice in the 18 ppm group but also in the 9 ppm-group. In addition, increased haematopoetic activity of the spleen was seen in both sexes of mice, particularly in females at 9 ppm and greater. The NOAEL for histopathologic injury is 4.5 ppm (28.8 mg/m³).

Oral

The repeated dose toxicity was also examined in a subacute feeding study with B6C3F1 mice according OECD Guideline 407 (Bayer 1991, 1993). The objective of the study was to recognize possible prae-neoplastic lesions by means of enzyme histochemistry.

12 mice/sex/dose received 0, 50, 500, 5000 ppm 1-chloro-2-nitrobenzene for 5 weeks. Additional 6 mice/sex/dose were used for interim kill and examination after one week of treatment. The calculated feed intake was 0, 16, 167, 1120 mg 1-chloro-2-nitrobenzene/kg bw/day for males and 0, 24, 220, 1310 mg/kg bw/day for females. Except of one male in the lowest dose group, no animal died during treatment. No clinical signs of toxicity up to and including 500 ppm were observed. At 5000 ppm narrowed palpebral fissures and corneal opacity in males were reported. From 5000 ppm reduced body weight gain and reduced food intake in both sexes and additionally in females from 500 ppm.

From 5000 ppm in both sexes reduced number of erythrocytes (change in morphology: anisocytosis, poikilocytosis and polychromasie), haematokrit- and haemoglobin-content and increased bilirubin-, methaemoglobin-(f: 2.8 %; m:1,7 %) MCV-, MCH- and MCHC-values. Increased spleen weights, dark red discoloration of the spleen and increased haemosiderin deposition could be seen.

No treatment related changes in the kidneys were observed.

From 500 ppm increase in cholesterin content in the blood, increased liver weights (differences of up to 89 % were noted in females) accompanied by hypertrophy of the centrolobular hepatocytes. From 5000 ppm gross changes in the liver, increase in the activity of ASAT and ALAT and alkaline phosphatase (male) was noted. In males, blood-urea was decreased.

Additional investigations demonstrate from 500 ppm increase in liver enzyme activities (EOD, ALD, EH, GSH-T,GLU-T) and disturbance of carbohydrate metabolism (decreased gluconeogenesis and glycogen, activated pentose phosphate cycle (at 5000 ppm), increased glycolysis (at 5000 ppm)).

At 5000 ppm males showed decreased testis weight without histopathological changes.

No other treatment-related functional disturbances or impairment of other organs were observed.

Thus, the NOAEL of 50 ppm (16 mg/kg bw/day for males and 24 mg/kg bw/day for females) could be derived.

Also in several other studies on rats and mice with oral or inhalational exposure for 2 and 4 weeks or 7 months, spleen, liver and kidneys were identified as target organs.

Effects on CNS function in rats were reported in a subchronic oral study with poor reliability (Davydova SG 1967). These effects cannot be evaluated because of the incomplete description of the results and the method used.

Conclusion

The repeated dose toxic ity was examined in rats and in mice for a period of 13 weeks via whole body inhalation. As target organs liver, kidney and spleen were identified in both species, and furthermore, in rats erythrocytes and the nasal cavity respiratory epithelium. The NOAEL in rats was not achieved, the LOAEL is 1.1 ppm (7 mg/m³). In mice, increased liver and kidney weights were observed even at 1.1 and 2.3 ppm, respectively. The NOAEL for histopathological injury in mice is 4.5 ppm (28.8 mg/m³).

In a subacute feeding study with mice target organs were blood, spleen and liver. The NOAEL was 50 ppm (males: 16 mg/kg bw/day ; females 24 mg/kg bw/day).

3.1.6 Mutagenicity

In vitro Studies

(A) Gene mutation

There are several Ames-tests which are mostly performed according to OECD Guideline 471 with and without metabolic activation. In every study at least the highest dose levels exhibit 100 % toxicity. For example 1-chloro-2-nitrobenzene was evaluated as mutagenic in the tests reported by Haworth et al. (1983) (doses: 6-600 resp. 10-1000 µg/plate) and by Bayer (1984) (doses: 833.3-2073.6 µg/plate). An additional Ames test, which was reported in JETOC (1996) (doses: 10-1000 µg/plate), yielded negative results. A repetition of the study (doses: 39.1-10000 µg/plate) showed

positive results in TA 100 and TA98. Investigations with E. coli yielded positive and negative results (JETOC 1997).

In a study with deficiencies in the description of results, 1-chloro-2-nitrobenzene showed mutagenic acitivity in *Salmonella typhimurium* TA98 with metabolic activation and norharman (Suzuki et al. 1983). In summary, the available tests with *Salmonella typhimurium* showed mostly negative results without the addition of a metabolic activation system in different strains. Only in strain TA98 and TA1538 there were obtained mostly negative and one resp. 2 positive results. In the presence of a metabolic activation system positive and negative results were obtained in TA 98 and TA 100 mostly at high but not bacteriotoxic concentrations.

In an HPRT Test which was performed with Chinese Hamster V79 lung cells according to OECD Guideline 476 1-chloro-2-nitrobenzene does not induce gene mutations. The doses used were 100-1200 ug/ml in the presence of S9-mix and 100-900 ug/ml without S9-mix. Cytotoxicity was noted in the highest concentration (TNO 1989).

Conclusion

1-Chloro-2-nitrobenzene yielded positive results only in 2 tester strains of Salmonella typhimurium and mostly at high but not bacteriotoxic concentrations. Therefore it can be regarded as a weak mutagen in bacterial test systems. It showed no mutagenic activity in mammalian cell test systems in vitro.

(B) Cytogenicity

There is a study on cytogenicity using Chinese Hamster Ovary (CHO) cells and doses ranging from 10-100 µg/ml without addition of a metabolic activation system (S9-mix) and from 25-250 µg/ml in the presence of S9-mix. Harvest times were 8, 12, 21 hours. The study was performed according to OECD Guideline 473 and yielded negative results (Huntingdon 1988).

NTP (1993) reported additional cytogenetic tests with Chinese Hamster Ovary cells using different harvest times: Without metabolic activation an equivocal result at the highest concentration was obtained when the harvest time was 14 hours (doses: 16-160 ug/ml) and a negative result with a harvest time of 18.5 hours (dose: 47-216 ug/ml). In the presence of an activation system negative results were obtained after a harvest time of 14 hours (doses: 50-500 µg/ml) and weak positive results at the highest concentration when the harvest time was 13.6 hours (doses: 101-465 and 125-500 µg/ml).

Conclusion

1-Chloro-2-nitrobenzene showed weak clastogenic activity in CHO cells in vitro at high concentrations only.

(C) Indicator Tests

1-Chloro-2-nitrobenzene did not increase Unscheduled DNA repair in rat hepatocytes using a dose range from 1.0 to 100 µg/ml DMSO. Cytotoxicity was determined in preliminary results (Monsanto 1984).

An increase in Sister Chromatid Exchange (SCE) rate was found in Chinese Hamster Ovary cells treated with 1-chloro-2-nitrobenzene in doses ranging from 5 to 500 µg/ml (NTP 1993). The biological relevance of SCE is not yet clear.

Conclusion

1-Chloro-2-nitrobenzene did not induce Unscheduled DNA repair. It induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear.

In vivo Studies

(A) Gene mutation

There are several Drosophila SLRL tests which are performed using different application routes: intraperitoneal injection, adult and larval feeding. Both dosing methods lead to negative results (Zimmering 1985, 1989).

Conclusion

1-Chloro-2-nitrobenzene showed no mutagenic activity in *Drosophila melanogaster*.

(B) Cytogenicity

Intraperitoneal injection of 60 mg 1-chloro-2-nitrobenzene/kg bw of unknown purity into CD-1 mice (n=8) induced single DNA strand breaks in liver and kidneys which were identified by alkaline elution technique (Cesarone et al. 1982). Intraperitoneal injection, however, is not the recommended exposure route of the respective OECD guideline because it could expose the organs directly rather than via the circulatory system.

Conclusion

Intraperitoneal injection of 1-chloro-2-nitrobenzene into mice resulted in DNA damage in the liver and kidney.

Conclusion

1-Chloro-2-nitrobenzene showed weak mutagenic activity in bacterial test systems but not in mammalian cell test systems in vitro. It was not mutagenic in *Drosophila melanogaster*. In mammalian cells in vitro, it showed weak clastogenic activity. The substance induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear. Intraperitoneal injection into mice resulted in DNA damage in the liver and kidney. The inconsistent results of the available genotoxic studies are typical for nitroaromatics. As a whole 1-chloro-2-nitrobenzene is suspected of being genotoxic, at least a weak clastogen.

3.1.7 Carcinogenicity

For evaluating carcinogenicity the only available studies in rats and mice don't meet the criteria of today (doses too high, number of animals too low, duration time too short) and are only reported in brief (Weisburger et al. 978).

25 male CD rats/group were given 1-chloro-2-nitrobenzene in the diet for 18 months (50 % of MTD, MTD): 0, 1000, 2000 mg/kg diet (approx. 0, 75, 150 mg/kg bw/day). After 6 months of treatment, dosage was reduced to 500, 1000 mg/kg diet (approx. 37.5, 75 mg/kg bw/day), because body weight gain was reduced by 10 % when compared to the control group or deaths occurred from toxicity (no further information). Reduced doses were given for the remaining 12 months. Following the 6-month-observation period, necropsy was performed and male rats with tumours were recorded: 1/22 in the simultaneous control group (pooled control: 14/111) and 7/22 resp 1/19 in the low resp. the high dose group. These tumours of the low dose group usually included

pituitary adenomas along with either a stomach papilloma, a tumour of the adrenals, a thyroid adenocarcinoma, a lymphosarcoma, a bile duct carcinoma or a subcutaneous fibroma.

25 male and female CD1 HaM/ICR mice/group were given 1-chloro-2-nitrobenzene in the diet for 18 months (50 % of MTD, MTD): 0, 3000, 6000 mg/kg diet (approx. 0, 450, 900 mg/kg bw/day). After 8 months of treatment dosage was reduced to 1500, 3000 mg/kg diet (approx. 225, 450 mg/kg bw/day) which was given for the remaining 10 months (see above). Following the 3-month-observation period, necropsy was performed and mice with tumours were recorded: 3/18 (m), 0/20 (f) in the simultaneous control group (pooled control: (m) 7/99, (f) 1/102) and 7/17 (m), 5/22 (f) resp 3/16 (m), 5/19 (f) in the low resp. the high dose group, identified as hepatocellular carcinomas.

The objective of a subacute **feeding** study with B6C3F1 mice was to recognize possible prae-neoplastic lesions by means of enzyme histochemistry.

12 mice/sex/dose received 0, 50, 500, 5000 ppm 1-chloro-2-nitrobenzene in the diet for 5 weeks. Additional 6 mice/sex/dose were used for interim kill and examination after one week of treatment. The calculated feed intake was 0, 16, 167, 1120 mg/kg bw/day for males and 0, 24, 220, 1310 mg/kg bw/day for females. Except of one male in the lowest dose group, no animal died during treatment.

The additional investigations demonstrate from 500 ppm increase in liver enzyme activities (EOD, ALD, EH GSH-T, GLU-T) and disturbance of carbohydrate metabolism (decreased gluconeogenesis and glycogen, activated pentose phosphate cycle (at 5000 ppm), increased glycolysis (at 5000 ppm)). These marked changes in the carbohydrate metabolism were evaluated as possible promotion activity of 1-chloro-2-nitrobenzene (Bayer 1991, 1993).

Conclusion

1-Chloro-2-nitrobenzene induced tumours in different organs of rats and in the liver of mice.
Overall taking into consideration the results of the genotoxicity tests, the analogy to other nitroaromatics and the results of the available limited studies in rats and mice, there is a concern for a carcinogenic potential of 1-chloro-2-nitrobenzene.

3.1.8 Toxicity for Reproduction

Effects on Fertility

There are no specific studies on toxicity to reproduction using inhalative exposure, but there is a 13 week inhalation study which also evaluated the reproductive organs and can therefore be taken into account for this exposure route.

Male and female F344/N rats were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (0, 7, 14.7, 28.8, 57.6, 115.2 mg/m^3), 6 hours per day, 5 days per week over a period of 13 weeks (NTP, 1993; see also chapter 3.2.7). At the end of the study sperm morphology and vaginal cytology evaluations were performed of animals in the 0, 4.5, 9 and 18 ppm groups (reproductive organs of animals of the two lower exposure groups were not evaluated).

There were no clear clinical signs of toxicity. All rats survived till the end of the study. Concentration-related increase in methaemoglobinaemia and oxidative damage to red blood cells occurred from the first days of exposure and resulted in a regenerative anaemia; target organs were kidneys, spleen, liver, erythrocytes and nasal cavity respiratory epithelium (for details see chapter 3.2.7). Males of the 18 ppm group showed decreases in cauda epididymis weights and in the spermatid count and spermatid heads/testis (NOAEL $_{\text{reproductive organs}}$ = 9 ppm). Females reproductive system was not affected by treatment (NOAEL $_{\text{reproductive organs}}$ = 18 ppm).

Male and female B6C3F1 mice were exposed to 0, 1.1, 2.3, 4.5, 9, 18 ppm (0, 7, 14.7, 28.8, 57.6, 115.2 mg/m^3), 6 hours per day, 5 days per week over a period of 13 weeks (NTP 1993). At the end of the study sperm morphology and vaginal cytology evaluations were performed of animals in the 0, 4.5, 9 and 18 ppm group (reproductive organs of animals of the two lower exposure groups were not evaluated): There were no clinical signs of toxicity. 2/10 male mice exposed to 18 ppm died; target organs were kidneys, spleen and liver (for further details see also Chapter 3.2.7). Male mice in all evaluated dose groups demonstrated a decrease in sperm motility (a NOAEL $_{\text{reproductive organs}}$ for male mice was not determined); in females no effects were observed (NOAEL $_{\text{reproductive organs}}$ = 18 ppm).

In a 5 week feeding study 12 B6C3F1 mice/sex/dose received 0, 50, 500 or 5000 ppm 1-chloro-2-nitrobenzene. Males of the highest dose group showed decreased testis weight without histopathological changes (Bayer 1991, 1993; for further details on general toxicity see chapter 3.2.7).

There is a carefully performed study on toxicity to reproduction in mice using oral treatment (NTP 1992):

Male and female Swiss CD-1 mice were exposed to 1-chloro-2-nitrobenzene dissolved in corn oil by gavage to assess reproduction and fertility using the NTP continuous breeding protocol:

Groups of 20 breeding pairs received 40, 80 or 160 mg/kg bw per day 2-chloronitrotoluene for 7 days prior to cohousing and for 98 days of continuous breeding. 40 breeding pairs received the corn oil vehicle only. The last litter born during the holding period following the continuous breeding phase from control and high dose groups was reared by the dam until weaning, after which time treatment of the F1 animals was initiated by the same route and at the same concentration as the F0 animals. These F1 animals were used for the assessment of second generation fertility.

Data from a 2-week dose-range-finding study were used to set exposure concentration. The highest dose used in the reproduction study was one-half of that caused mortality in the dose-range-finding study.

In the F0-generation mortality occurred in 2, 2, 2 and 3 mice in the control to the high dose groups, respectively, which was suggested not to be treatment related. There was a slight increase in male and post partum dam terminal weights. 3 females in the high dose group appeared cyanotic. No other clinical signs were observed. Necropsy of the high dose mice showed increased spleen weights by 50-100 % and 4-6 fold increased methemoglobin level. No other necropsy data were collected.

Reproductive performance and function of the F0-mice was not affected by treatment: number of litters, pup weight, and viability were all unchanged; live pups per litter and proportion of pups born alive were increased (15% resp. 10%) in the high dose group.

In the final litter of the holding period following the continuous breeding phase, pup weight gain during suckling was lower in the treated groups. At weaning, pups of the high dose group weighed 12% less than control. None of the pups showed clinical signs of toxicity.

Mating of the adult F1 mice (only control and high dose group) revealed no difference between the groups in terms of proportion of mated pairs, number of litters per group, number of live pups per litter and pup weight or viability. Treated F1 male and female mice had 3-fold increased methaemoglobin level compared to the control and were approximately 7 and 5 % heavier than their control counterparts. At necropsy, liver and spleen weights were increased by 40 to 60 %. In male mice, abs. right epididymis and kidney/adrenals weights were increased, seminal vesicle-to-body weight was reduced compared to controls. Sperm measured were unaffected by 1-chloro-2-nitrobenzene exposure (epididymal sperm motility, sperm count, percentage of abnormal sperm). In

females, oestrous cycle were unaffected by 1-chloro-2-nitrobenzene exposure. Thus, NOAEL for fertility is 160 mg/kg bw/day.

Conclusion

Following inhalational exposure of F344/N rats and B6C3F1 mice for 13 weeks, only in males 1-chloro-2-nitrobenzene affects the reproductive organs. Performance of a specific study on toxicity to reproduction (NTP Continuous Breeding Protocol) reveals that 1-chloro-2-nitrobenzene was without reproductive toxicity in a different mice strain following oral treatment by gavage despite of significant changes in liver and spleen weights and despite elevated methemoglobin levels. The $NOAEL_{fertility}$ in Swiss CD-1 mice after oral application is 160 mg/kg bw/day whereas the dams showed general toxicity effects at this concentration.

Because 1-chloro-2-nitrobenzene affected the reproductive organs in systemic toxic doses in male rats and in males of one strain of mice after subchronic inhalation there is a concern for a reproductive toxicity potential, even if an impairment of reproduction after oral administration in males of a second strain of mice could not be detected.

Developmental Toxicity

25 female Sprague-Dawley rats per group received 0, 25, 75 or 150 mg/kg bw/day 1-chloro-2-nitrobenzene dissolved in corn oil by gavage from d6 to d15 of gestation. Due to severe toxicity and high mortality rate of the dams in the 150 mg/kg bw/day group, all females of the 150 mg-group were terminated prior to scheduled sacrifice. One year later, in another laboratory, a third dose group was examined together with a concurrent control group (see later).

No evidence of maternal toxicity was exhibited at the 25 mg/kg level.

For gestation d 6-10 a slight, but not significant reduction in maternal body weight gain at the 75 mg/kg level, urinary staining and alopecia were noted in some dams when compared to the respective control groups. The difference in maternal body weight gain was accompanied by reductions in food consumption for d 6-10. The reductions noted at 75 mg/kg were recovered later in gestation.

Maternal reproductive parameters and fetal body weight in the treatment groups were similar to the respective control groups except for the mean number of early resorptions and postimplantation loss at the 75 mg/kg level. However, postimplantation loss in the respective control group was very low compared to the historical control value.

No differences in the number of the litters exhibiting malformations were evident in the treatment groups compared to the control group. Increased incidences of variations were seen in the 25 and 75 mg/kg group: cervical #7 rib (sign. at 75 mg/kg); and 13 full pairs of ribs with lumbar #1 rudimentary rib; in the 25 mg/kg group: 12 full pair ribs with #13 unilateral full rib and/or rudimentary rib(s). No historical control data were given. Thus, $NOAEL_{maternal\ toxicty}$ is 25 mg/kg bw/day, a $NOAEL_{developmental\ toxicity}$ could not be conclusively derived (Monsanto 1990).

In an additional study which was performed in a different laboratory one year later and which was intended to clarify the observation of the first study, mated female rats received 0, or 100 mg 1-chloro-2-nitrobenzene/kg bw in corn oil by gavage from d6 to d15 of gestation. For gestation d 6-10 slight reduction in maternal body weight loss accompanied by reduction in food consumption for days 6-16 was noted. Maternal reproductive parameters and fetal body weights in the treatment group was comparable to the respective control group. No teratogenic effect nor statistically significant increase of skeletal variations like in the first experiment were observed (IRDC 1984).

Conclusion

Developmental toxicity was examined by two studies with Sprague Dawley rats which both have methodological deficiencies. In one study, due to high mortality rate at the highest dose level, only two doses could be evaluated: $NOAEL_{maternal\ toxicity}$ is 25 mg/kg bw/day, a $NOAEL_{developmental\ toxicity}$ could not be conclusively derived since there was an increase in the number of litters exhibiting specific skeletal variations. In the second study only one dose was applied: $NOAEL_{developmental\ toxicity}$ is 100 mg/kg bw/day, a $NOAEL_{maternal\ toxicity}$ could not be derived. Based on the available studies the overall conclusion is, that there is no indication of developmental toxicity, although there are some limitations within the studies.

3.2 Initial Assessment for Human Health

All available reports relate to mixed exposure, frequently in combination with 4-chloronitrobenzene and/or nitrobenzene. A critical aspect in this context is that the chemical is rapidly absorbed via skin and the respiratory tract. The signs of acute intoxication include methaemoglobinaemia, vomiting, headache and, in severe cases, collapse (Gerbis 1932, Renshaw and Ashcroft 1926, Linch 1974, Sekimpi and Jones 1986)

No allergenic potential had been indicated although 1-chloro-2-nitrobenzene has been used for decades (BUA 1985, BG-Chemie 2000)

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and Chronic Toxicity Test Results

The lowest valid test concentrations of acute and chronic testing are presented in the following.

Acute toxicity to fish (*Brachydanio rerio*) has been tested in a flow through system according to OECD Guideline 203 with analytical monitoring. The 96 h-LC_{50} was determined to be 34.8 mg/l (Röderer 1990). In a semi static test with *Cyprinus carpio* according to OECD Guideline 203 as well, the 96 h-LC_{50} was determined to be 25.5 mg/l (no information about analytical monitoring) (Zhao 1997). An Early Life Stage Test was conducted in an analytically monitored flow through system with Pimephales promelas. In a first step 50 embryos were tested on hatchability and development after 4 - 5 days of incubation. In a second step 15 randomly selected frys from the initial egg cups where observed on their further development for 33 days. The 33 d-NOEC was determined by the authors Call & Geiger (1992) to be 0.264 mg/l based on the endpoint 'normal larvae' related to the hatched larvae. The review of the raw data of the study shows that at the next higher test concentration of 0.530 mg/l a statistically significant effect compared to the control could be observed, however, there is no dose-effect relation for this endpoint at higher test concentrations. The highest test concentration of 3.9 mg/l shows less normal larvae after hatch with a deviation of 7% compared to the control. Apart from that regarding the endpoint 'normal larvae related to initial embryos' no effect at any concentration can be seen. Regarding 'weight' and 'length' of the fry, at both endpoints a deviation to the control of > 5 % can be seen at a concentration of 2.04 mg/l. Also for this endpoint there is no dose-effect relationship seen at the next higher concentration. As statistically significant effects for the endpoint "normal larvae" were seen at concentrations above 0.264 mg/l, the NOEC derived by the authors is used for the hazard assessment for reasons of precaution.

With Daphnia three valid acute tests are available. A test according to a Dutch standard test showed a 48 h-EC_{50} of 23.9 mg/l for *Daphnia magna* (Deneer et al. 1989). A second test on *Daphnia carinata*, comparable to OECD guideline 202 part I, showed a 48 h-EC_{50} of 21.3 mg/l (Zhao 1997). For both tests there is no information about analytical monitoring given. The pretest to the reproduction test showed a lower 24 h-EC_{50} of 12 mg/l (nominal). The long-term study revealed a 21 d-NOEC of 3.0 mg/l (measured concentration) for reproduction of *Daphnia magna* (Kühn et al. 1988).

The lowest effect value for algae has been found for *Chlorella pyrenoidosa*. A 96 h-EC_{50} on biomass is reported with 6.9 mg/l (no information about analytical monitoring), but there is no EC_0 value given (Deneer 1989). With the green alga *Scenedesmus subspicatus* the following effect values were found:

48h-E_bC_{50}:	34 mg/l
48h-E_bC_{10}:	11 mg/l
48h-E_rC_{50}:	75 mg/l
48h-E_rC_{10}:	19 mg/l

The lowest available long-term test value without effects, a NOEC of 0.264 mg/l found in the early life stage test with *Pimephales promelas*, is used as basic value for the derivation of the PNECaqua. Since long-term tests with species from three trophic levels are available, an assessment factor of 10 is proposed.

Therefore: PNECaqua = 0.264 mg/l / 10 = 0.026 mg/l.

4.2 Terrestrial Effects

In a test according to OECD-Guideline 208 (Terrestrial plant growth test) a 14 d-EC50 in the range of 3.2 - 10 mg/kg soil dry weight was determined for *Lactuca sativa* regarding the endpoint of growth (Hulzebos 1993). The soil has an organic matter content of 1.8 %. In a second soil with an organic matter content of 1.4 % a 14d-EC50-value of 5.4 mg/kg soil dry weight was found. Both values are related to nominal concentrations.

With an assessment factor of 1000 a PNECsoil of 3.2 µg/kg dw can be derived from this test.

4.3 Other Environmental Effects

No data available.

5 CONCLUSIONS

Production and processing

The world wide production of 1-chloro-2-nitrobenzene amounted to 111,800 tons in 1995 by approximately 30 producers, excluding production in East European countries. 1-Chloro-2-nitrobenzene is a basic chemical for processing intermediates which are further processed mainly to dyestuffs, pigments, pesticides, and pharmaceuticals within the chemical industry. Direct use of 1-chloro-2-nitrobenzene is not known. Releases into the environment may occur during production and processing. Emission data are only available for Bayer AG. During normal operation no 1-chloro-2-nitrobenzene is emitted into the atmosphere. Following the Official German Emission Declaration in year 2000, less than 25 kg/a 1-chloro-2-nitrobenzene were emitted. Regular monitoring data at the industrial sewage treatment plant showed the substance to be eliminated to less than 5 µg/l. As worst case for the receiving water a PEC of < 0.007 µg/l is calculated taking the 10 percentile of the river flow into account. There is no information on releases into the environment from other production and processing sites. A significant exposure to the terrestrial compartment could not be identified.

Environmental behavior

The favourite target compartments for 1-chloro-2-nitrobenzene are water with 65.4 %, followed by air with 32.9 % according to a Mackay calculation level I. In air, the substance is indirectly photodegradable with $t_{1/2}$ = 187 days. 1-Chloro-2-nitrobenzene is not readily biodegradable. According to the model Simpletreat a removal in sewage treatment plants of 4.8 % can be estimated. Under the conditions of industrial waste water treatment plants removal to > 95 % was observed at one production/processing site. However, this removal cannot be transferred to other sewage treatment plants. Special tests showed adapted cultures to be able to degrade 1-chloro-2-nitrobenzene in a cometabolic pathway.

Measured bioconcentration factors in fish are in the range of 7.0 - 22.3 at a 1-chloro-2-nitrobenzene concentration of 0.25 to 0.025 mg/l. A calculated Koc suggests the substance to have a medium geoaccumulation potential.

The lowest valid acute test results of aquatic testing were determined for fish (*Cyprinus carpio*) with a 96 h-LC_{50} of 25.5 mg/l, for *Daphnia magna* with a 24 h-EC_{50} of 12 mg/l and 48 h-EC_{50} of 23.9 mg/l, and for algae (*Chlorella pyrenoidosa*) with a 96 h-EC_{50} of 6.9 mg/l. With another algae species (*Scendesmus subspicatus*) a 48h-ErC50 of 75 mg/l and a 48h-ErC10 of 19 mg/l was found. Chronic toxicity has been tested for fish (*Pimephales promelas*) in an Early Live Stage Test with a 33 d-NOEC of 0.264 mg/l (endpoint number of normal larvae; measured concentration), and for *Daphnia magna* with a 21 d-NOEC of 3.0 mg/l on reproduction (measured concentration). A PNECaqua of 0.026 mg/l is derived from the fish NOEC of 0.264 mg/l using an assessment factor of 10.

In a test with terrestrial plants a 14 d-EC_{50} in the range of 3.2 - 10 mg/kg soil dry weight was determined for *Lactuca sativa* regarding the endpoint of growth. A PNECsoil of 3.2 µg/kg dw was derived from this test.

Human health

After single oral application 1-chloro-2-nitrobenzene is toxic to moderate toxic (LD_{50}, oral: rat, male: 144, 251 or 560 mg/kg bw; rat, female: 263 or 560 mg/kg bw). The acute inhalative toxicity and dermal toxicity is moderate (LC_{50} (rat) ca. 3200 mg/m³ (= 495 ppm, vapor/aerosol mixture) for 4 hours; LD_{50}, dermal, rat: male: 655 mg/kg bw, female: 1320 mg/kg bw; LD_{50} dermal rabbit: 400 mg/kg bw (male: 445 mg/kg bw, female: 355 mg/kg bw)).

Cyanotic appearance was the predominant appearance for all three routes of application.

The documentation of the available studies on skin irritation is incomplete in one case and in the two other cases the test substance was applied undissolved or respectively diluted. However, the studies gave no evidence of a skin irritating potential of 1-chloro-2-nitrobenzene.

1-Chloro-2-nitrobenzene caused slight irritational effects to the eyes of rabbits which were reversible within 24 hours.

Due to the limited and poor quality information available regarding skin sensitization it cannot be concluded whether or not the chemical has a sensitizing activity.

The repeated dose toxicity was examined in rats and in mice for a period of 13 weeks via whole body **inhalation**. As target organs liver, kidney and spleen were identified in both species, and furthermore, in rats erythrocytes and the nasal cavity respiratory epithelium. The NOAEL in rats was not achieved, the LOAEL is 1.1 ppm (7 mg/m³); In mice, increased liver and kidney weights were observed even at 1.1 ppm and 2.3 ppm, respectively. The NOAEL for histopathological injury in mice is 4.5 ppm (28.8 mg/m³).

In a subacute **feeding** study with mice target organs were blood, spleen and liver. The NOAEL was 50 ppm (males: 16 mg/kg bw/day ; females 24 mg/kg bw/day)

1-Chloro-2-nitrobenzene showed weak mutagenic activity in bacterial test systems but not in mammalian cell test systems in vitro. It was not mutagenic in Drosophila melanogaster. In mammalian cells in vitro, it showed weak clastogenic activity. The substance induced increased rates of Sister Chromatid Exchanges, whereas the biological relevance of this effect is not yet clear. Intraperitoneal injection into mice resulted in DNA damage in the liver and kidney. The inconsistent results of the genotoxic tests as described above are typical for nitroaromatics. As a whole 1-chloro-2-nitrobenzene is suspected of being genotoxic, or at least a weak clastogen.

1-Chloro-2-nitrobenzene showed tumours in different organs of rats and in the liver of mice. Overall taking into consideration the results of the genotoxicity tests,and the resulsts of the available lmited studies in rats and mice, there is a concern for a carcinogenic potential of 1-chloro-2-nitrobenzene.

Following inhalative exposure of F344/N rats and B6C3F1 mice for 13 weeks, only in males 1-chloro-2-nitrobenzene affects the reproductive organs. Performance of a specific study on toxicity to reproduction (NTP Continuous Breeding Protocol) reveals that 1-chloro-2-nitrobenzene was without reproductive toxicity in a different mice strain following oral treatment by gavage despite of significant changes in liver and spleen weight and despite of elevated methemoglobin levels. Thus, the $NOAEL_{fertility}$ in Swiss CD-1 mice after oral application is 160 mg/kg bw/day whereas the dams showed general toxicity effects at this concentration. Because 1-choro-2-nitrobenzene affected the reproductive organs in systemic toxic doses in male rats and in males of one strain of mice after subchronic inhalation there is a concern for a reproductive toxicity potential, even if an impairment of reproduction after oral administration in males of a second strain of mice could not be detected.

Developmental toxicity was examined by two studies with Sprague-Dawley rats which have methodology deficiencies. In one study, due to high mortality rate at the highest dose level, only two doses could be evaluated. $NOAEL_{maternal\ toxicity}$ is 25 mg/kg bw/day, a $NOAEL_{developmental\ toxicity}$ could not be conclusively derived, since there was an increase in the number of litters exhibiting specific skeletal variations. In the second study only one dose was applied: $NOAEL_{developmental\ toxicity}$ is 100 mg/kg bw/day, a $NOAEL_{maternal\ toxicity}$ could not be derived. Based on the available studies the overall conclusion is, that there is no indication of developmental toxicity, although there are some limitations within the studies.

6 RECOMMENDATIONS

Environment: The substance is a candidate for further work. Environmental exposure at the sponsor company is adequately controlled. However, as there are no information on environmental releases from other production / processing sites, national or regional exposure information gathering and risk assessment may need to be considered. This is justified because the substance is not readily biodegradable and has a PNECaqua of 26 µg/l.

Human Health: The substance is a candidate for further work. Due to possible hazards (haemotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity) the exposure situation in occupational settings and consumer settings should be clarified and, if then indicated, a risk assessment should be performed.

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I U C L I D Data Set

Existing Chemical	ID: 88-73-3
CAS No.	88-73-3
EINECS Name	1-chloro-2-nitrobenzene
EC No.	201-854-9
TSCA Name	Benzene, 1-chloro-2-nitro-
Molecular Formula	C6H4ClNO2
Producer Related Part	
Company:	Bayer AG
Creation date:	08-JUN-1993
Substance Related Part	
Company:	Bayer AG
Creation date:	08-JUN-1993
Memo:	OECD HPV Chemicals Programme, SIDS Dossier, approved at SIAM 13 (6-9 November 2001)
Printing date:	26-NOV-2003
Revision date:	02-JUN-1994
Date of last Update:	26-NOV-2003
Number of Pages:	96
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: cooperating company
Name: ACNA C.O.
Town: 17010 Cengio (SV)
Country: Italy

Type: cooperating company
Name: Chemie AG Bitterfeld-Wolfen
Town: 06749 Bitterfeld-Wolfen
Country: Germany

Type: cooperating company
Name: Hoechst AG
Town: 65903 Frankfurt/Main
Country: Germany

Type: cooperating company
Name: Monsanto
Town: 1150 Brussels
Country: Belgium

Type: cooperating company
Name: Rhone-Poulenc Chimie
Street: 25 quai Paul Doumer
Town: 92408 Courbevoie Cedex
Country: France

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

1.1.1 General Substance Information

Substance type: organic
Physical status: solid
Purity: > 99 - % w/w

Remark: cooperating companies for the Existing Chemical Regulation:
Hoechst AG, Germany
Chemie AG Bitterfeld-Wolfen, Germany
Monsanto Europe S.A., Belgium
Rhone-Poulenc Chimie, France
ACNA Chimica Organica, Italy
Flag: Critical study for SIDS endpoint
16-NOV-2000

1.1.2 Spectra

1.2 Synonyms and Tradenames

1-CHLORO-2-NITROBENZOL

Flag: Critical study for SIDS endpoint
27-JUL-2001

1-NITRO-2-CHLORBENZOL

Flag: Critical study for SIDS endpoint

2-CHLOR-1-NITROBENZOL

Flag: Critical study for SIDS endpoint

2-CHLORNITROBENZOL

Flag: Critical study for SIDS endpoint

2-NITRO-1-CHLORBENZOL

Flag: Critical study for SIDS endpoint

2-NITROCHLORBENZOL

Flag: Critical study for SIDS endpoint

BENZENE, 1-CHLORO-2-NITRO-

Flag: Critical study for SIDS endpoint

CHLOR-O-NITROBENZOL

Flag: Critical study for SIDS endpoint

O-CHLORNITROBENZOL

Flag: Critical study for SIDS endpoint

O-NITROCHLORBENZOL

Flag: Critical study for SIDS endpoint

OCNB

Flag: Critical study for SIDS endpoint

ONCB

Flag: Critical study for SIDS endpoint

1.3 Impurities

Remark: Dinitrochlorobenzene : max. 0.01 %
p-Nitrochlorobenzene : max. 0.2 %
water : max. 0.1 %

1.4 Additives

1.5 Total Quantity

1.6.1 Labelling

Labelling:	provisionally by manufacturer/importer
Symbols:	(T) toxic (N) dangerous for the environment
R-Phrases:	(24/25) Toxic in contact with skin and if swallowed (40) Possible risks of irreversible effects (43) May cause sensitization by skin contact (51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment (62) Possible risk of impaired fertility
S-Phrases:	(28) After contact with skin, wash immediately with plenty of water and soap, if possible with Polyethylenglykol 400, too (36/37) Wear suitable protective clothing and gloves (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible) (61) Avoid release to the environment. Refer to special instructions/Safety data sets
Remark:	Classification by EEC is required
Flag:	Critical study for SIDS endpoint

18-JUN-2001

1.6.2 Classification

Classified:	provisionally by manufacturer/importer
Class of danger:	carcinogenic, category 3
R-Phrases:	(40) Possible risks of irreversible effects
Flag:	Critical study for SIDS endpoint

28-MAR-2000

Classified:	provisionally by manufacturer/importer
Class of danger:	dangerous for the environment
R-Phrases:	(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Flag:	Critical study for SIDS endpoint

28-MAR-2000

Classified:	provisionally by manufacturer/importer
Class of danger:	harmful
R-Phrases:	(62) Possible risk of impaired fertility
Remark:	due to classification according to TRGS 905 (DE): risk of impaired fertility, category 3
Flag:	Critical study for SIDS endpoint

25-JUN-2001

Classified:	provisionally by manufacturer/importer
Class of danger:	irritating
R-Phrases:	(43) May cause sensitization by skin contact
Flag:	Critical study for SIDS endpoint

03-APR-2000

Classified:	provisionally by manufacturer/importer
Class of danger:	toxic
R-Phrases:	(24/25) Toxic in contact with skin and if swallowed
Remark:	Classification by EEC is required

Flag: Critical study for SIDS endpoint
28-MAR-2000

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Use in closed system

Flag: Critical study for SIDS endpoint

Type: industrial
Category: Chemical industry: used in synthesis

Flag: Critical study for SIDS endpoint

Type: use
Category: Intermediates

Flag: Critical study for SIDS endpoint

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)

Remark: carcinogenic category 3
risk of cutaneous absorption
risk of impaired fertility, category 3
Source: TRGS 905 (DE)
Flag: Critical study for SIDS endpoint
18-JUN-2001

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: KBwS (DE)
Labelled by: KBwS (DE)
Class of danger: 2 (water polluting)

1.8.4 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: yes

Remark: Appendix I, No. 2
16-JUL-2001

1.8.5 Air Pollution

Classified by: other: producer according to TA-Luft (DE)

Number: 3.1.7 (organic substances)
Class of danger: I

1.8.6 Listings e.g. Chemical Inventories

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

1.11 Additional Remarks

1.12 Last Literature Search

Type of Search: Internal and External

Remark: Environmental, ecotoxicology : November 2000
Toxicology: April 1999
25-JUN-2001

1.13 Reviews

Memo: BUA Report No. 2 (o-Chloronitrobenzene), VCH, Weinheim, Oct. 1985

25-JUN-2001

2.1 Melting Point

Value: 32 degree C

Remark: solidifying point
Flag: Critical study for SIDS endpoint
27-JUL-2001 (11)

Value: 31.7 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

Value: >= 31.7 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (37)

Value: 33 degree C

25-JUN-2001 (103)

2.2 Boiling Point

Value: 245.5 degree C at 1000 hPa

Flag: Critical study for SIDS endpoint
25-JUN-2001 (103)

Value: 243 degree C at 1013 hPa

12-JUL-2001 (12)

Value: 245 degree C at 1013 hPa

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

Value: 370 degree C
Decomposition: yes

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

2.3 Density

Type: density
Value: 1.368 g/cm³ at 22 degree C

Flag: Critical study for SIDS endpoint
27-JUL-2001 (103)

Type: density
Value: 1.32 g/cm³ at 70 degree C

Source: Hoechst AG Frankfurt/Main (reference not available)
11-JUL-2001 (37)

Type: density
Value: 1.294 g/cm³ at 90.5 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .04 hPa at 20 degree C
Decomposition: no

Method: Directive 84/449/EEC, A.4 "Vapour pressure"
Year: 2001
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: 0.07 hPa at 25 °C
Flag: Critical study for SIDS endpoint
27-JUL-2001 (10)

Value: .0575 hPa at 20 degree C

25-JUN-2001 (16)

Value: 6 hPa at 20 degree C

24-NOV-2000 (25)

Value: 2 hPa at 67.6 degree C

14-SEP-2000 (1)

Value: 49.8 hPa at 150 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

2.5 Partition Coefficient

log Pow: 2.24

Method: other (measured)

Flag: Critical study for SIDS endpoint
30-JUL-2001 (58)

log Pow: 2.46

Method: other (calculated)
Year: 2000

Remark: Calculation KOWWIN v1.66 (2001)
Flag: Critical study for SIDS endpoint
25-JUN-2001 (94)

2.6.1 Solubility in different media

Solubility in: Water
Value: .441 g/l at 20 degree C

Flag: Critical study for SIDS endpoint
27-JUL-2001 (27)

Solubility in: Water
Value: .43 g/l at 20 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
27-JUL-2001 (37)

Solubility in: Water
Value: .59 g/l at 20 degree C

27-JUL-2001 (16)

2.6.2 Surface Tension

2.7 Flash Point

Value: 127 degree C
Type: closed cup

Flag: Critical study for SIDS endpoint
25-JUN-2001 (103)

Value: 124 degree C

27-JUL-2001 (16)

Value: 124 degree C

Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

Value: 128 degree C
Type: closed cup

Method: other: DIN 51758

12-JUL-2001 (12)

2.8 Auto Flammability

Value: 470 degree C

Method: other: DIN 51794

Remark: ignition temp.
Flag: Critical study for SIDS endpoint
12-JUL-2001 (12)

Value: > 450 degree C

Source: Hoechst AG Frankfurt am Main, (Reference not available)
25-JUN-2001 (37)

Value: 487 degree C

Remark: Zuendtemperatur
Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

2.9 Flammability

2.10 Explosive Properties

2.11 Oxidizing Properties

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Remark: Untere Explosionsgrenze: 1.15 Vol-%
Obere Explosionsgrenze: 13.1 Vol-%
Gefährliche Zersetzungsprodukte: Nitrose Gase, Chlorwasserstoff
Unverträgliche Substanz: Chlornitrobenzole reagieren mit Reduktionsmitteln.
Source: Hoechst AG Frankfurt/Main, (Reference not available)
25-JUN-2001 (38)

3.1.1 Photodegradation

Type:	other: air, indirect photolysis
Method:	Calculation of the atmospheric oxidation of 1-chloro-2-nitrobenzene by hydroxyl radicals (AOPWIN v1.90, 2001)
Result:	OH rate constant: 0.1714 E-12 cm3/molecule x sec Half-life : 187.2 days (12 h day; 0.5 E6 OH/cm3)
Reliability:	(2) valid with restrictions Accepted calculation method
Flag:	Critical study for SIDS endpoint

12-JUL-2001 (93)

Type:	water
Light source:	other: mercury high pressure lamps
Light spect.:	> 290 nm
DIRECT PHOTOLYSIS	
Degradation:	= 0 % after 180 minute(s)
Method:	other (measured)
Year:	1987
GLP:	no
Test substance:	other TS: 1-chloro-2-nitrobenzene
Method:	irradiation of TS in aqueous solution in the absence and in the presence of TiO2; HPLC analysis
Result:	quantitative degradation of TS was observed only in the presence of TiO2
Reliability:	(3) invalid no detailed description of method, test conditions, and results

12-JUL-2001 (48)

3.1.2 Stability in Water

Remark:	Based on the chemical structure of the compound hydrolysis is not expected under environmental conditions
Flag:	Critical study for SIDS endpoint

25-JUN-2001

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

3.3.2 Distribution

Media:	air - biota - sediment(s) - soil - water
Method:	Calculation according Mackay, Level I
Year:	1991

Remark: Mackay, Calculation of the environmental distribution of 1-chloro-2-nitrobenzene according to fugacity model level I (1991)
Input parameter:
Temperature: 20°C
Vapor pressure: 4.0 Pa
Water solubility: 441 mg/l
log Kow: 2.24
Entry of chemical: 1 mol
Result: Calculated distribution between environmental compartments: water 65.4 %, air 32.9 %, soil 0.9 %, sediment: 0.8 %, susp. sediment: < 0.1 %, fish: < 0.1 %
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
26-NOV-2003

Media: water - air
Method: other (calculation): Henry constant

Result: H = 1.43 Pa m3 mol-1
Flag: Critical study for SIDS endpoint
27-JUL-2001 (61)

Media: water - soil
Method: other (calculation): SCR-PCKOCWIN v1.66
Year: 2000

Result: Koc = 315.5
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (95)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: other: sludge samples from different sewage plants, rivers, bays and a lake, non adapted
Concentration: 30 mg/l related to Test substance
Degradation: 8.2 % after 14 day(s)

Method: other: Japanese Guideline by MITI of 1974; corresp. OECD 301 C Modified MITI Test I
GLP: no data
Test substance: other TS: no purity given

Remark: Inoculum added: 30 mg/l; BOD measurement
Difference to OECD 301C: Initial test substance concentration 30 mg/l instead of 100 mg/l
Reliability: (1) valid without restriction
Test procedure according to national standards
Flag: Critical study for SIDS endpoint
15-JUL-2002 (64)

Type: aerobic
Inoculum: activated sludge, industrial, non-adapted
Concentration: 200 mg/l related to DOC (Dissolved Organic Carbon)

Kinetic: 5 day(s) 80 %
10 day(s) > 90 %

Method: OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year: 1982
GLP: no

Remark: Elimination by Stripping
Source: Hoechst AG Frankfurt/Main
Reliability: (4) not assignable
Original reference not available
25-JUN-2001 (39)

Type: aerobic
Inoculum: activated sludge
Concentration: 200 mg/l related to DOC (Dissolved Organic Carbon)
Degradation: < 10 % after 15 day(s)
Kinetic: 5 day(s) < 10 %
10 day(s) < 10 %

Method: other: Respirometer Test
Year: 1982
GLP: no

Source: Hoechst AG Frankfurt/Main
Reliability: (4) not assignable
Original reference not available
25-JUN-2001 (39)

Type: aerobic
Inoculum: predominantly domestic sewage, adapted
Degradation: 0 % after 20 day(s)
Result: under test conditions no biodegradation observed

Method: other: comparable to OECD Guide-line 301 D
Year: 1977
GLP: no

Remark: related to BOD
Reliability: (4) not assignable
Original reference not available
12-JUL-2001 (9)

Type: aerobic
Inoculum: other: activated sludge, non-adapted and adapted

Method: other: see remarks
GLP: no
Test substance: other TS: > 99.9 % purity

Method: 3 methods were applied:
1) Revised OECD test, 1971 (Determination of the Biodegradability of Anionic Surface Active Agents)
2) Repetitive Die Away Test: Blok, 1979 (A repetitive Die Away test combining several biodegradability test procedures; Int. Biodeterior. Bull. 15, 57-63)
3) Pitter test (Pitter (1976): Determination of biological degradability of organic substances, Water Res. 10, 231-235.)
Result: t1/2 >> 4 weeks

Reliability: (3) invalid
Insufficient documentation: no details on origin and density of inoculum, and on tested concentrations and test conditions
12-JUL-2001 (18)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 56 day(s) at 25 degree C
Concentration: .025 mg/l
BCF: = 7.4 - 22.3

Method: other: Japanese Guideline by MITI of 1974; corresp. OECD 305 C Bioaccumulation (1981)
GLP: no data
Test substance: other TS: o-chloronitrobenzene (CAS-No. 88-73-3)

Method: Flow-through system;
Weight/length of exposed fish: 30g / 10cm, lipid content: 2-6 %; water analyzed twice a week, fish every two weeks
Remark: At a o-chloronitrobenzene concentration of 0.25 mg/l and the same test conditions as already described, a BCF of 7.0 - 20.8 was determined
Test condition: flow-rate of test water: 200-800 ml/min
Reliability: (1) valid without restriction

Test procedure according to national standards
Flag: Critical study for SIDS endpoint
12-JUL-2001 (64)

Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 3 day(s) at 22 degree C
Concentration: 6 mg/l
BCF: 11.6 - 19.4

Method: other: comparable to OECD 305B (Bioaccumulation: Semi Static Fish Test) (1981)
Year: 1986
GLP: no data
Test substance: other TS: > 99 %

Remark: Test temperature 21-23 °C
Mean fat content of fish: 8 +/- 2 %
Difference to Guideline 305 B: only 1 test concentration at 1/5 of 14 d-LC50 tested
Result: The test result in the publication is given on fat weight basis with BCFfat = 194. The BCF values of 11.6 - 19.4 are calculated from this data to the whole fish for reason of

comparability to other test results.
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions (see remark)
27-JUL-2001 (24)

Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 36 day(s)

Method:	other: fish exposed to a mono- to pentachloronitrobenzene isomer mixture at the same time in a flow-through system
Year:	1989
GLP:	no
Test substance:	other TS: mono- to pentachloronitrobenzenes
Method:	30 fish exposed to 720 +/-130 mg TS/l in a flow-through system; acetone used as solvent; samples of 6 fish each analyzed at 5, 12, 20, 28 and 36 days of exposure; duplicate water samples taken every 3 or 4 days; GC analysis
Remark:	significant differences among sample intervals: BCF decreasing from 134 mg/l (day 5) to 89 mg/l (day 20) and then increasing again to 179 mg/l (day 36)
Result:	as the higher chlorinated nitrobenzenes are possibly dechlorinated by metabolism in fish a BCF for o-chloronitrobenzene cannot be derived within this test design
Reliability:	(3) invalid Unsuitable test system (more than one substance tested in the same test vessel)

27-JUL-2001 (78)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type:	flow through
Species:	Brachydanio rerio (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l Analytical monitoring: yes
LC50:	34.8 -
Method:	other: OECD Guide-line 203 (1984)
Year:	1990
GLP:	no data
Test substance:	other TS: no purity given
Test condition:	10 fish per concentration step; fish length: 2 cm; temperature: 23 °C; pH (dilution water) 8.15; 16 h light / 8 h dark
Reliability:	(1) valid without restriction Guideline study
Flag:	Critical study for SIDS endpoint
02-AUG-2001	(86)

Type:	other: static or semistatic, no details given
Species:	Oryzias latipes (Fish, fresh water)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: no data
LC50:	28 -
Method:	other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water" (1986)
GLP:	no data
Test substance:	other TS: o-chloronitrobenzene (CAS-No. 88-73-3)
Test condition:	25 +/- 2 degree C
Reliability:	(2) valid with restrictions Test procedure according to national standards but only basic data given
10-AUG-2001	(64)

Type:	other: semistatic, renewal at 12 hours
Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l Analytical monitoring: no data
LC50:	25.5 -
Method:	other: comparable to OECD 203 (Fish: Acute Toxicity Test, 1992)
Year:	1996
GLP:	no data
Test substance:	other TS: purity not given (commercial TS)
Remark:	Deviation to OECD 203: higher fish load in test vessel (about 50 g in 16 l test water)
Test condition:	60 fish used in each test; fish weight/length: 5 g/5 cm; temperature: 20°C
Reliability:	(2) valid with restrictions According to guideline study with acceptable restrictions
Flag:	Critical study for SIDS endpoint
27-JUL-2001	(114)

Type: semistatic
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: yes
LC50: 30 -

Method: other: comparable to OECD 204 (fish, prolonged toxicity test, 1984)
Year: 1987
GLP: no data
Test substance: other TS: > 99 %

Reliability: (2) valid with restrictions
Basic data given: comparable to guideline
02-AUG-2001 (24)

Type: flow through
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: 2.9 -
LOEC: 5.9 -

Method: other: OECD 204: Fish, Prolonged Toxicity Test: 14-day Study (4 April 1984)
Year: 1990
GLP: no data

Remark: The 14 d-LOEC of 5.9 mg/l corresponds to the feeding behaviour of the fish. A 14 d-LOEC concerning lethal effect was determined to be 24.8 mg/l.
Reliability: (1) valid without restriction
Guideline study
27-JUL-2001 (86)

Type: static
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 30 -

Method: other: according to OECD Proposal (1979:) Report on the Assessment of Potential Environmental Effects of Chemicals
Year: 1984
GLP: no data
Test substance: other TS: 1-chloro-2-nitrobenzene; purity > 99.9 %

Reliability: (3) invalid
Documentation insufficent for assessment
12-JUL-2001 (18)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 5 -
LC100: 10 -

Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15

Year: 1974
GLP: no

Reliability: (3) invalid
Range-finding test with two fish only
Original report not available
12-JUL-2001 (9)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: other: Daphnia carinata
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 21.3 -

Method: other: comparable to OECD 202 part I (Daphnia, Acute Toxicity, 1984)
Year: 1996
GLP: no data
Test substance: other TS: purity not given

Reliability: (2) valid with restrictions
Basic data given: comparable to guideline
Flag: Critical study for SIDS endpoint
12-JUL-2001 (114)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 5 -
EC50: 12 -

Method: other: Daphnien-Schwimmunfaehigkeits-Test, UBA-Verfahrensvorschlag Mai 1984, Bestimmung der Schwimmunfaehigkeit beim Wasserfloh Daphnia magna, EC0, EC50, EC100 24h, statisches System
Year: 1987
GLP: no data

Remark: Pretest to reproduction test
Reliability: (2) valid with restrictions
Basic data given
Flag: Critical study for SIDS endpoint
27-JUL-2001 (57)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 23.9 -

Method: other: according to the Protocol of the Dutch Standards Organisation, NEN 6501 (1980)
Year: 1988
GLP: no data
Test substance: other TS: no purity given

Test condition: Daphnids < 24 h old; temperature: 20 °C; illumination 12 h/day; hardness: 200 mg/l as CaCO3; pH 8.4; dissolved oxygen > 7.9 mg/l
Reliability: (2) valid with restrictions
Basic data given
Flag: Critical study for SIDS endpoint
27-JUL-2001 (23)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: 3.2 -
LC50 : 49 -

Method: other: OECD Proposal (1979: Report on the assessment of Potential Environmental Effects of Chemicals I)
Year: 1979
GLP: no data
Test substance: other TS: 1-chloro-2-nitrobenzene; purity > 99.9 %

Remark: no data on test conditions
Reliability: (3) invalid
Documentation insufficent for assessment
11-JUL-2001 (18)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella pyrenoidosa (Algae)
Endpoint: biomass
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 6.9 -

Method: other: According to Modified OECD 201 (Algae, growth inhibition test, 1984)
Year: 1988
GLP: no data
Test substance: other TS: purity not given

Reliability: (2) valid with restrictions
Basic data given: comparable to guideline
Flag: Critical study for SIDS endpoint
07-SEP-2001 (23)

Species: Scenedesmus subspicatus (Algae)
Endpoint: biomass

Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC10: 11 -
EC50: 34 -

Method: other: Scenedesmus-Zellvermehrungs-Hemmtest, DIN 38412 Teil 9, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen (1988)
Year: 1988
GLP: no data
Test substance: other TS: purity not given

Remark: modification of test procedure: bottles with ground glass stoppers were used

Result: Effect levels determined for the endpoint growth rate:
EC10: 19 mg/l
EC50: 75 mg/l
Reliability: (2) valid with restrictions
Test procedure according to national standards, but only basic data given
Flag: Critical study for SIDS endpoint
10-AUG-2001 (56)

Species: other algae: Scenedesmus obliquus
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 18.1 -

Method: other: comparable to OECD 201 (Algae, Growth inhibition test, 1984)
Year: 1996
GLP: no data
Test substance: other TS: purity not given

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
12-JUL-2001 (114)

Species: Scenedesmus pannonicus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = 24 -

Method: other: OECD Proposal (1979: Report on the Assessement of Potential Environmental Effects of Chemicals I
Year: 1984
GLP: no data
Test substance: other TS: 1-chloro-2-nitrobenzene; > 99.9 % purity

Reliability: (3) invalid
Documentation insufficent for assessment
12-JUL-2001 (18)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: no
EC0: 100 -

Method: other: Bewertung toxischer Wasserinhaltsstoffe aus ihrer Inhibitorwirkung auf die Substratoxydation von Pseudomonas Stamm Berlin mit Hilfe polarographischer Sauerstoffmessungen. Robra, K.H.: gwf wasser/abwasser 117 (2), 80-86 (1976)
Year: 1983
GLP: no
Test substance: other TS: no purity given

Reliability: (4) not assignable
Original reference not available
12-JUL-2001 (9)

Type: aquatic
Species: anaerobic bact. from a domestic water treatment plant
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: ca. 80 -

Method: ETAD Fermentation tube method "Determination of damage to effluent bacteria by the Fermentation Tube Method"
Year: 1982
GLP: no
Test substance: other TS: no purity given

Source: Hoechst AG Frankfurt/Main
Reliability: (4) not assignable
Publication/report not available
27-JUL-2001 (39)

Type: other: phytopathogen
Species: other fungi: Pythium ultimum
Exposure period: 88 hour(s)
Unit: mg/l Analytical monitoring: no data
ED 50 : 157.6 -

Year: 1961
GLP: no
Test substance: other TS: recrystallized

Method: Growth inhibition test: test substance incorporated in agar medium which is filled into a growth tube; inoculation after solidification of agar with 8 mm plug of an 48 h fungi culture. Evaluation of linear growth.
Reliability: (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles
12-JUL-2001 (27)

Type: other: phytopathogen
Species: other fungi: Rhizoctonia solani
Exposure period: 88 hour(s)
Unit: mg/l Analytical monitoring: no data
ED 50 : 48.9 -

Year: 1961
GLP: no
Test substance: other TS: recrystallized

Method: Growth inhibition test: test substance incorporated in agar medium which is filled into a growth tube; inoculation after solidification of agar with 8 mm plug of an 48 h fungi culture. Evaluation of linear growth.
Reliability: (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles
13-JUL-2001 (27)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species: Pimephales promelas (Fish, fresh water)
Endpoint: other: weight and length of juveniles

Exposure period: 33 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: 1.02 -
LOEC: 2.04 -

Method: other: comp. to OECD 210 (Fish, Early-life Stage Toxicity Test, 1992)
Year: 1992
GLP: no data
Test substance: other TS: 99 %

Remark: In a first step 50 embryos were tested on hatchability and development after 4 - 5 days of incubation. In a second step 15 randomly selected frys from the initial egg cups where observed on their further development for 33 days. The 33 d-NOEC was determined by the authors Call & Geiger (1992) to be 0.264 mg/l based on the endpoint 'normal larvae' related to the hatched larvae. The review of the raw data of the study shows, that at the next higher test concentration of 0.530 mg/l a statistically significant effect compared to the control could be observed, however, there is no dose-effect relation for this endpoint at higher test concentrations. The highest test concentration of 3.9 mg/l shows less normal larvae after hatch with a deviation of 7 % compared to the control. Apart from that regarding the endpoint 'normal larvae related to initial embryos' no effect at any concentration can be seen. Regarding 'weight' and 'length' of the fry, at both endpoints a deviation to the control of > 5 % can be seen at a concentration of 2.04 mg/l. Also for this endpoint there is no dose-effect relationship seen at the next higher concentration. As statistically significant effects for the endpoint "normal larvae" were seen at concentrations above 0.264 mg/l, the NOEC derived by the authors is used for the hazard assessment for reasons of precaution.
Test condition: Flow through system
Photoperiod: 16 h light / 8 h dark
Temperature, mean: 24.81 degree C
O2, mean: 6.32 mg/l
pH, mean: 7.42
Total hardness: 54.35 mg/l CaCO3
Total alkalinity, mean: 45.09 mg/l CaCO3
Reliability: (2) valid with restrictions
Well-documented study, comparable to guideline
Flag: Critical study for SIDS endpoint
07-SEP-2001 (17)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = 3 -

Method: other: UBA-Verfahrensvorschlag (vorlaeufiger) "Verlaengerter Toxzitaetstest bei Daphnia magna" (Bestimmung der NOEC fuer Reproduktionsrate, Mortalitaet und den Zeitpunkt des ersten Auftretens von Nachkommen; 21d) (1984)
Year: 1987
GLP: no data
Test substance: other TS: no purity given

Remark: semistatic test system
Reliability: (1) valid without restriction
Test procedure according to national standards
Flag: Critical study for SIDS endpoint
27-JUL-2001 (57)

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day(s)
Unit: mg/l Analytical monitoring: no data
LOEC: 9.9 -

Method: other: According to the Protocol of the Dutch Standards Organisation, NEN 6502 (1980)
Year: 1988
GLP: no data
Test substance: other TS: no purity given

Remark: semi static test system
Reliability: (2) valid with restrictions
Basic data given
27-JUL-2001 (23)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Species:	other terrestrial plant: Lactuca sativa Ravel R2
Endpoint:	growth
Expos. period:	14 day(s)
Unit:	mg/kg soil dw
EC50:	= 3.2 - 10
Method:	other: OECD Guide-line 208 (1984)
Year:	1991
GLP:	no data
Test substance:	other TS: purity >= 95 % (summarized information for all test substances)
Remark:	Two different natural soils at different testing facilities were used. Both soil characteristics corr. to OECD advice of an Entisol soil (organic matter content 1.4 % and 1.8 % resp., and clay content 12 % and 24 % resp., pH 7.5). Nominal concentrations given
Test condition:	10 seeds per tray, trays covered with glas plates, temperature 21 °C, photoperiod 16 h light / 8 h dark; light intensity 6,500 Lux; humidity 40-80 %
Reliability:	(2) valid with restrictions Guideline study with acceptable restrictions; only one type of soil tested
Flag:	Critical study for SIDS endpoint

10-AUG-2001 (46)

Species:	other terrestrial plant: Cucumis sativus var. National Pickling
Endpoint:	growth
Expos. period:	6 day(s)
Unit:	mg/l
Method:	other: germination and growth of seedlings in sand
Year:	1961
GLP:	no
Test substance:	other TS: recrystallized
Remark:	A definite amount of test solution was added to sand. Three concentrations were tested (20, 50, and 100 ppm) by weight in to water.
Result:	A 6 d-ED 50 of 18.1 mg/l was determined for sand.
Reliability:	(3) invalid Unsuitable test system (no soil tested)

11-JUL-2001 (27)

Species:	Phaseolus aureus (Dicotyledon)
Endpoint:	growth
Expos. period:	6 day(s)
Unit:	mg/l
Method:	other: germination and growth of seedlings in sand
Year:	1961
GLP:	no
Test substance:	other TS: recrystallized

Remark: A definite amount of test solution was added to sand. Three concentrations were tested (20, 50, and 100 ppm) by weight in to water.
Result: A 6 d-ED 50 of 29.9 mg/l was determined for sand.
Reliability: (3) invalid
Unsuitable test system (no soil tested)
11-JUL-2001 (27)

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex: male
No. of Animals: 15
Vehicle: other: polyethylene glycol 400
Value: = 219 mg/kg bw

Method: other: 15 rats/dose group, 7 doses dissolved in polyethylenglycol 400, given by gavage, observation time: 14 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	conc. %	result m /s /n	signs of intoxication start	signs of intoxication end	time of death
50	1	0/ 0/15	-	-	-
100	2	0/15/15	49 min.	5 d	-
150	3	2/15/15	20 min	7 d	2 d
200	4	4/15/15	16 min	7 d	24 h
250	5	10/15/15	36 min	11 d	1-2 d
300	6	14/15/15	13 min	9 d	24 h
500	10	15/15/15	18 min	-	24 h

m: number of rats which died;
n: number of animals put in test
s: number of animals with signs of intoxication:
reduced general condition, cyanotic appearance

Reliability: (2) valid with restrictions
no histopathological examination performed, individual animal data and information on GLP is missing
21-MAR-2003 (6)

Type: LD50
Species: rat
Sex: female
No. of Animals: 15
Vehicle: other: polyethylene glycol 400
Value: = 457 mg/kg bw

Method: other: 15 rats/dose group, 8 doses dissolved in polyethylenglycol 400, given by gavage, observation time: 14 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	conc. %	result m /s /n	signs of intoxication start	signs of intoxication end	time of death
25	0.5	0/ 0/15	-	-	-
50	1	0/15/15	24 h	3 d	-
100	2	0/15/15	24 h	7 d	-
250	5	1/15/15	90 min	7 d	8 d
350	7	2/15/15	11 min	7 d	1-2 d
500	10	10/15/15	2 h	13 d	1-2 d
650	13	12/15/15	8 min	12 d	1-2 d
850	17	15/15/15	2 h	-	1-2 d

m: number of rats which died;
n: number of animals put in test
s: number of animals with signs of intoxication:
reduced general condition, cyanotic appearance

Reliability: (2) valid with restrictions
no histopathological examination performed, individual animal data and information on GLP is missing

21-MAR-2003 (6)

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 10
Vehicle: other: Lutrol
Value: = 251 mg/kg bw

Method: other: 10 rats/dose, 5 doses, test subst. dissolved in lutrol, gavage: application volume: 20 ml/kg bw., observation time: 14 d, some of the rats, that died, and some of the survivors were dissected
Year: 1982
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	result m /s /n	signs of intoxication start	time of death
100	0/ 0/10	-	-
200	2/10/10	1 h	8 - 24 h
250	5/10/10	1 h	4 - 24 h
300	7/10/10	1 h	8 h - 3 d
400	10/10/10	1 h	4 h - 2 d

m: number of rats which died;
n: number of animals in test
s: number of animals with signs of intoxication:
reduced general condition, cyanotic appearance, rough fur, sedation, narcosis, no macroscopic effects in dissected animals

Reliability: (2) valid with restrictions
study meets criteria of today, but information on GLP is missing
Flag: Critical study for SIDS endpoint
21-MAR-2003 (7)

Type: LD50
Species: rat
Strain: Wistar

Sex: female
No. of Animals: 10
Vehicle: other: Lutrol
Value: = 263 mg/kg bw

Method: other: 10 rats/dose, 5 doses, test subst. dissolved in lutrol, gavage: application volume: 20 ml/kg bw., observation time: 14 d, some of the animals, that died, and some of the survivors were dissected
Year: 1982
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	result m /s /n	signs of intoxication start	time of death
100	0/ 0/10	-	-
200	3/10/10	2 h	8 h - 3 d
300	5/10/10	2 h	24 h - 3 d
400	9/10/10	1 h	24 h - 3 d
500	10/10/10	1 h	4 h - 3 d

m: number of rats which died;
n: number of animals in test
s: number of animals with signs of intoxication:
reduced general condition, cyanotic appearance, rough fur, sedation, narcosis, paralysis of the hind limb

no macroscopic effects in dissected animals
Reliability: (2) valid with restrictions
study meets criteria of today, but information on GLP is missing
Flag: Critical study for SIDS endpoint
21-MAR-2003 (8)

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 10
Vehicle: other:sesame oil
Value: = 144 mg/kg bw

Method: other: 10 rats/dose, males were more sensitive in a pre-test, starved 16 hrs prior appl. and 2 hrs post appl., 4 doses, dissolved in sesame oil, single application by gavage, observation time: 14 d
Year: 1975
GLP: no
Test substance: other TS: no data on purity

Remark: doses and mortality rate (death occurred within 3 days):
63 mg/kg: 0/10; 100 mg/kg: 2/10;
160 mg/kg: 5/10; 250 mg/kg: 10/10
signs of intoxication: imbalance, rough fur, diarrhea, slight tremor
section of survivors: no findings
section of rats, that had died, was not possible because of autolytic changes.
Reliability: (2) valid with restrictions
individual animal data of signs of intoxication and information on GLP is missing

Flag: Critical study for SIDS endpoint
25-MAR-2003 (40)

Type: LD50
Species: rat
Sex: no data
Vehicle: no data
Value: = 350 mg/kg bw

Method: other: no information
Year: 1967
GLP: no data
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (22)

Type: LD50
Species: rat
Sex: no data
Vehicle: no data
Value: = 339 mg/kg bw

Method: other: no information given
Year: 1982
GLP: no data
Test substance: other TS: no data on purity

Remark: clinical signs: central nervous system affected, methaemoglobin former (no further information)
Reliability: (4) not assignable
lack of information
16-JUN-2003 (50)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
Vehicle: other: corn oil
Value: = 560 mg/kg bw

Method: other: 2 or 3 rats/dose, single oral dose as 10 % warm solution, observation time: 7 d
Year: 1983
GLP: no data
Test substance: other TS: purity: 99.71 %

Remark: doses and mortality:
398 mg/kg: males 1/2 females 0/3
501 mg/kg: males 1/3 females 1/2
631 mg/kg: males 2/2 females 2/3
794 mg7kg: males 3/3 females 2/2
signs of intoxication: reduced appetite and activity(2-3 days in survivors), increasing weakness, ocular discharge, collapse and death
time to death: 1-4 days with most deaths within 2 days
gross autopsy:
decedents: hemorrhagic lungs, jaundiced liver, darkened kidneys and spleen, and gastrointestinal inflammation
survivors: lung congestion and darkened kidneys and spleen

Reliability: (2) valid with restrictions
individual animal data and information on GLP is missing
Flag: Critical study for SIDS endpoint
21-MAR-2003 (68) (113)

Type: LD50
Species: rat
Sex: no data
Vehicle: no data
Value: = 288 mg/kg bw

Method: other: observation time: 14 d (no further information)
Year: 1972
GLP: no
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (2)

Type: LD50
Species: rat
Value: = 510 mg/kg bw

Method: other: no details given

Reliability: (4) not assignable
lack of information
16-JUN-2003 (106)

Type: LD50
Species: rat
Sex: male
Value: = 270 mg/kg bw

Method: other: according to Smyth, Am. Ind. Hyg. Ass. J. 30, 470 (1962)
Year: 1977
GLP: no
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (107)

Type: LD50
Species: rat
Sex: male
Value: = 300 mg/kg bw

Method: other: no further information given
Year: 1988
GLP: no data
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (65)

Type: LD50
Species: rat
Sex: male

No. of Animals: 5
Vehicle: other: none
Value: ca. 630 mg/kg bw

Method: other: 3 rats/dose, single oral application of undiluted substance, observation time: 14 d
Year: 1975
GLP: no
Test substance: other TS: o-nitrochlorobenzene residue

Remark: dose / mortality / time of death:
50 mg/kg / 0/5 / -;
500 mg/kg / 2/5 / one day;
5000 mg/kg / 5/5 / one day
signs of intoxication: reduced appetite and activity (2-4 days in survivors, increasing weakness, collapse, and death
gross autopsy:
decedents: haemorrhagic areas of the lungs, slight liver discoloration, acute gastrointestinal inflammation
survivors: viscera appeared normal
Reliability: (4) not assignable
o-nitrochlorobenzene residue used, no information for o-nitrochlorobenzene itself
21-MAR-2003 (111)

Type: LD50
Species: mouse
Sex: no data
Vehicle: no data
Value: = 440 mg/kg bw

Method: other: no information given
Year: 1982
GLP: no data
Test substance: other TS: no data on purity

Remark: clinical signs: central nervous system affected, methaemoglobin former (no further information)
Reliability: (4) not assignable
lack of information
16-JUN-2003 (50)

Type: LD50
Species: mouse
Sex: no data
Vehicle: no data
Value: = 135 mg/kg bw

Method: other: observation time: 14 d (no further information)
Year: 1972
GLP: no
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (2)

Type: LD50
Species: mouse
Value: = 340 mg/kg bw

Method: other: no details given

Reliability: (4) not assignable
lack of information
16-JUN-2003 (106)

Type: LD50
Species: mouse
Value: = 140 mg/kg bw

Method: other: according to Smyth, Am. Ind. Hyg. Ass. J. 30, 470 (1962)
Year: 1977
GLP: no
Test substance: other TS: no data on purity

Reliability: (4) not assignable
lack of information
16-JUN-2003 (107)

Type: LD50
Species: rabbit
Sex: no data
Vehicle: no data
Value: = 280 mg/kg bw
Method: other: no information given
Year: 1982
GLP: no data
Test substance: other TS: no data on purity
Remark: clinical signs: central nervous system affected, methaemoglobin former (no further information)
Reliability: (4) not assignable
lack of information
16-JUN-2003 (50)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Strain: other: CD
Sex: male
No. of Animals: 10
Exposure time: 4 hour(s)
Value: ca. 3200 mg/m³
Method: other: 10 male rats/conc., head-only exposure, 6 conc., heated vapour was diluted with humidified and O2-enriched air and thus converted to a mixture of vapour and liquid aerosol, post exposure observation time: 14 d
Year: 1981
GLP: no data
Test substance: other TS: purity: 99.8 %
Remark:

Concentration (mg/l)	Mortality	Time to death 0, 1, 2, 3, 5, 7 (d)
1.56	1/10	1
1.83	3/10	2 1
2.46	2/10	1 1
2.64	10/10	1 1 7 1
3.23	1/10	1
3.33	6/10	1 2 2 1

signs of intoxication during exposure: slight to moderate cyanosis, semi-prostration, lethargy and reddish brown nasal discharge to 24 hours, slight to moderate corneal opacity, tachypnea, some rats with partial hind-leg paralysis, abnormal arched-back posture
signs of intoxication post exposure: weight loss of 8 to 16 % from 1 to 3 days with normal gains thereafter, pallor, stained perineal area, lethargy, some rats with salivation, lacrimation and corneal opacity, chromodacryorrhea
gross autopsy not reported
LD50: 495 ppm
Mortalities were not strictly dose-dependant, stat. analysis showed a non significant regression

Result: value: LD50: 495 ppm
Reliability: (2) valid with restrictions
gross autopsy not reported, no information about GLP
Flag: Critical study for SIDS endpoint
21-MAR-2003 (31)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 10
Vehicle: other: polyethylene glycol 400
Value: = 655 mg/kg bw

Method: other: 10 rats/dose, 6 doses, subst.(solved in polyethylene glycol 400) appl. on the shaved back for 24 hours, covered by alu and a plaster, then rinsed with water and soap, post exposure observ.-time: 14 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	conc. %	result m /s /n	signs of intoxication start	signs of intoxication end	time of death
250	25	1/10/10	18 h	14 d	4 d
350	25	1/10/10	18 h	7 d	18 h
500	50	3/10/10	18 h	9 d	1-3 d
750	50	7/10/10	24 h	13 d	1-3 d
1000	50	7/10/10	18 h	4 d	18 h - 3 d
1500	75	9/10/10	18 h	14 d	18 h - 4 d

m: number of rats which died;
n: number of animals put in test
s: number of animals with signs of intoxication:
reduced general condition, difficulties in breathing, cyanotic appearance, some animals showed lacrimation

Reliability: (2) valid with restrictions
no pathologic examination performed, individual animal data and information on GLP are missing
Flag: Critical study for SIDS endpoint
21-MAR-2003 (6)

Type: LD50
Species: rat

Strain: Wistar
Sex: female
Vehicle: other: polyethylene glycol 400
Value: ca. 1320 mg/kg bw
Method: other: 10 or 20 rats/dose, 3 doses, subst.(solved in polyethylene glycol 400) appl. on the shaved back for 24 hours, covered by alu and a plaster, then rinsed with water and soap, post exposure observ.-time: 14 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark:

dosis mg/kg	conc. %	result m /s /n	signs of intoxication start	signs of intoxication end	time of death
750	50	0/10/10	24 h	6 d	-
1000	50	5/20/20	18 h	14 d	2 - 3 d
1500	75	6/10/10	18 h	10 d	2 - 6 d

m: number of rats which died;
n: number of animals in test
s: number of animals with signs of intoxication: reduced general condition, difficulties in breathing, cyanotic appearance, some animals showed lacrimation
Reliability: (2) valid with restrictions
no pathologic examination performed, individual animal data and information on GLP are missing
Flag: Critical study for SIDS endpoint
21-MAR-2003 (6)

Type: LD50
Species: rat
Sex: female
No. of Animals: 6
Vehicle: other: diluted in sesame oil to give a concentration of 40 %
Value: = 1796 mg/kg bw

Method: other: 6 rats/dose, single application to the clipped intact skin, covered by alu and a plaster, exposure time: 24 h, then rinsing, postexposure observation time: 14 d
Year: 1975
GLP: no
Test substance: other TS: no data on purity

Remark: doses and mortality:
500 mg/kg: 0/6 ; 1000 mg/kg: 1/6 ; 1600 mg/kg: 3/6;
2000 mg/kg: 3/6
no signs of toxicity, necropsy of the survivors: no pathological findings
Reliability: (2) valid with restrictions
no data on purity and information on GLP is missing
21-MAR-2003 (42)

Type: LD50
Species: rabbit
Value: = 450 mg/kg bw

Method: other: 5 rabbits/dose, trunks were clipped free of hair, 3 doses (warm to melting point), exposure time 24 h (rabbits immobilized during exposure), then rinsing and wiping dry, observation time: 14 d
Year: 1975

GLP: no
Test substance: other TS: no data on purity

Remark: dose / mortality / individual reactions
330 mg/kg/ 20 % / slight discoloration of the skin and eyes; normal < 48 hrs
560 mg/kg/ 80 % / death 48 to 96 hours preceded by lethargy, loss of motor coordination, sometimes coma
750 mg/kg/ 80 % / death 2 to 5 days, other reaction similar

general reaction:
manifestation of methaemoglobinaemia symptoms evident in < 20 minutes
Reliability: (2) valid with restrictions
no data on purity, no pathologic examination, information on GLP is missing
16-JUN-2003 (104)

Type: LD50
Species: rabbit
Sex: male/female
No. of Animals: 2
Vehicle: other: undissolved
Value: = 400 mg/kg bw
Method: other: 2 rabbits/sex/dose, 5 doses, single dermal application (intact skin), undiluted (warmed to make suitable for dosing), no further information, exposure time: 24 hrs, post exp.observation time: 14 d
Year: 1983
GLP: yes
Test substance: other TS: purity: no data
Remark: Dose and mortality: 251 mg/kg: Males: 0/2; Females: 0/2
316 mg/kg: 0/2 1/2
398 mg/kg: 0/2 2/2
501 mg/kg: 2/2 1/2
631 mg/kg: 2/2 2/2

observations: toxic signs: lethargy (lasting up to 3 days); increasing weakness; collapse; death
Gross necropsy:
decedents: haemorrhagic areas of the lungs;
liver, kidney, spleen discoloration; enlarged gall bladder, gastrointestinal inflammation
survivors(14 d): viscera appeared normal
LD50 (male): 445 mg/kg bw
LD50 (female): 355 mg/kg bw
Reliability: (2) valid with restrictions
no data on purity, no individual pathologic data
Flag: Critical study for SIDS endpoint
21-MAR-2003 (69) (112)

Type: LD50
Species: rabbit
Sex: male/female
No. of Animals: 1
Vehicle: other: none
Value: > 79.4 mg/kg bw
Method: other:1 rabbit/dose, 6 doses, single application of undiluted, warmed substance, exposure time. 24 hrs, postexposure observation time: 14 d (no further information)
Year: 1975
GLP: no

Test substance: other TS: no data on purity

Remark: dose, sex, mortality, time to death:
31.6 mg/kg, male, 0/1, -; 50.0 mg/kg, female, 0/1, -;
79.4 mg/kg, male, 0/1, -; 126.0 mg/kg, female, 1/1, 2 d;
200.0 mg7kg, male, 1/1, 1 d; 398.0 mg/kg, female, 1/1, 1 d

signs of intoxication: slight lethargy (1-2 d in survivors), increasing weakness, collapse, death

gross autopsy: decedents: haemorrhagic areas of the lungs, slight liver discoloration, enlarged gall bladder, gastrointestinal inflammation;
survivors: viscera appeared normal

Reliability: (2) valid with restrictions
no data on purity, information on GLP is missing, only 1 animal/dose, no individual pathologic data

16-JUN-2003 (113)

Type: LDLo
Species: rabbit
Sex: male/female
No. of Animals: 1
Vehicle: other: none
Value: 316 mg/kg bw

Method: other: 1 rat /dose, single application of undiluted substance, exposure time: 24 hrs, post exposure observation time: 14 d
Year: 1975
GLP: no
Test substance: other TS: orthonitrobenzene residue

Remark: dose, sex, mortality, time to death:
126 mg/kg, male, 0/1, -; 200 mg/kg, female, 0/1, -;
316 mg/kg, male, 1/1, 2 days; 794 mg/kg, 1/1, 3 days
signs of intoxication: reduced appetite and activity (2-4 days in survivors), increasing weakness, collapse, death
gross autopsy: decedents: haemorrhagic areas of the lungs, mottled liver, slight enlarged gall bladder, blackened spleen, gastrointestinal inflammation
survivors: viscera appeared normal

Reliability: (4) not assignable
o-chloronitrobenzene residue used, no information of o-chloronitrobenzene itself

21-MAR-2003 (111)

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: 500 other: mg
Exposure Time: 24 hour(s)
No. of Animals: 2
Result: not irritating

Method: other: ear, dose: 500 mg/animal, undissolved TS, covered by cellulose pads and plaster, a rolled gauze pad was put on it, all together was bandaged, exposure time: 24 h, semi-occlusive, observation time 7 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
only a few animals used, no information on GLP
Flag: Critical study for SIDS endpoint
21-MAR-2003 (6)

Species: rabbit
Concentration: 10 %
Exposure: Semiocclusive
Exposure Time: 24 hour(s)
No. of Animals: 6
Result: not irritating

Method: other: appl. to intact and abraded skin, flank, test substance diluted in sesame oil, dose: 0.5 ml/animal, observation time: 72 hrs, reading: 24, 48 and 72 hours, evaluated according Fed.Reg.38, No.187, p.27019, 1973, § 1500.41
Year: 1975
GLP: no
Test substance: other TS: no data on purity

Remark: intakt skin (score 0-4):
24 hrs: 4/6 erythema: score: 1; 0/6 oedema
48 hrs: 0/6 erythema: score: ; 0/6 oedema
72 hrs: 0/6 erythema: score: ; 0/6 oedema
abraded skin:
24 hrs: 4/6 erythema: score: 1; 0/6 oedema
48 hrs: 0/6 erythema: score: ; 0/6 oedema
72 hrs: 0/6 erythema: score: ; 0/6 oed
Reliability: (2) valid with restrictions
sesame oil as vehicle, no data on purity
16-JUN-2003 (41)

Species: rabbit
Concentration: undiluted
Exposure: no data
Exposure Time: 24 hour(s)
No. of Animals: 3
Result: corrosive

Method: other: 0.5 ml undiluted, exposure: 24 hrs
Year: 1974
GLP: no
Test substance: other TS: o-nitrochlorobenzene residue (not the original substance, no further information on chemical characteristics)

Reliability: (4) not assignable
o-chloronitrobenzene residue used, no information of o-chloronitrobenzene itself
21-MAR-2003 (111)

Species: rabbit
Concentration: other: undissolved
Exposure: no data

Exposure Time: 24 hour(s)
No. of Animals: 6
Result: not irritating

Method: other: 0.5 ml/rabbit, warmed, observation time: 168 hours (no further information)
Year: 1973
GLP: no
Test substance: other TS: purity: 99.71 %

Remark: time of reading up to 168 hours: no erythema or oedema
Reliability: (2) valid with restrictions
no GLP, no information on exposure
Flag: Critical study for SIDS endpoint
21-MAR-2003 (113)

5.2.2 Eye Irritation

Species: rabbit
Dose: 50 other: mg
No. of Animals: 2
Result: not irritating

Method: other: undissolved test substance, dose: 50 mg/animal,observation period: 7 d
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Slight redness (score 1/3) observed in 1/2 animals, disappeared within 24 hours, the other animal was without effects
Reliability: (2) valid with restrictions
no GLP, only a few animals used
Flag: Critical study for SIDS endpoint

21-MAR-2003 (6)

Species: rabbit
Concentration: other: undissolved
Dose: 100 other: mg
Exposure Time: 24 hour(s)
Comment: no data
No. of Animals: 6
Result: slightly irritating

Method: other: according Fed.Reg.38, No.187, 1973: undissolved test substance, dose: 100 mg/animal, observation time: 24 hrs
Year: 1975
GLP: no
Test substance: other TS: no data on purity

Remark: 1 hr post appl: 4/6 with conjunctival injections, score: 1/0-3; and 2/6 with conjunctival injections, score: 2/0-3; 7 hr post appl: 2/6 with conjunctival injections, score: 1/0-3; 24 hr post appl: no findin
Reliability: (2) valid with restrictions
no data on purity, no GLP
Flag: Critical study for SIDS endpoint
16-JUN-2003 (41)

Species: rabbit

Concentration: undiluted
Dose: .1 ml
Exposure Time: 24 hour(s)
No. of Animals: 3
Result: corrosive

Method: other: 0.1 ml, undiluted, 24 hrs exposure
Year: 1974
GLP: no
Test substance: other TS: o-nitrochlorobenzene residue (not the original substance, no further information on chemical characteristics)

Reliability: (4) not assignable
o-chloronitrobenzene residue used, no information of o-chloronitrobenzene itself
21-MAR-2003 (111)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 24 hour(s)
No. of Animals: 6
Result: not irritating

Method: other: 0.1 ml/rabbit, warmed, observation time: 168 hours
Year: 1973
GLP: no
Test substance: other TS: purity: 99.71 %

Remark: Time of reading:
24 hrs: 6/6 slight erythema, Score 9.6/110
48 hrs: 5/6 slight erythema, Score 2.3/110
72 hrs: 1/6 slight erythema, Score 0.3/110
168 hrs: no findings
Reliability: (2) valid with restrictions
no GLP
21-MAR-2003 (113)

Species: rabbit
Concentration: 10 %
Dose: .1 ml
No. of Animals: 6
Result: slightly irritating
Method: other: according Fed.Reg.38, No.187, 1973: observation time: 24 hrs
Year: 1975
GLP: no
Test substance: other TS
Remark: 1 hr post appl: 3/6 conjunctival injection, score: 1/0-3; 7 and 24 hrs post appl: no findings
Reliability: (2) valid with restrictions
no data on purity, no GLP
21-MAR-2003 (41)

5.3 Sensitization

Type: no data
Species: human

Remark: experience with human exposure: o-chloronitrobenzene

has been used for decades, but there have been no indications of an allergenic potential in man

(16)

Type: other: modified Draize test
Species: guinea pig
Concentration 1st: Induction 1 %
2nd: Challenge 1 %
No. of Animals: 10
Vehicle: other: Aceton
Result: not sensitizing

Method: other: 3 drops of a 1 % solution to the clipped area of the skin for 5 d; on the 7th d 3 drops of the 1 % solution to an untreated area of the skin; reading time not mentioned
Year: 1973
GLP: no
Test substance: other TS: no data on purity

Remark: The study documentation is incomplete and the methology employed is no longer in use.
Reliability: (3) invalid
no data on purity, study documentation incomplete, no data on GLP
16-JUN-2003

(88)

Type: other: modified Freunds complete adjuvant test
Species: guinea pig
Concentration 1st: Induction 10 %
2nd: Challenge 10 %
No. of Animals: 10
Vehicle: other: aceton
Result: sensitizing

Method: other: 3 drops(10% sol.) to the clipped area of the skin;22nd inj.of Freund-adjuvans and TS into the hind paw (0.5 mg/kg bw), 28th d 3 drops(10 % sol.) to an untreated clipped area of the skin; reading time not mentioned
Year: 1973
GLP: no
Test substance: other TS: no data on purity

Remark: The allergenic activity of o-chloronitrobenzene is less marked than that of p-chloronitrobenzene; 2,4-dinitro-chlorobenzene provokes even stronger sensitization effects than p-chloronitrobenzene
The study documentation is incomplete and the methology employed is no longer in use.
Reliability: (3) invalid
no data on purity, study documentation incomplete, no data on GLP
16-JUN-2003

(88)

Type: other: the rats were exposed via inhalation to o-chloronitrobenzene for 5 months
Species: rat
Result: sensitizing
Year: 1973
GLP: no
Test substance: other TS: no data on purity

Reliability: (3) invalid
no data on purity, study documentation incomplete, no data

on GLP
16-JUN-2003 (88)

5.4 Repeated Dose Toxicity

Species:	rat	Sex: male/female
Strain:	other: F344/N	
Route of administration:	inhalation	
Exposure period:	13 w	
Frequency of treatment:	6 h/d, 5 d/w	
Post exposure period:	no	
Doses:	0, 1.1, 2.3, 4.5, 9 or 18 ppm (approx. 0, 7, 14.7, 28.8, 57.6, 115.2 mg/m3)	
Control Group:	yes	
LOAEL:	ca. 1.1 ppm	

Method: other: see freetext: method
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Method: 10 rats/sex/group, whole body expos., clin.chem., hematol., bw., org.weight, compl. histopathol. in all control rats and 18ppm gr. and rats that died, gross lesions and selec. organs of rats < 18-ppm-groups, add. 10 rats/sex/conc: clin. pathol. at d1, d4, d23

histopathol. evaluations on reproductive organs: see chapter 5.8

Remark: although a no-observed-effect level (NOEL) for histopathological findings was not found in this study, observations among rats exposed to 4.5 ppm or less were limited to minimal effects on nasal tissues

Result: clinical signs:
no clear signs of toxicity (no other information), no deaths, no differences in body weight gain or terminal body weight compared to controls;
haematology, male and female:
concentration-related increase in methaemoglobinaemia (m sign: from 1.1 ppm at d23; from 2.3 ppm at all time points with max of 1.14 g/dl at 18 ppm; f sign.: from 1.1 ppm at week 13 and from 2.3 ppm at all time points with max of 1.04 g/dl at 18 ppm), reticulocyte count (sign. at all dose groups at week 13), nucleated erythrocytes, leucocyte count (predominantly at the highest dose groups of male and females); concentration-related decrease in haematocrit, haematoglobin, RBC (m. sign.: 1.1 ppm(d23), 4.4 ppm (week13), 9 ppm (d4,week13),18 ppm (at all time points); f. sign.: at every dose group at week13), MCH and MCHC (only in females)
clinical chemistry, male and female:
increase in serum activities of sorbitol dehydrogenase and alanine aminotransferase in different male and female exposure groups at various time points, decrease in alkaline phosphatase
pathology: dark spleen (1 female, 2 males, 18 ppm)
concentration-related increases in liver, spleen and right kidney weight
Histopathologic changes:
liver: basophilia of centrilobular hepatocytes, kidney: pigmentation and regeneration of the proximal convoluted

tubules, spleenic congestion was observed in all exposed and control rats: in males with dose-dependent increase in severity and in females with dose-dependent increase in incidences; nose: hyperplasia of the nasal cavity respiratory epithelium
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
21-MAR-2003 (45) (80) (102)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 w
Frequency of treatment: 6 h/d, 5 d/w
Post exposure period: no
Doses: 0, 10, 30 or 60 mg/m3
Control Group: yes, concurrent no treatment
LOAEL: ca. .01 mg/l

Method: other: 15 rats/sex/group, whole body exposure, haematology, clinical chemistry, gross and microscopic examination, statistical analysis
Year: 1986
GLP: no data
Test substance: other TS: purity: 99.71%
Result: all concentration groups:
no deaths, mean body weights comparable to controls, microscopic changes of the spleen: increased degree of haemosiderosis
0.01 mg/l: slight, but statistically significant increase in relative liver weights in male rats
0.03 and 0.06 mg/l: increases in liver, kidneys and spleen weight, significant increase in blood methaemoglobin levels and decrease in haemoglobin, haematocrit and red blood cell count values; increases in liver, kidney, and spleen weights, microscopic changes of the spleen:
slight increase in degree of extramedullary haematopoiesis
Reliability: (2) valid with restrictions
Histopathologic evaluation not performed from all animals, no information on GLP
21-MAR-2003 (73) (74)

Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: inhalation
Exposure period: 2 weeks
Frequency of treatment: 6 h/d, 5 d/w
Post exposure period: no
Doses: 0, 1.1, 2.3, 4.5, 9, 18 ppm (approx. 0, 7, 14.7, 28.8, 57.6, 115.2 mg/m3)
Control Group: yes
LOAEL: ca. 1.1 ppm

Method: other: 5 rats/sex/group, whole body exposure, complete necropsies on all rats, histopatholologic evaluation of all rats in the controls and the highest exposure group
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Result: clinical signs:
18 ppm, males: hypoactivity, ataxia, pallor
18 ppm, males, females: dehydration, nasal discharge, decreased urination and defecation
all concentration groups:
no deaths, body weight gain was not affected
pathology:
males and females: exposure-related increases in liver weights,
18 ppm, males, females: increased spleen weights
18 ppm-group, males: slight increased relative kidney weights
histopathologic findings:
18 ppm, all rats:
hemosiderin deposition in liver (minimal) and spleen (mild severity)
Reliability: (2) valid with restrictions
dose-finding study
21-MAR-2003 (80)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 3 days
Frequency of treatment: 6 hours/day, daily
Post exposure period: none
Doses: 0.045 mg/l
Control Group: yes
NOAEL: < .045 mg/l
LOAEL: = .045 mg/l

Method: other: no information
Year: 1982
GLP: yes
Test substance: other TS: as prescribed in 1.1-1.4 of the Monsanto datasheet

Result: 0.045 mg/l blood, methaemoglobin (3%), incr.; m.f.
Source: Monsanto
Reliability: (3) invalid
information on method and no. of animals is missing
21-MAR-2003 (70)

Species: rat Sex: male
Strain: other: Crl:CD
Route of administration: inhalation
Exposure period: 2 weeks
Frequency of treatment: 6 hrs/d, 5 d/week
Post exposure period: 13 d
Doses: 0, 0.03, 0.15, 0.53 mg/l
Control Group: yes, concurrent no treatment
NOAEL: ca. .03 mg/l

Method: other
Year: 1984
GLP: no data
Test substance: other TS: purity: 99.8 %

Result: haemolytic anemia, methaemoglobinemia
Reliability: (2) valid with restrictions
no information of GLP
21-MAR-2003 (32)

Species: rat Sex: no data
Strain: no data
Route of administration: oral unspecified
Exposure period: 20 d
Frequency of treatment: daily
Post exposure period: no data
Doses: 70 mg/kg bw/d
Control Group: other: no data

Method: other: 20 rats, no further information
Year: 1967
GLP: no
Test substance: other TS: no data on purity

Result: no deaths (thus, the test substance may be regarded as lacking any marked cumulative properties)
Reliability: (3) invalid
only one dose used, lack of information (e.g. unspecified route of oral administration)
16-JUN-2003 (22)

Species: rat Sex: no data
Strain: no data
Route of administration: oral unspecified
Exposure period: 7 months
Frequency of treatment: daily
Post exposure period: no data
Doses: 0.0025, 0.005, 0.025. 0.25 or 5 mg/kg bw/d
Control Group: yes
NOAEL: ca. .25 mg/kg bw

Method: other: CNS function evaluated according Cherkinskii, 1949: method of conditioned reflexes (time required for appearance, establishment,latent period,magnitude, frequency of occurrence), no further information
Year: 1967
GLP: no
Test substance: other TS: no data on purity
Remark: o-, m-, and p-chloronitrobenzene were tested: the para-isomer was found to be most toxic
Result: 0.0025, 0.005, 0.025, 0.25 mg/kg bw/d: no toxic effects

5 mg/kg bw/d:
hemapoetic system, last month of the experiment:
increase in the methaemoglobin content in the blood,
decrease of the haemoglobin content,
increase in the reticulocyte count (up to 78 %) and presence of Heinz bodies in the erythrocytes(up to 47 %);

liver function test: slight increase in blood alkaline phosphatase (no detail given)
effects on CNS function: some slowing down of fixation of the positive conditioned reaction and of the development of the differentiation reaction; liver function tests: increase in the blood alkaline phosphatase activity; rise in the level of bilirubin in the urine

urine: slight increase in bilirubin level
Reliability: (4) not assignable
lack of relevant information
16-JUN-2003 (22)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 13 w
Frequency of treatment: 6 h/d, 5 d/w
Post exposure period: no
Doses: 0, 1.1, 2.3, 4.5, 9 or 18 ppm (0, 7, 14.7, 28.8, 57.6,115.2 mg/m3)
Control Group: yes

Method: other: 10 mice/sex/group, whole body exposure, body/organ weight, gross and microscopic pathology , statistical analysis; histopathological evaluations on reproductive organs: see chapter 5.8
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Result: No clinical signs related to 2-chloronitrobenzene exposure
Mortality: 18 ppm, week 12, 2/10 males (livers darkly discoloured, defuse, severe sinusoidal congestion with hepatocellular degeneration and necrosis);
males: no significant different in body weight gain between control and treated mice; females: from 2.3 ppm body weight greater than in control mice
pathology:
2.3, 4.5, 9 and 18 ppm: increases in right kidney weight and liver weight (all groups, females)
9 and 18 ppm: increase in liver weights(males), hepatocytomegaly in all males; spleen enlargement among females due to hematopoietic cell proliferation
18 ppm: incidence of mild hepatic mineralization and/or necrosis, pale discoloration of the liver (1/10 females, 6/10 males), chronic inflammation in the liver (especially males), incidence of hematopoietic cell proliferation in the spleens of the males; histopathologic changes in the liver, notably hepatocytomegaly observed among females
NOAEL: 4.5 ppm (histopathological injury)
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
30-AUG-2001 (44) (80) (102)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 2 weeks
Frequency of treatment: 6 h/d, 5 d/w
Post exposure period: no
Doses: 0, 1.1, 2.3, 4.5, 9, 18 ppm (approx. 0, 7, 14.7, 28.8, 57.6, 115.2 mg/m3)
Control Group: yes
NOAEL: ca. 2.3 ppm

Method: other: 5 mice/sex/group, whole body exposure, complete necropsy on all mice, histopathological evaluation on all mice
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Result: clinical signs:

18 ppm, esp. males: hypoactivity, abnormal posture, dyspnea
mortality, 18 ppm: 1/5 male on day 2 (diffusely dark,
discoloured liver, severe centrilobular congestion,
necrosis)
body weight gain was not affected,
pathology:
concentration-related increases in liver weights,
18 ppm, all rats: increased spleen and kidney weights
histopathologic findings:
18 ppm, all rats: liver: coagulative necrosis with
associated inflammation; spleen: haemosiderin deposition
18 ppm, esp. males: haematopoietic cell proliferation,
increased haematopoietic activity
9,18 ppm: hepatocytomegaly of the centrilobular cells
4.5, 9, 18 ppm, females: increasing incidence and severity
of haematopoietic activity
Reliability: (2) valid with restrictions
dose-range finding study
21-MAR-2003 (80)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: oral feed
Exposure period: 5 weeks
Frequency of treatment: daily
Post exposure period: no
Doses: 0, 50, 500, 5000 ppm (calc. intake: (m):0,16,167,1120
mg/kg bw; (f):0,24,220,1310 mg/kg bw)
Control Group: yes, concurrent no treatment
NOAEL: ca. 50 ppm

Method: other: according to OECD Guideline 407, 1981; 12
mice/sex/group and additional 6 mice/sex/group for the interim
sacrifice
Year: 1990
GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Result: except one male in the low dose group no deaths,
5000 ppm(m)/500, 5000 ppm(f): reduced food intake,
sign. clin. findings only in the male 5000 ppm gr.: narrowed
palpebral fissure and corneal opacity;
500/5000 ppm, m/f: centrilobular hepatocytomegaly
5000 ppm, m/f: reduced body weight gain, increased spleen
weight, discolored spleen, deposition of hemosiderin in the
spleen; increased liver weight (differences up to 89% were
noted in females)
5000 ppm,m: reduced tested weight, decreased urea;
5000 ppm, m/f: reduced erythrocyte count(change in morphology:
anisocytosis, poiklocytosis and polychromasie), reduced HK-
and HB-content, increased MetHb (2.8 % f; 1.7% m), MCV, MCH,
MCHC, bilirubin,
500 and 5000 ppm, after 1 week, m/f: increased cholesterin
content, sign. changes in the activity of cytochrome
450-dependent EOD (7-Ethoxycoumarin deethylase), EH (Epoxide
Hydroxylase) and ALD (Aldrin epoxidase) and Phase II enzymes:
GSH-T(Glutathion-S-transferase), GLU-T
(UDP-Glucuronyltransferase), and decreased gluconeogenesis
and glycogen;
after 5 weeks:
f: normal ALD activity, increased activity of EOR, EH,
Glu-T, slight increase in EOD, strong increase in GSH-T
activity; m: increased activities of EOD, EOR, GLU-T, ALD,

GSH-T, EH
5000 ppm: increased activity of ASAT, ALAT, alkaline phosphatase(m), activated pentose phosphate cycle, increased glycolysis
no signs of nephrotoxicity
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
30-AUG-2001 (4) (5)

Species: mouse Sex: male/female
Strain: other: Swiss CD-1
Route of administration: gavage
Exposure period: 14 d
Frequency of treatment: daily
Post exposure period: no data
Doses: 0, 20, 40, 80, 160 or 320 mg/kg bw/d dissolved in corn oil
Control Group: yes, concurrent vehicle
NOAEL: ca. 40 mg/kg bw

Method: other: 8 mice/sex/dose, statistical analysis
Year: 1992
GLP: yes
Test substance: other TS: purity: > 99 %

Remark: type: dose-setting study
Result: mortality due to gavage trauma: control, f: 2/8, 20 mg-group, f: 1/8, 40-mg-group, f: 1/8
20 and 40 mg/kg bw/d: no clinical signs
80 mg/kg bw/d: all animals were inactive after the first two daily doses but appeared normal post-dosing throughout the rest of the exposure period
160 mg/kg bw/d: during the first week, animals were slightly weak and inactive; during the second week, these animals became slightly cyanotic, but remained active
320 mg/kg bw/d: during the first 2 days of treatment, all mice died or were moribund and sacrificed; clinical signs of toxicity: recumbency, trembling, inactivity, weakness and cyanosis
Reliability: (2) valid with restrictions
dose-setting study, histopathologic examination not performed
21-MAR-2003 (75) (80)

Species: rabbit Sex: no data
Strain: no data
Route of administration: inhalation
Exposure period: up to 18 d
Frequency of treatment: 8 h/d
Post exposure period: no
Doses: 0.1 mg/l
Control Group: other: no data

Method: other: no information
Year: 1910
GLP: no
Test substance: other TS: no data on purity

Result: deaths occurred after exposure for 8-18 d (no further data)
Reliability: (3) invalid
lack of information

16-JUN-2003 (26)

Species: cat Sex: no data
Strain: no data
Route of administration: inhalation
Exposure period: up to 14 d
Frequency of treatment: 8 h/d
Post exposure period: no
Doses: 0.1 mg/l
Control Group: other: no data

Method: other: no data
Year: 1910
GLP: no
Test substance: other TS: no data on purity

Result: deaths occurred after exposure for 8-14 d (no further data); 1 animal survived (total number of animals not mentioned)
Reliability: (3) invalid
lack of information

16-JUN-2003 (26)

Species: cat Sex: no data
Strain: no data
Route of administration: inhalation
Exposure period: all together 17.5 h during 3 consecutive d
Frequency of treatment: no data
Post exposure period: no
Doses: 0.05-0.18 mg/l
Control Group: other: no data

Method: other: no details given
Year: 1908

Result: mortality: 100 % (no further data)
Reliability: (3) invalid
lack of information: secondary literature

16-JUN-2003 (96)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration: 0, 833.3, 1000.0, 1200.0, 1440.0, 1728.0, 2073.6 ug/plate in DMSO; from 1000 ug/plate bacteriotoxicity
Metabolic activation: with and without
Result: positive

Method: other: s. freetext
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: suspensions of bacterial cells were incubated with the TS with and without S9-mix from rat liver for 48 hours at 37 celsius, the number of revertant colonies were counted; positive (2-aminoanthrazene, trypaflavine, endoxan) and negative controls

Remark: on strain TA 100, a marked dose-dependent increase in mutation rate (up to 4 times higher than in control) was found with metabolic activation
Reliability: (2) valid with restrictions
only 4 strains used
Flag: Critical study for SIDS endpoint
25-MAR-2003 (3)

Type: Ames test
System of testing: S. typhimurium TA 100
Concentration: no data
Metabolic activation: with
Result: positive

Method: other: no data
Year: 1981
GLP: no data
Test substance: other TS: no data on purity
Reliability: (4) not assignable
documentation insufficient for assessment
16-JUN-2003 (21)

Type: Ames test
System of testing: S. typhimurium TA 78, TA 100, TA 1535, TA 1538
Concentration: no data
Metabolic activation: with and without
Result: negative

Method: other: no data
Year: 1983
GLP: no data
Test substance: no data

Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (30)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration: (1): 0.0, 6.0, 20.0, 60.0, 200.0, 600.0:
TA98,TA100,TA1535,TA1537
(2): 0.0, 6.0, 20.0, 60.0, 200.0, 600.0: TA100,TA98
(3): 0.0, 62.5, 125.0, 250.0, 500.0, 1000.0: TA100
see RM
Metabolic activation: with and without
Result: positive

Method: other: s. freetext
Year: 1983
GLP: no data
Test substance: other TS: purity 99 %
Method: preincubation method, solvent: DMSO, S9 prepared from rat liver and hamster liver, positive controls (2-AA, NOPD, 9-AAD), solvent control, performed in triplicate and repeated twice, highest dose: cytotoxic, statistical method according to Margolin et al. 1981

Remark: (4): 0.0, 10.0, 33.3, 100.0, 333.3, 1000.0 : TA98,TA100,TA1535,TA1587
(5): 0.0, 10.0, 33.3,100.0, 333.3, 1000.0: TA100
the test substance was mutagenic only in strain TA 100 with metabolic activation from hamster and rat
Reliability: (2) valid with restrictions
only 4 strains used, no information about GLP
Flag: Critical study for SIDS endpoint
25-MAR-2003 (33) (80)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100
Concentration: no information
Metabolic activation: with and without
Result: negative

Method: other: preincubation method (only engl. abstract available)
Year: 1987
GLP: no data
Test substance: no data

Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (54)

Type: Ames test
System of testing: S. typhimurium TA 97, TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538
Concentration: no data
Metabolic activation: with and without
Result: positive
Method: other: no data
Year: 1985
GLP: no data
Test substance: no data
Remark: the strain(s) on which the test substance induced an increase in the mutant count is (are) not mentioned in the description of the test results
Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (55)

Type: Cytogenetic assay
System of testing: Chinese Hamster Ovary cells
Concentration: without: 0, 16, 50, 160 ug/ml DMSO;
with: 0, 50, 160, 500 ug/ml DMSO
Metabolic activation: with and without
Result: ambiguous
Method: other: protocol in Galloway Environm. Mol. Mutagen. 10 [Suppl 10],1-175, 1987; solvent control, positive control, harvest time: 14 hours
Year: 1993
GLP: no data
Test substance: other TS: purity: 99 %

Remark: type: chromosomal aberration test
Result: without S9: equivocal, cell with aberrations (control, low to high doses): 2, 7, 8, 9%
with S9: negative
Reliability: (2) valid with restrictions
no information about GLP

Flag: Critical study for SIDS endpoint
25-MAR-2003 (77) (80)

Type: Sister chromatid exchange assay
System of testing: Chinese Hamster Ovary cells
Concentration: without S9:
(1) 0, 5, 16, 50 ug/ml DMSO
(2) 0,30, 40, 50, 60, 75ug/ml DMSO;
with S9:
0, 50,160,500 ug/ml DMSO
Metabolic activation: with and without
Result: positive

Method: other: s. freetext
Year: 1993
GLP: no data
Test substance: other TS: purity: 99 %

Method: protocol in Galloway Environm. Mol. Mutagen. 10 [Suppl 10],1-175, 1987; solvent control, positive control (mitomycin C, cyclophosphamide), S9-mix of induced rat liver, incubation time without S9: 26 hours, with S9: 2 hours, after removal of TS 26 hours
Remark: the test substance exhibited a mutagenic response only in the absense of S9-mix (up to 29% increase over solvent control)
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
25-MAR-2003 (77) (80)

Type: other: mutation assay in Actinobacteria
System of testing: spores of Actinomyces sphaeroides
Concentration: 0, 0.63 g/l (= 0.004 M)
Metabolic activation: no data
Result: positive

Method: other: no details given
Year: 1971
GLP: no
Test substance: no data

Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (87)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration: 0, 25.6, 51.2, 102.4, 204.8, 409.6, 819.2, 1638.4, 3276.8 ug/plate in DMSO
Metabolic activation: without
Result: positive

Method: other: according to: OECD Guide-line 471: pour plate method, highest dose cytotoxic, performed in duplicate and repeated at least 2 times, solvent and positive control
Year: 1983
GLP: no data
Test substance: other TS: purity: 99 %

Remark: increased mutation rate only in strains TA 98 and TA 1538

Reliability: (2) valid with restrictions
study meets criteria of today but is only performed without metabolic activation, no information about GLP
25-MAR-2003 (92)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100
Concentration: 0, 1, 5, 10, 15, 20 ug/plate in DMSO
Metabolic activation: with and without
Result: positive

Method: other: according to OECD Guide-line 471, preincubation method, without S9-mix, and with S9-mix and 200 ug/plate Norharman
Year: 1983
GLP: no data
Test substance: other TS: chromatographically pure

Remark: the test substance exhibited no mutagenicity to the tester strains in the absence of S9 mix, without norharman;
in the presence of S9 mix, without norharman, o-chloronitrobenzene was not mutagenic to S. typhimurium TA 98;
in the presence of norharman and S9-mix, the test substance exhibited mutagenicity only to S. typhimurium TA
Reliability: (3) invalid
special study, only performed in the presence of metabolic activation, cytotox concentration not determined, no information on GLP, no exact data on purity
25-MAR-2003 (98)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 98 NR and TA 98/1,8-DNP6
Concentration: 0, 5, 10, 15, 20 ug/plate in DMSO
Metabolic activation: with
Result: positive

Method: other: according to OECD Guide-line 471, preincubation method, addition of S9-mix and norharman
Year: 1987
GLP: no data
Test substance: other TS: no dataon purity

Remark: the test substance exhibited weak mutagenicity towards TA 98 NR; the mutagenic activity, however, was much lower than that of o-chloronitrobenzene towards TA 98; the difference in the mutagenicities (test results: positive) of the test compound towards TA 98 and TA 98/1,8-DNP6 could not be regarded as significant
Reliability: (3) invalid
special study, only performed in the presence of metabolic activation, cytotox concentration not determined, no information on GLP, no exact data on purity
16-JUN-2003 (97) (99)

Type: other: SOS chromotest
System of testing: E. coli PQ 37
Concentration: 3-5 different concentrations (no further information)
Metabolic activation: with and without
Result: negative

Method: other
Year: 1988

GLP: no data
Test substance: other TS: no data on purity

Remark: o-chloronitrobenzene did not induce SOS-repair in the chromotest with and without S9 mix (without norharman); it was tried to increase the sensitivity of the SOS chromotest by addition of norharman to the S9 mix: a negative result was obtained again with the test substance
Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (108)

Type: HGPRT assay
System of testing: V 79 Chinese Hamster lung cells
Concentration: without S9-mix: 0,100,300,400,500,600,700,800,900 ug/ml DMSO;
with S9-mix: 0,100,200,450,600,750,900,1050,1200 ug/ml DMSO
Cytotoxic Concentration: without: 800 ug/ml; with: 750 ug/ml
Metabolic activation: with and without
Result: negative

Method: other: OECD Guide-line 476, rat liver S9-mix (induced), toxicity test prior to testing, exposure duration 5 hours, positive controls (EMS, DMN)
Year: 1989
GLP: yes
Test substance: other TS: purity: 99.8%

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
25-MAR-2003 (101)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: without S9-mix: 0, 10, 50, 100 ug/ml DMSO; with S9-mix: 0, 25, 125, 250 ug/ml DMSO
Metabolic activation: with and without
Result: negative
Method: other: OECD Guide-line 473, harvest time: 8, 12, 21 hours, cytotoxicity was tested prior to testing, positive controls: mitomycin C, cyclophosphamide
Year: 1988
GLP: yes
Test substance: other TS: purity: 99.8 %

Remark: type: chromosomal aberration test
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
25-MAR-2003 (47)

Type: Ames test
System of testing: Salmonella typhimurium TA 100, TA 1535, TA 1537, TA 1538, TA 98, Escherichia coli WP2uvrA
Concentration: 0, 4, 20, 100, 500, 2500 ug/plate, dissolved in 100 ul DMSO, additionally:TA100 with S9-mix: 2000 ug/plate, dissolved in 100 ul DMSO
Metabolic activation: with and without
Result: positive
Method: other: OECD Guideline 471, rat S9-mix, positive controls
Year: 1984

GLP: yes
Test substance: other TS: purity: 99 %
Remark: mutagen with metabolic activation in TA100 and without in TA 1538
Source: Hoechst AG Frankfurt/Main
Reliability: (1) valid without restriction
25-MAR-2003 (43)

Type: Unscheduled DNA synthesis
System of testing: Rat Hepatocytes
Concentration: 0, 1.0, 5.0, 10, 50, 75, 100 ug/ml DMSO, 500 ug/ml DMSO was cytotoxic
Metabolic activation: with and without
Result: negative
Method: other: in accordance with OECD Guide-line 482, no detailed data available
Year: 1983
GLP: yes
Test substance: other TS: as prescribed in 1.1-1.4 of the Monsanto dataset

Remark: Cytotoxicity observed at 100 ug/ml in preliminary, but not replicate assay
Cytotoxicity at 500 ug/ml
Source: Monsanto
Reliability: (2) valid with restrictions
no details on results given
25-MAR-2003 (72)

Type: other: UMU test
System of testing: Salmonella typhimurium TA1535/pSK1002
Concentration: 100 ug/ml
Metabolic activation: with and without
Result: negative

Method: other: incubation time: 4 hours; determination of ß-galactosidase activity
Year: 1992
GLP: no data
Test substance: no data

Reliability: (4) not assignable
documentation insufficient for assessment
25-MAR-2003 (81)

Type: Bacterial reverse mutation assay
System of testing: S. typhimurium TA98, TA100, TA1530, TA1532, TA1535, TA1537, TA1538, TA1950, TA1975, G46
Concentration: no data
Metabolic activation: with and without
Result: negative

Method: other: OECD guideline 471: plate incorporation method: aerobic and anaerobic condition; fluctuation method
Year: 1980
GLP: no data
Test substance: other TS: purest grade available
Reliability: (3) invalid
no details given, special study
25-MAR-2003 (29)

Type: Sister chromatid exchange assay
System of testing: Chinese Hamster Ovary cells

Concentration: without S9:
0,5,16,50 ug/ml DMSO;
with S9:
(1): 0, 50, 167, 500 ug/ml DMSO
(2): 0, 63, 125, 250 ug/ml DMSO
Metabolic activation: with and without
Result: positive

Method: other: s. freetext
Year: 1993
GLP: no data
Test substance: other TS: purity: 99 %

Method: protocol in Galloway Environm. Mol. Mutagen. 10 [Suppl 10],1-175, 1987; solvent control, positive control (mitomycin C, cyclophosphamide), S9-mix of induced rat liver, incubation time without S9: 26 hours, with S9: 2 hours, after removal of TS 26 hours
Result: without S9-mix: negative; with S9-mix: positive (up to ca. 40% increase over solvent control)
Reliability: (2) valid with restrictions
no information about GLP
Flag: Critical study for SIDS endpoint
25-MAR-2003 (80)

Type: Cytogenetic assay
System of testing: Chinese Hamster Ovary (CHO) cells
Concentration: without S9: 0,47,101,216 ug/ml DMSO; with S9: 0, 101,125,216,250,465,500 ug/ml DMSO
Metabolic activation: with and without
Result: positive

Method: other: protocol in Galloway Environm. Mol. Mutagen. 10 [Suppl 10],1-175, 1987; solvent control, positive control, harvest time: without S9: 18.5 hours, with S9: 13.6 hours
Year: 1993
GLP: no data
Test substance: other TS: purity: 99 %

Result: with S9-mix: poitive;
without S9-mix: negative
Reliability: (2) valid with restrictions
no information about GLP
Flag: Critical study for SIDS endpoint
25-MAR-2003 (80)

Type: HGPRT assay
System of testing: Chinese Hamster Ovary cells
Concentration: with S9-mix: 0, 10,30,100,300,400 ug/ml DMSO; without S9-mix: 0, 6.6, 20, 66.6, 200, 300 ug/ml DMSO
Metabolic activation: with and without
Result: negative
Method: other: in accordance with OECD Guide-line 476
Year: 1984
GLP: yes
Test substance: other TS: as prescribed in 1.1-1.4 of the Monsanto dataset
Reliability: (2) valid with restrictions
only summarized report available
16-JUN-2003 (71)

Type: Bacterial reverse mutation assay

System of testing: Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2uvrA
Concentration: 0, 10, 20, 50, 100, 200, 500, 1000 ug/plate dissolved in DMSO, highest dose cytotoxic
Metabolic activation: with and without
Result: negative

Method: other: OECD Guide-line 471, preincubation method, S9-mix from induced rat liver, solvent and positive controls (AF2, NaN3, 9AA)
Year: 1996
GLP: no data
Test substance: other TS: purity: 99 %

Reliability: (2) valid with restrictions
no information about GLP
Flag: Critical study for SIDS endpoint
25-MAR-2003 (51)

Type: Bacterial reverse mutation assay
System of testing: S. typhimurium TA100, TA1535, WP2uvrA, TA98, TA1537
Concentration: 0, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000 ug/plate dissolved in DMSO and TA100, TA1535, WP2uvrA: 500 ug/plate dissolved in DMSO
Metabolic activation: with and without
Result: positive

Method: other: OECD Guide-line 471, preincubation method, S9-mix from rat and from hamster, highest dose cytotoxic, solvent and positive controls
Year: 1997
GLP: no data
Test substance: other TS: purity: 99 %

Result: positive: TA100 with rat and hamster S9, TA98 with hamster S9
WP2uvrA: positive and negative with hamster S9-mix
Reliability: (2) valid with restrictions
no information about GLP
25-MAR-2003 (52)

Type: Ames test
System of testing: S. typhimurium TA100, TA98
Concentration: (1)0,10,33,100,133,166,250,333,666,1000,1666 ug/plate
(2)0,3,10,33,66,100,166,333,666 ug/plate
Metabolic activation: with and without
Result: positive

Method: other: praeincubation assay, S9-mix from hamster and rat liver
Year: 1983
GLP: no data
Test substance: other TS: purity: 98 %

Remark: TS was positive only in TA98 in presence of 30 % hamster S9-mix and in TA100 in presence of induced hamster or rat mix
Reliability: (2) valid with restrictions
no information on GLP only two strains used
25-MAR-2003 (80)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton-S wild type
Route of admin.: i.p.
Exposure period: once
Doses: 0, 10000 ppm in peanut oil
Result: negative

Method: other: males(1-3d old), mated with 3x with Basc virgin females brood1: 3d, brood2: 2d, brood3: 2d;
Year: 1985
GLP: no data
Test substance: other TS: purity:>99 %

Reliability: (2) valid with restrictions
no information about GLP
25-MAR-2003 (80) (116)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton-S wild type
Route of admin.: oral feed
Exposure period: 72 hours
Doses: 0, 125 ppm in 10 % ethanol and 5 % sucrose solution
Result: negative

Method: other: males(24 hrs old), mated with 3x with Basc virgin females brood1: 3d, brood2: 2d, brood3: 2d;
Year: 1985
GLP: no data
Test substance: other TS: purity: > 99 %

Reliability: (2) valid with restrictions
no information about GLP
Flag: Critical study for SIDS endpoint
25-MAR-2003 (80) (116)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton S wild type
Route of admin.: oral feed
Doses: 0, 60 ppm in 4 % ethanol
Result: negative

Method: other: see ME
Year: 1989
GLP: no data
Test substance: other TS: purity: > 99 %

Method: In order to obtain individuals for larval treatment Canton-S females and males were mated and eggs exposed in vials with standard cornmealfood containing the chemical plus solvent alone. Adult males emerging from the treatrment were mated at approximately 24 hours of age with two successive harems of three to five Basc females to establish two single day broods. Males were then discarded and two conventional SLRL assay were carried out.

Reliability: (2) valid with restrictions
no information about GLP
25-MAR-2003 (80) (115)

Type: other: single-strand DNA-breaks
Species: mouse Sex: male
Strain: CD-1
Route of admin.: i.p.
Exposure period: single application
Doses: 60 mg/kg bw
Result: positive
Method: other: 8 mice, 4 h post appl. nuclei were isolated from liver and kidney cells, DNA damage was evaluated by alkaline elution technique was used, coupled with a microfluorometric method for DNA assay
Year: 1982
GLP: no data
Test substance: other TS: no data on purity
Result: effects: an increased elution rate in alkali of DNA from liver and kidney was obtained
Reliability: (2) valid with restrictions
no data on purity and GLP, only 1 dose used
Flag: Critical study for SIDS endpoint
25-MAR-2003 (19)

5.7 Carcinogenicity

Species: rat Sex: male
Strain: other: CD
Route of administration: oral feed
Exposure period: 18 months
Frequency of treatment: daily
Post exposure period: 6 months
Doses: 0, 500, 1000 or 2000 ppm (= ca. 0, 37.5, 75 or 150 mg/kg bw/d) ; see method
Control Group: yes, concurrent no treatment

Method: other: s. freetext
Year: 1978
GLP: no data
Test substance: other TS: purity: 97-99 %

Method: 25 rats/group,1000 or 2000 ppm for 6 mo., 500 or 1000 ppm for another 12 mo; complete gross necropsy and histology on certain organs (lung, liver, spleen, kidney, adrenal, heart, bladder, stomach, intestines, reproductive organs, pituitaries), on all grossly abnormal organs and tumour masses, statistical methods: Fisher Exact Test, Bonferroni correction
Remark: pathological examination was not performed of animals that died within the first six months
Result: no information on body weight gain
multiple tumours at the low dose only and late in life: usually a pituitary adenoma along with either a stomach papilloma, adrenal tumour, thyroid adenocarcinoma, lymphosarcoma, choliangosarcoma of the liver or subcutaneous fibroma
incidences: low dose level:7/22, high dose level:1/19, simultaneous control: 1/22, pooled control: 14/111

Reliability: (2) valid with restrictions
study doesn't meet the criteria of today (number of animals too low, time of duration too short, doses too high), reported in brief
Flag: Critical study for SIDS endpoint
16-JUN-2003 (110)

Species: mouse Sex: male/female
Strain: CD-1
Route of administration: oral feed
Exposure period: 18 months
Frequency of treatment: daily
Post exposure period: 3 months
Doses: 0, 1500, 3000 or 6000 ppm (= ca.0, 225, 450 or 900 mg/kg bw/d)
Control Group: yes, concurrent no treatment

Method: other: s. freetext
Year: 1978
GLP: no data
Test substance: other TS: purity: 97-99 %

Method: 25 mice/sex/group,3000 or 6000 ppm for 8 mo., 1500 or 3000 ppm for another 10 mo; complete gross necropsy, histology on certain organs (lung, liver, spleen, kidney, adrenal, heart, bladder, stomach, intestines, reproductive organs), on all grossly abnormal organs and tumour masses , statistical methods: Fisher-Exact Test, Bonferroni correction
Remark: pathological examination was not performed of animals that died within the first six months
Result: no information on body weight gain
significant increase in hepatocellular carcinomas in female mice at both dose levels and in male mice at the low dose level
incidences of hepatocellular carcinomas:
male mice:
low dose level: 7/17, high dose level: 3/16, simultaneous control: 3/18, pooled control: 7/99;
female mice:
low dose level: 5/22, high dose level: 5/19, simultaneous control: 0/20, pooled control: 1/102
Reliability: (2) valid with restrictions
study doesn't meet the criteria of today (number of animals too low, time of duration too short, doses too high), reported in brief
Flag: Critical study for SIDS endpoint
16-JUN-2003 (110)

5.8.1 Toxicity to Fertility

Type: Two generation study
Species: mouse
Sex: male/female
Strain: other: Swiss CD-1
Route of administration: gavage
Exposure Period: see type and remarks
Frequency of treatment: daily
Premating Exposure Period
male: 7 d
female: 7d
Duration of test: 34 weeks

Doses: 0, 40, 80 or 160 mg/kg bw/d dissolved in corn oil
Control Group: yes, concurrent vehicle
NOAEL F1 Offspring: ca. 160 mg/kg bw
NOAEL F2 Offspring: ca. 160 mg/kg bw

Method: other: NTP Continuous Breeding Protocol, see also ME
Year: 1992
GLP: yes
Test substance: other TS: purity: > 99 %
Method: NTP Continuous Breeding Protocol: 20 ps/group, 40 ps (contr.), exposure period: F0: 7d prior to cohousing, 98d of continuous breeding. Last litter from F0, control and high dose groups were reared, weaned, and kept until mating. Siblings received the same treatment as their parents. At sexual maturity, 20 non-sibling males and females were cohabited for 7 days and housed singly through delivery, until sacrifice. Exam.: symtoms, bw gain, water consumption; F0,F1: contr,160 mg-gr.: spleen weight, methb; F0,F1:

fertility indices; F1(m): testes,epididymis, F1(f): vaginal cytolo
Result: Conclusion:
In the presence of altered somatic and selected organ weights 2-chloronitrobenzene (2CNB) did not alter reproductive function in either generation (NOEL 160 mg/kg bw); thus, 2CNB is not a selective reproductive toxicant.
F0 mice:
Mortality: 2,2,2,3 control to high dose gr., 160 mg-group: increased terminal bw and spleen weights; 80 mg-gr.(1m), 160 mg-gr.(3m): with hepatocellular degeneration;
160 mg-gr.: methaemoglobinaemic, during the first 10 d mice were slightly inactive post dosing, 3 lactating females were cyanotic for up to 2 weeks; no other signs of clin.l toxicity
F0-fertility and reproductive parameters were not affected
F1-pups:
in the final litter of the holding period following the continuous breeding phase, F1 pup weight gain during suckling was lower in all treated groups;
at weaning, F1 pups in the 160 mg/kg bw/d group weighed 10-13% less than controls, all other fertility and reproductive parameters were not affected;
F1 mice (only control and high dose group):
no signs of clin. tox. observed, 160 mg/kg bw/d:
significantly lowered body weights at weaning but sign. heavier than controls at mating and at terminal necropsy;
right epididymis, kidney/adrenals(m), spleen and liver weights increased, seminal vesicle-to-body weight ratio was sign. decreased, sign. methaemoglobinaemia;
none of the fertility and reproductive parameters examined were affected in F1 mice, i.e., epididymal sperm parameters (motility, count and percentage of abnormal sperms) and estrous cycle length and estrual cyclicity
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
27-AUG-2001 (20) (76) (80)

Type: other:
Species: rat
Sex: male/female
Strain: other: F344/N
Route of administration: inhalation

Exposure Period: 13 w
Frequency of treatment: 6 h/d, 5 d/w
Doses: 0, 4.5, 9 or 18 ppm (approx. 0, 28.8, 57.6, 115.2 mg/m3)
Control Group: yes, concurrent no treatment

Method: other: 10 rats/sex/group, reproduct. system evaluation: vaginal cytology, sperm morphology, necropsy body and reproductive tissue weights, sperematozoal data, spermatogenesis, oestrous cycle length, percent of cycle spent in various
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Remark: see chapter 5.4.
Result: females: no effects observed
males, 18 ppm: decreases in cauda epididymis weights (6.8%), and in the spermatid count and spermatid heads/testis (ca. 13%)
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
25-MAR-2003 (44) (80)

Type: other:
Species: rat
Sex: male
Strain: Fischer 344
Route of administration: gavage
Exposure Period: single application
Frequency of treatment: once
Doses: 150 mg/kg bw
Control Group: yes

Method: other: 5or 6 rats, sacrifice on d1 and d25 post application, evaluation of testes weight, testicular histopathology, sperm production
Year: 1988
GLP: no data
Test substance: other TS: no data

Result: no effect on testicular histopathology (at 1 d) or testes weight and daily sperm production (at 25 d)
Reliability: (4) not assignable
lack of information
25-MAR-2003 (65)

Type: other:
Species: mouse
Sex: male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure Period: 13 w
Frequency of treatment: 6 h/d, 5 d/w
Doses: 0, 4.5, 9 or 18 ppm (approx. 0, 28.8, 57.6, 115.2 mg/m3)
Control Group: yes, concurrent no treatment

Method: other: 10 rats/sex/group, reproductive system evaluation: vaginal cytology, sperm morphology, necropsy body and reproductive tissue weights, spermatozoal data, spermatogenesis, estrous cycle length, percent of cycle spent in various
Year: 1993
GLP: yes
Test substance: other TS: purity: 99 %

Remark: see chapter 5.4
Result: male, 4.5, 9, 18 ppm: decreased sperm motility
females: increased terminal body weight; no reproductive effects observed
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
03-SEP-2001 (20) (44) (80)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: days 6-15 of gestation
Frequency of treatment: daily
Duration of test: 21 d
Doses: 0, 25, 75, or 150 mg/kg bw/d dissolved in corn oil
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: ca. 25 mg/kg bw

Method: other: 25 females/group, due to severe mat. tox. and mortality the 150 mg-level was terminated prior to scheduled sacrifice
Year: 1986
GLP: yes
Test substance: other TS: purity: commercial

Result: mortality:
150 mg-gr.: due to severe toxicity and high mortality rate of the dams, all females were terminated prior to sheduled sacrifice, 75 mg-group: 1/25;
general toxicity:
75 mg/kg bw/d: gest.-d. 6-10: reduced body weight gain (slight but not significant) and
reduced food consumption; recovery later in gestation;
urinary staining, alopecia; maternal reproductive parameters comparable to controls, mean number of early resorptions and post implantation loss slightly increased (post implantation loss in the respective control very low when compared to historical control; values range: 0-0.9)
25 mg/kg bw/d: no evidence of maternal toxicity
developmental toxicity:
fetal body weight comparable to control
variations: cervical #7 ribs at 25 mg-gr (1.1%) and sign. at 75 mg-gr (2%); 13 full pair of ribs with lumbar #1 rudimentary ribs in controls, at 25 mg-, 75 mg-gr increased, but not sign.;
12 full pair of ribs with #13 unilateral full rib and/or rudimentary rib(s) in controls and in 25 mg-gr. increased, but not sign.
Reliability: (2) valid with restrictions
highest dose was too high
Flag: Critical study for SIDS endpoint
25-MAR-2003 (67) (105)

Species: rat Sex: female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: d6-d15
Frequency of treatment: daily
Doses: 0, 100 mg/kg bw in corn oil
Control Group: yes, concurrent vehicle
other: NOAEL developmental toxicity : ca. 100 mg/kg bw

Method: other: 25 females/group, only one dose
Year: 1984
GLP: yes
Test substance: other TS: purity: commercial

Remark: The study was intended to clarify the observations of the study of Monsanto,1986
Result: d6-10: slight maternal body weight loss accompanied by reduction in food consumption for d6-16, maternal reproductive parameters were not affected, fetal body weight comparable to the respective controls; no teratogenic effects were observed
Reliability: (2) valid with restrictions
only one dose used
Flag: Critical study for SIDS endpoint
25-MAR-2003 (49)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: based on clinical and laboratory evaluation of cyanosis cases during a 10-year period a number of cyanogenic aromatic nitro compounds were ranked in descending order of relative hazard relating to their cyanogenic potential observed in exposed industrial workers (rank 1 = most potent, rank 13 = least potent): o-chloronitrobenzene was classified in rank 7; laboratory evaluation showed that total oxygenatable haemoglobin in some cases, notably after be expected from methaemoglobin analysis (unspecified route of absorption)
Flag: Critical study for SIDS endpoint
(59)

Remark: experience with human exposure: a number of the more important aromatic nitrocompounds were ranked showing their commparative hazard ratings for cyanosis, anaemia and overall toxicity (the degree of hazard ranges from 1 = slight hazard to 6 = severe hazard): for o-chloronitro-benzene, the degree of hazard is 4 concerning cyanosis hazard, 2 concerning anaemia hazard and 3 concerning over-all toxic hazard (no further data)
(60)

Remark: all 325 records of industrial chemical cyanosis poisoning in Britain notified to the inspectorate from 1961 to 1980 were scrutinised: the cases occurred mainly during chemical or dyestuff manufacture; a total of 50 cases of chemical cyanosis syndrome due to chloronitrobenzene were reported; 23 (46 %) cases were "early cases", i.e., the symptoms developed while at work on the same day of exposure, and 27 (54 %) cases were "delayed cases", i.e., the symptoms developed insidiously or some definite time after the "working" day on which the poisoning occurred (the route of absorption is not described in detail for each test compound, the most cases resulted from skin absorption and/or inhalation; in this study, the isomer(s) of chloronitrobenzene is/are not clearly specified)
Flag: Critical study for SIDS endpoint
14-AUG-2001 (91)

Remark: experience with human exposure: in chloronitrobenzene poisoning cardiac complications appear to be more frequent and more serious than in aniline poisoning and gastrointestinal irregularities (anacidity) also appear to be quite common (no further data, isomer(s) of chloronitrobenzene not specified)
(13) (14)

Remark: experience with human exposure: four workmen were reported who were hospitalized as the result of exposure to a mixture of o- and p-chloronitrobenzene; these cases resulted from two to four days exposure and all were cyanotic; headache and weakness accompanied the cyanoses
Flag: Critical study for SIDS endpoint
(84)

Remark: The exposition against a mixture of 2-chloro- and 4-chloronitrobenzene caused severe intoxications which exceeds the signs of intoxication during repair of a unit for isolation of the isomers. As symtoms cyanotic appearance and collapse were described. Hb-content was decreased up to 65 % of the normal value. During the recovery period the patients suffered from difficulty in breathing and sensation of dizziness. Within 7 weeks Hb content increased to 80 % of the normal value.
Flag: Critical study for SIDS endpoint
14-AUG-2001 (28)

5.11 Additional Remarks

Type: other

Remark: the level of lipid peroxidation, content of vitamine E and its metabolites as well as antioxidative activity in the blood serum, liver and spleen of white rats were studied. Toxicological efects of nitrochlorobenzenes were decreased by vitamine E (no further information) .

23-FEB-1998 (82) (83)

Type: other: Haematotoxizitaet

Remark: Ergebnis: 10 mg/kg Kgw. zeigte (2 Katzen): keine Letalitaet, leichte Veraenderungen im weissen Blutbild, leichten Anstieg der Zahl der Heinz'schen Innenkoerper und leichte Methaemoglobinaemie, nach 48 Stunden p.a. weitgehend reversibel.
Source: Hoechst AG Frankfurt/Main
Test substance: technisch rein

(36)

Remark: an attempt to vaporize o-chloronitrobenzene by passing air (2 l of air/min. for 1 h) through a tower of dust was not successful in that no weighable amounts of the test substance were vaporized; rats and mice in an inhalation chamber were exposed to the generated atmosphere for 1 h: no symptoms of toxicity were observable and no deaths occurred at the end of the exposure period or within an observation period of 7 d

(6)

Remark: 48 h after a single oral administration of 100 mg/kg bw of o-chloronitrobenzene to rabbits, 0.3 % of the administered dose was found in faeces as unabsorbed material which was completely reduced to the chloroaniline; in the urines collected each 24 h for 48 h the following metabolites of o-chloronitrobenzene were detectable (expressed as percentages of the administered dose): ether glucuronide (42 %), ethereal sulphate (24 %), mercapturic acid (7 %), free chloroaniline (9 %) (total accounted for: 82 %)
Flag: Critical study for SIDS endpoint

(15)

Remark: metabolism in vitro: radiolabelled (14 C) o-chloronitrobenzene (concentration not specified) was incubated with isolated rat hepatocytes for up to 90 min.: after 90 min., 71 % of the o-chloronitrobenzene had been metabolized; the primary metabolic pathway for o-chloronitrobenzene was reduction to o-chloroaniline (19.2 % of the total radioactivity after 90 min.); o-chloronitrobenzene was also conjugated with glutathione; two other very polar metabolites, com- prising 14.2 % of the total 14 C from o-chloronitrobenzene, have not been identified

23-FEB-1998 (34) (35)

Remark: in order to identify the specific enzymes involved in the metabolism of o-chloronitrobenzene by isolated rat hepatocytes, hepatic subcellular fractions were isolated from rats; microsomes incubated with radiolabelled (14 C) o-chloronitrobenzene in the presence of NADPH produced o-chloroaniline under aerobic conditions and SKF 525 A and metyrapone had no effect on the metabolism to o-chloroaniline: these findings suggest that cytochrome P-450 reductase is responsible for o-chloronitrobenzene reduction; radiolabelled o-chloronitrobenzene was also incubated with or without microsomes, cytosol and/or glutathione: o-chloronitrobenzene was converted to S-(2-nitrophenyl)glutathione in the presence of cytosol and glutathione suggesting that cytosolic glutathione transferase is involved in this conjugation (concentration of the test substance un-

specified)

(34)

Remark: the effect of o-chloronitrobenzene on heme synthesis was determined in vitro by studying its influence on delta-aminolevulinic acid synthetase (ALAS) and ferrochelatase (FC) activities in rat liver homogenates; at 0.001 mol/l concentration, o-chloronitrobenzene did not significantly affect the enzyme activities

(53)

Remark: o-chloronitrobenzene was administered by gavage to adult and geriatric rats at 65 mg/kg bw/d for 11 d; 14 C-o-chloronitrobenzene was administered on days 1, 5 and 9; 14 C was determined in urine and faeces up to 96 h after each 14 C-dose and in tissues at 72 h after the day 9 dose: in adult rats, at all treatment intervals, 71-74 % of each dose was excreted in urine and 20-27 % in faeces and the rates of excretion increased with pretreatment; 5 % of the day 9 dose was in tissues, the highest concentrations were in liver and kidney; 24 urinary metabolites were found; pattern, rate and extent of excretion of 14 C were similar in geriatric and adult rats, except that urinary excretion by unpretreated geriatrics was more extensive (85 %) and the rates of urinary and faecal excretion did not increase with pretreatment; tissue distribution of 14 C was also similar and 8 % of the day 9 dose was in tissues

Flag: Critical study for SIDS endpoint

27-AUG-2001 (62)

Remark: 14 C-o-chloronitrobenzene was administered by gavage to rats at 2, 20 or 200 mg/kg bw (single administration); radioactivity was determined in urine and faeces up to 72 h and in tissues at 24 and 72 h: at 2 and 20 mg/kg bw 58-60 % of the dose was excreted in urine, 26-28 % in faeces, primarily during the first 24 h, 6 % was in 24-h and 3 % in 72-h tissues; at 200 mg/kg bw 74 % was in urine and only 7 % in faeces and it was excreted more slowly with 21 % in 24-h and 4 % in 72-h tissues; at 2 and 20 mg/kg bw o-chloronitrobenzene equivalent concentrations in tissues

were proportional to dose, whereas at 200 mg/kg bw they were disproportionately higher in all tissues, especially in fat, and disproportionately lower in liver; at all doses the highest concentrations were in liver and kidney and at 200 mg/kg bw in fat; up to 23 metabolites were in urine

Flag: Critical study for SIDS endpoint

27-AUG-2001 (63)

Remark: After a single non-occlusive, protective dermal application of 14 C-o-chloronitrobenzene at doses of ca. 0.65, 6.5 or 65 mg/kg bw to male rats, 33-40 % of the doses of o-chloronitrobenzene was absorbed from the skin within 72 h; the absorbed 14 C was excreted in urine (21-28 %) and faeces (11-15 %). The extent absorption increased with an increase in dose from 0.65 to 6.5 mg/kg bw but increased only neglibly when the dose was increased to 65 mg/kg bw.

The extent of urinary excretion of radioactivity was not significantly affected by dose over the range studied. The initial rate of urinary excretion was also unaffected by dose. The initial rate of faecal exretion increased with dose over the 0.65 to 6.5 mg/kg range, but decreased notably at the high dose.

Flag: Critical study for SIDS endpoint

27-AUG-2001 (66) (79)

Remark: metabolism of o-chloronitrobenzene by hepatic subcellular fractions from rats: to determine the enzyme systems involved in the metabolism of o-chloronitrobenzene by rat isolated hepatocytes, radiolabelled (14 C) o-chloronitrobenzene (100 uM) was incubated with hepatic microsomes (incubation mixture containing microsomes and NADPH, some incubations also containing UDP-glucuronic acid) or with cytosol (incubation mixture containing GSH and cytosolic protein): reduction of o-chloronitrobenzene to o-chloroaniline occurred readily in microsomal incubations; substitution of NADH for NADPH or incubation of microsomes under a carbon monoxide atmosphere significantly inhibited nitroreduction, boiling the microsomes completely abolished reduction of o-chloronitrobenzene; addition of SKF 525-A or metyrapone significantly inhibited the microsomal reduction of o-chloronitrobenzene to o-chloroaniline (the inhibition of nitroreduction by carbon monoxide, SKF 525 A and metyrapone suggests that cytochrome P-450 catalyzes this reaction); incubation of o-chloronitrobenzene with rat hepatic cytosol and glutathione resulted in the formation of S-(2-nitrophenyl)glutathione

Flag: Critical study for SIDS endpoint

(85)

Remark: in vitro study of metabolism: after 90 min. incubation of isolated rat hepatocytes with radiolabelled (14 C) o-chloronitrobenzene (100 uM final concentration), 46.7 % of the added o-chloronitrobenzene was metabolized; the calculated half-life for disappearance of o-chloronitrobenzene from the incubations was 84 min.; a major metabolic pathway for o-chloronitrobenzene was reduction to o-chloroaniline (19.2 % of the total radioactivity after 90 min. incubation); o-chloroaniline was further metabolized to form the N-glucuronide accounting for 14.2 % of the total radioactivity; o-chloronitrobenzene was conjugated with glutathione and S-(2-nitrophenyl)glutathione accounted for 13.3 % of the total radioactivity

Flag: Critical study for SIDS endpoint

(85)

Remark: in vitro assay: the reduction of chloronitrobenzenes was investigated in purified milk xanthine oxidase-xanthine system: o-chloronitrobenzene was less readily reduced by the enzyme than the corresponding para and meta isomers, indicating the steric hindrance effect at ortho position

Flag: Critical study for SIDS endpoint

(100)

Remark: in an in vivo study, 100 umoles/kg bw (= 15.7 mg/kg bw) of o-chloronitrobenzene was given i.p. to male rats, the animals were killed 5 h after the injection to examine methaemoglobin levels: formation of methaemoglobin was observable (methaemoglobin level: 20.6 %)
Flag: Critical study for SIDS endpoint

(109)

Remark: in vitro methaemoglobin formation was studied by incubating haemolyzate (obtained from rats and containing 0.1 umole of haemoglobin) with 0.5 umole of o-chloronitrobenzene at pH 6.6 and 37 degrees centigrade for 5 h: formation of methaemoglobin (concentration: 4.8 %) was not significantly increased compared with the control

(109)

Remark: Single oral administration of 0.1 ml/100 g bw of a 0.5 M tricaprylinsolution of 1-chloro-2-nitrobenzene (o-CNB) to female Wistar rats resulted in hemoglobin binding: 2.1 (mmol TS/mol Hb)/(mmol TS/kg bw)
Flag: Critical study for SIDS endpoint

23-FEB-1998 (89) (90)

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FOREWORD INTRODUCTION

TRIETHYLENE TETRAMINE
CAS N°: 112-24-3

SIDS Initial Assessment Report for SIAM 8

(Paris, 28-30 October 1998)

Chemical Name: Triethylenetetramine

CAS No: 112-24-3

Sponsor Country: Germany

National SIDS Contact Point in Sponsor Country: Dr Jan Ahlers

HISTORY:

The SIDS Initial Assessment Report was discussed at SIAM 5 & 6 and adopted at SIAM 8.

COMMENTS:

Date of Circulation: July 1998

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	112-24-3
Chemical Name	Triethylene tetramine
Structural Formula	H_2N-CH_2-CH_2-NH-CH_2-CH_2-NH-CH_2-CH_2-NH_2

CONCLUSIONS AND RECOMMENDATIONS

Environment

The chemical is toxic to algae, but PEC/PNEC ratios are lower than 1. It is currently considered of low potential risk and low priority for further work.

Human Health

The chemical is genotoxic *in vitro*, a severe irritant to skin and eyes and a skin sensitiser, but exposure is low and well-controlled. Therefore, it is currently considered of low potential risk and low priority for further work. However due to its hazard character appropriate classification and labelling are recommended.

SHORT SUMMARY WHICH SUPPORTS THE REASONS FOR THE CONCLUSIONS AND RECOMMENDATIONS

The production volume of triethylenetetramine (TETA) in 1990 is 1200-1500 t/a in Germany, ca. 6000 t/a in the Netherlands, >11000 t/a in the USA and ca. 1800 t/a in Japan. TETA is mostly used as intermediate in chemical synthesis. Ca. 160 t/a are directly used as curing agent for epoxy resins in Germany. For Sweden, a similar use pattern was described. TETA is stable in neutral solution and is classified as "non biodegradable". The most sensitive environmental species to TETA is the alga *Scenedesmus subspicatus* (72h-EC10 = 0.67 mg/l). A PNEC of 13.4 µg/l is determined.

TETA has a moderate acute toxicity: LD50 (oral, rat) > 2000 mg/kg bw, LD50 (dermal, rabbit) = 550-805 mg/kg bw. The NOAEL for repeated dose toxicity is 600 ppm (92 (male), 99 (female) mg/kg bw) for mice (oral, 90 days). In *in vitro* tests the substance showed genetic toxicity whereas in *in vivo* test negative results were found. There are no animal data on reproductive toxicity available. From experience with humans TETA reveals no effects on reproduction. TETA is a severe irritant to skin and eyes. TETA induces skin sensitisation in guinea pigs, mice and man.

The highest aquatic local PEC during processing as an intermediate was estimated to be 4.5 µg/l.

The estimated human exposure at the workplace is estimated at < 0.143 resp. < 0.0143 mg/kg bw. Data on consumer exposure are not available.

NATURE OF FURTHER WORK RECOMMENDED

Appropriate classification and labelling are recommended.

FULL SIDS SUMMARY

CAS-NO.: 112-24-3			PROTOCOL	RESULTS
PHYSICAL CHEMICAL				
2.1	Melting-Point		NA	12 °C
2.2	Boiling-Point		NA	ca. 280°C (at kPa)
2.3	Density		NA	ca. 980 kg/m^3
2.4	Vapour Pressure		NA	1.3 Pa at 20°C
2.5	Partition Coefficient (Log Pow)		(calc.)	- 1.4
2.6 A	Water solubility		NA	completely miscible
B	pH		NA	10.7. at 10 g/l
	pKa		20 °C	pKa1 = 3.32 pKa2 = 6.67 pKa3 =9.2 pKa4 = 9.92
2.12	Oxidation : Reduction potential		/	mV
ENVIRONMENTAL FATE / BIODEGRADATION				
3.1.1	Photodegradation		calc. (Atkinson)	In air T1/2 = 1.7 hour
3.1.2	Stability in water		NA	no hydrolysis
3.2	Monitoring data			In air = /mg/m^3 In surface water= /µg/l In soil / sediment=/µg/g In biota= / µg/g
3.3	Transport and Distribution		calculated (fugacity level 1 type)	In air / % In water / % In sediment / % In soil / % In biota / %
3.5	Biodegradation		OECD 301 D	not readily biodegradable
			OECD 302 B	not inherently biodegradable

CAS-NO.:112-24-3		SPECIES	PROTOCOL	RESULTS
ECOTOXICOLOGY				
4.1	acute/prolonged toxicity to fish	Poecilia reticulata	84/449/EEC, C.1	LC_{50} (96 hr) =570mg/l
4.2	acute/prolonged toxicity to aquatic invertebrates (daphnia)	Daphnia magna	84/449/EEC, C.2	EC_{50} (24hr) =31.1mg/l
4.3	toxicity to aquatic plants e. g. algae	Scenedesmus subspicatus	DIN 38412 part 9	EC_{50} (72hr) =2.5mg/l EC_{10} (72hr) =0.67mg/l
4.4	toxicity to microorganisms	Pseudomonas fluorescens	DEV, L 8	EC_0 (24 hr) =500mg/l
4.5.2	chronic toxicity to aquatic invertebrates (daphnia)	Daphnia magna	OECD 202 part 2	NOEC (21d) =1mg/l
(4.6.3)	toxicity to other non mammalian terrestrial species (including birds)	Agelaius phoeniceus	NA	LD_{50} (18hr) => 101mg/kg
TOXICOLOGY				
5.1.1	acute oral toxicity	rat mouse rabbit	NA NA NA	LD_{50} =2500 mg/kg LD_{50} =1600 mg/kg LD_{50} =5500 mg/kg
5.1.2	acute inhalation toxicity			LC_{50} =mg/m^3
5.1.3	acute dermal toxicity	rabbit	NA	LD_{50} =550 mg/kg
5.4	repeated dose toxicity	mouse	NA	NOAEL =92mg/kg bw
5.5	genetic toxicity in vitro			
	bacterial test (gen mutation)	S. typhimurium	Ames test	positive (with and witout metabolic activation)
	non-bacterial in vitro test (chromosomal abberations)	CHO cells		positive (with and witout metabolic activation)
5.6	genetic toxicity in vivo	mouse	Micronucleus assay	negative
5.8	toxicity to reproduction			NOEL =mg/kg (general toxicity) NOEL =mg/Kg (rep. tox. parental) NOEL =mg/Kg (rep. tox. F1)
5.9	developmental toxicity / teratogenicity			NOEL =750mg/kg (general toxicity) NOEL =750mg/Kg (pregnancy/litter) NOEL =750mg/Kg (foetal data)
5.11	experience with human exposure			

SIDS Initial Assessment Report

1.Identity

Name: Triethylenetetramine (TETA)

CAS Nr.: 112-24-3

Empirical Formula: $C_6H_{18}N_4$

Structural Formula: $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}NH_2$

Purity of industrial product: 60 - 70 %

Major impurities:

N,N'-Bis-(2-aminoethyl)piperazine	11 - 13 %
N-[1-(2-Piperazin-1-yl-ethyl)]-ethane-1,2-diamine	10 - 13 %
Tris-(2-aminoethyl)-amine	4 - 6 %
Diethylenetriamine	<= 3 %
Water	<=0.5 %

2. Exposure

2.1 General discussion

Triethylenetetramine is produced by the reaction of aqueous ammonia with 1,2-dichloroethane. This process yields the entire family of ethyleneamines: ethylenediamine, piperazine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, pentaethylenehexamine and aminoethylpiperazine. These polyamines are produced as their hydrochloride salts, and must be neutralized, typically with aqueous caustic soda, to obtain the free amines. The by-product salt produced in the neutralisation step is separated and the individual products are isolated by fractional distillation (8).

TETA can be used as an intermediate in a number of production processes (10):

- The reaction with polyisobutenylsuccinic anhydride yields the corresponding polybutenylsuccinimides, which are ashless, dispersant-detergent additives for motor oil.
- Polyamide-epichlorohydrin resins are produced by the reaction of epichlorohydrin with a polyamide, such as those formed by polymerisation of adipic acid and TETA. These are used in the paper industry as wet-strength additives for liner board, toweling, tissue and sanitary applications.
- The ethoxylated products of TETA are curing agents for epoxy resins. The largest application is surface coatings (35%).
- Imidazolines from the condensation of TETA with two moles of fatty acid are cationic surfactants used as fabric softeners, asphalt emulsifiers, oil field corrosion inhibitors, ore flotation agents and epoxy curing agents.
- Reactive polyamides from the polymerisation of dimer acids with TETA are mostly used as curing agents for epoxy surface coatings.

In 1989 - 1991, 1200 - 1500 t/a were produced in Germany. Production capacities as of 1990 for other countries are available as well (8):

Netherlands	ca. 6000 t/a	(2 sites)
USA	> 11000 t/a	(3 sites)
Japan	ca. 1800 t/a	(1 site)

According to the German producer, ca. 40 to 50% are sold in Germany (> 10 clients) and ca. 40 - 50 % are exported; the rest is further processed by the same producer. Import volumes are estimated by the producer at ca. 1500 t/a. The total consumption in Germany amounts to ca. 2200 t/a.

In Germany, triethylenetetramine (TETA) is mainly used as

- intermediate for curing agents for epoxy resins (ca.1600 t/a)
- direct curing agent for epoxy resins (ca. 160 t/a)
- intermediary for auxiliary agents used in the paper industry, the textile industry and in glues (ca. 330 t/a)
- intermediate for asphalt emulsifiers (ca. 110 t/a)

Ca. 100 t/a are used by the producer as an intermediate. No information is available on the processing at other chemical manufacturers.

In Sweden, the use pattern of TETA is similar to the use pattern described for Germany:

- intermediate for transport, fertilizer and plastics industry (200 - 533 t/a)
- adhesive, binding agent (4 - 6 t/a)
- hardener for plastic (1 - 4 t/a)
- others (max 5 t/a)

The use pattern for other countries is not available.

2.2 Environmental exposure

2.2.1 General/Environmental fate

TETA is completely miscible with water forming an alkaline solution (pH 10 at 10 g/l). The technical product has a vapour pressure of ca. 1 Pa at 20 °C. The calculated Log Pow (unprotonated form) amounts to ca. -1.4 and indicates a low potential for bioaccumulation. There are no measured Koc-values available. For ethylenediamine (CAS Nr. 107-15-3) and diethylenetriamine (CAS Nr. 111-40-0), Koc-values of 4766 and 19111 were measured respectively (1). The high adsorption is most likely due to electrostatic interaction. A comparable Koc can be expected for TETA, which would suggest a high potential for geoaccumulation.

Based on the physical-chemical properties the target compartment of TETA in the environment is the hydrosphere (the estimation of the distribution with a Fugacity model is not opportune due to the protophile behaviour of TETA).

TETA is not readily biodegradable (0% after 20 days, OECD GL 301 D; same result with adapted inoculum). Also, in a test on inherent biodegradability with industrial sludge, TETA was not degraded (0 % DOC removal after 28 days, OECD GL 302 B). TETA has therefore to be regarded as **non biodegradable.** Adsorption onto sewage sludge was not observed.

In a test on hydrolysis, TETA was not found to have undergone hydrolysis after 36 days.

Direct photolysis of TETA in the hydrosphere is not to be expected (molar extinction coefficient < 10 l / (mol·cm) at > 240 nm). The half-life due to photooxidative degradation by OH-radicals in the atmosphere is estimated to be 1.7 hours. As TETA does have a low tendency to pass from water to air, this does not represent a significant removal process from the environment.

Based upon the physical-chemical and biodegradation properties of TETA, no elimination in waste water treatment plants is assumed.

2.2.2 Exposure assessment

a) Local concentrations

Considering the above described use pattern, point releases are to be expected during production and processing.

production

According to the German producer, no continuous releases occur during the production process to waste water. During cleaning operations of the production facility and the distillation column, the releases are estimated by the German producer at ca. 1 g/t related to the production capacity (8). For a production capacity of 5000 t/a (worst case assumption) a release of 5000 g TETA during one day (assuming one cleaning operation per year) can be estimated. Assuming no elimination in the WWTP, 5000 g are released into a river with a flow of 60 m^3/s, according to the generic release scenario for production in (3). A **PEC_{local} of 1 µg/l** is calculated.

processing

Many processes involving TETA as intermediate with different release rates are to be expected.
Specific data are available only from one German producer, using ca. 100 t TETA per year for processing with fatty acids: a maximum of 2.4 kg/a are released to the waste water (8).

For a generic estimation, the following worst case situation according to the release scenario for intermediates described in (3) is used.
For a processing site using 1000 t/a of TETA, a release factor of 0.7 % is assumed. Considering no elimination in the WWTP, 7 t/a are released into a river with a flow of 60 m^3/s. Assuming release over 300 days per year, a concentration of **PEC_{local} = 4.5 µg/l** is calculated.

b. Regional concentrations

Diffuse release into the environment would occur through the direct use of TETA as a curing agent. Also, the curing agents produced from TETA contain residual concentrations of TETA (approx. 7.9%).
The final extent of conversion of TETA during curing reactions is not known. On the other hand, the conversion of diethylenetriamine was determined to be 60 to 80 % (2) (related to the total NH-functions). As TETA presents 6 NH-functions, a molecular conversion rate of > 90% can be assumed.

About 160 t/a of TETA are used directly as curing agent. With a conversion factor of 90%, ca. 16 t are available as free molecules in the resins. On the worst case assumption , that 10% are released through migration from the matrix (3), a maximum of 1.6 t/a are released into the environment through this path.

About 1600 t/a are processed to yield curing agents containing an average of 7.9% free TETA. For a rough estimate, it is assumed that TETA reacts with the same amount of chemicals so that 3200 t of curing agents with ca. 250 t of free TETA result. Of these, max. 10% (see above) remain unreacted in the curing process and 10 % of these may be released through migration, i.e. a maximum of 2.5 t/a.

For the calculation of the regional PEC the use of a fugacity model is not opportune due to the ionic nature of TETA. The regional concentration can be estimated in a first approach with the following formula (9):

$$PEC_{regional} = \frac{EMIS}{FLOW + V \cdot k}$$

with: EMIS: emission into surface water = 1.6 + 2.5 = 4.1 t/a
FLOW: flow through the water compartment
V: Volume of water compartment
k: first order biodegradation rate constant

The default values described in (3) will be used for the calculation:
- a small but densely populated area is considered: 200x200 km with 20 million inhabitants;
- with an area fraction of water of 0.02 and a mixing depth of 3 m, $V = 2.4 \cdot 10^9$ m^3
- with an average residence time of the water of 40 days, $FLOW = 6 \cdot 10^7$ m^3/d
- TETA being non-biodegradable, k = 0

=> $\mathbf{PEC_{regional} = 0.18\ \mu g/l}$

2.3 Consumer exposure

Where epoxy resins are cured in do-it-yourself applications (e.g. in coatings, adhesives, and epoxy-fiber composites), consumers may come into contact with TETA or TETA-derived curing agents, either when mixing the ingredients, or when grinding and polishing the solidified product whereby unreacted TETA may be set free.

2.4 Occupational exposure

The production unit simultaneously produces ethylenediamine, diethylenetriamine, triethylenetetramine and other substances from ammonia and 1,2-dichloroethane.

To date, exposure to triethylenetretamine (TETA) has not been measured directly. Instead, exposure is estimated on the basis of measurements of ethylenediamine (according to TRGS 402) - the end product with the lowest boiling point.

The MAK-value of 25 mg/m^3 for ethylenediamine is consistently met. All measurements indicate that exposure is below 1 mg/m^3.

Substance	Boiling Point	Vapour Pressure
Ethylenediamine	116.5 °C	12.1 hPa
TETA	approx. 280 °C	< 0.1 hPa

Due to ethylenediamine's significantly lower boiling point and its greater vapour pressure (by a factor of 100) it can be concluded with certainty that the concentration of TETA in the air during synthesis and processing does not exceed 0.1 mg/m^3.

Exposure is, therefore, clearly below the actual occupational exposure limit of 6 mg/m^3 in Sweden.

3. Toxicity

3.1 Human Toxicity

a) Acute Toxicity

Triethylene tetramine is of low acute toxicity on oral administration (LD_{50} rat > 2000 mg/lkg bw) and moderate toxicity on dermal application (LD_{50} rabbit 550-805 mg/kg bw). Exposition to saturated vapour was tolerated without impairment whereas the exposition to aerosol leads to reversible irritations of the mucous membranes in the respiratory tract. According to EC Directive 67/584/EEC triethylene tetramine is labelled as harmful in contact with skin (R 21).

Conclusion:
Moderate acute toxicity
Priority setting: low priority or concern

b) Repeated Dose Toxicity

In a subacute study (rat, oral, up to 2980 mg/kg bw) retarded body weight gain and elevated liver and kidney weights were observed in the highest dose groups. From this study, a NOAEL of 500 mg/kg was derived.

In a subacute study, undiluted test substance was rubbed into the skin of pregnant and non-pregnant guinea pigs (4 mg/guinea pig and day = ca. 9 mg/kg bw) daily for 55 days. In the course of the experiment the death of test animals (2/9) as well as of the control animals (6/11) occurred (11). In another study, dermal application to pregnant and non-pregnant guinea pigs (4 mg/animal = ca. 9 mg/kg bw) daily for the first 10 days and every second day for next 45 days resulted in reduced weight gain, and from the 5th day of treatment in inflammatory alterations at the application site with subsequent erosions. In the course of the experiment 7/11 pregnant and 7/11 non-pregnant animals died (12). It is unclear whether the death of the animals is due to the strong irritant and/or the skin sensitization potential of the test substance.

In an additional study F344 rats and B6C3F1 mice received triethylenetetramine dihydrochloride in the drinking water at concentrations of 0, 120, 600, 3000 ppm (target concentration) for up to 92 days. Each dose group were fed either cereal based (NIH-31) or purified (AIN-76A) diet both containing nutritionally adequate levels of copper. An additional control group of rats and mice received a Cu-deficient AIN-76A diet. Sign of triethylenetetramine dihydrochloride toxicity were noted only in B6C3F1 mice fed AIN-76A diet given 3000 ppm triethylenetetramine dihydrochloride. These toxic signs included inflamation of the lung interstitium, hemapoetic cell proliferation of the spleen, liver periportal fatty infiltration, kidney weight reduction, reduced renal cytoplasmatic vacuolization and body weight gain reduction. From this study a NOAEL of 600 ppm for mice was derived. According to the authors, the signs observed in F344 rats appear to be related to copper deficiency (13).

Lifelong dermal application to mice (1.2 mg/mouse and application) caused no skin tumours or any tumours.

In a former inhalation study with rats, mice, guinea pig and rabbit (aerosol: 0.4 ml in 0.5 ml ethanol in a 400 l chamber, 10 d), no irritations or other toxic effects were observed.

Conclusion:
Signs of impairment only in mice following subchronic oral dosing of 3000 ppm triethylenetetramine dihydrochloride. NOAEL: 600 ppm [92 (male), 99 (female) mg/kg bw].
Priority setting low priority or concern

c) Reproductive/Developmental Toxicity

In rabbits, triethylene tetramine does not cause embryotoxic and teratogenic effects, even at maternally toxic dose levels (4).
In rats, there are several studies concerning developmental toxicity. The oral treatment of rats with 75, 375 and 750 mg/kg resulted in no effects on dams and fetuses, except slight increased fetal body weight (5). After oral treatment of rats with 830 or 1670 mg/kg bw only in the highest dose group increased fetal abnormalities in 27/44 fetus (69,2 %) were recorded, when simultanously the copper content of the feed was reduced. Copper-supplementation in the feed reduced significant the fetal abnormalities of the highest dose group to 3/51 (6,5 % fetus. These findings suggest that the developmental toxicity is produced as a secondary consequence of the chelating properties of triethylene tetramine (6).
In chapter 3.1.b) 2 studies on pregnant guinea pigs dermally treated with 4 mg/animal = ca. 9 mg/kg bw daily for 55 days or daily for 10 days and every second day for the next 45 days, respectively, were described (11, 12). Beside the clear mortality rate and the local effects, necrotic changes of the placenta and miscarriage or mortification of the fetuses and stillbirth of malformed fetuses were observed. Due to the clear maternal toxicity and due to the lack of dose-response relationship the reported studies are not suitable to evaluate developmental toxicity.

There are no data on effects on fertility with triethylene tetramine .In the subchronic toxicity studies with mice and rats, which were described in chapter 3.1.b, the reproductive organs are examined. In mice, there were no treatment related effects on the reproductive organs. According to the authors the only finding which may be attributable to trien-2HCl occured in AIN-76A-fed females rats. There was a significant dose-related trend toward an increased prevalence of uterine dilatation (13). There are no changes of the vagina and the ovaries. Therefore dilatation of uterus in isolation cannot be regarded as hormonal effects. Thus, this finding is not suitable to evaluate any reproductive toxicity. In addition, oral treatment of rats with the analogue diethylene triamine caused no adverse effects respective mating index, fertility index and number of live and dead pups.

Triethylene tetramine is used in the therapy of Wilsons' disease. While taking 400 to 800 mg triethylene tetramine 3 times a day for about 120 months, there have been six pregnancies in four female patients. There were no miscarriages and no fetal abnormalities. All six children developed normally (7).

Conclusion:
From experiences with humans (substance given as a drug) there is no reason to assume that the substance reveals effects on reproduction.

Priority setting: low priority or concern

d) Genetic Toxicity

The results of the genetic toxicity testing are not uniform. In vitro, triethylene tetramine has clear genotoxic activity in the Ames-test and in mammalian cytogenetic tests. Whereas in vivo, triethylene tetramine is not clastogenic in the mouse micronucleus test following intraperitonal injections of 130 to 600 mg/kg bw. The study was conducted in accordance with GLP standards. In addition, there is a further micronucleus test using oral application (14) which yielded a negative result as well. In this study, mice received once 1500, 3000 and 6000 mg/kg bw. These doses are within the range of and/or greater than the LD50 value for mice, which is cited in the basic data set: LD50(mice) = 1600 mg/kg bw (15). The test design and test performance was carried out according to W. Schmid and coworkers who developed the test (see references).
Following 1500 and 3000 mg/kg bw the percentage of erythrocytes containing micronuclei corresponds with the percentage of those in the concurrent solvent control. Following 6000 mg/kg bw a decrease in erythrocytes containing micronuclei was noted and was thus lower than those in the concurrent solvent control.
Triethylene tetramine revealed no mutagenic activity in the SLRL test in Drosophila melanogaster.

Conclusion:
As triethylene tetramine revealed no mutagenic activity in relevant in-vivo tests there is no reason to assume genotoxicity.
Priority setting: low priority or concern

e) Sensitizatio

The sensitization potency of triethylene tetramine was investigated in the Guinea Pig Maximization Test (GPMT) and in the Mouse Ear Swelling Test (MEST).
One of the GPMTs (16) used triethylene tetramine as a commercial product (no further information on purity of the substance). The method used was in accordance with the original description of the GPMT by Magnusson and Kligman (20, 21). Control animals received vehicle only. Induction concentration was 0.5 % in water and challenge concentration was 2 %. 12/15 animals (80 %) showed positive reactions 24 hours after removal af the patch. In the second GPM test, carried out according to OECD Guideline 406, purified TETA (purity: 99.5 %) was used and the applied concentrations were for induction 0.5 % and for challenge 2 % as well. As positive control served dinitrochlorobenzene. 9/10 animals (90 %) showed positive reactions (17). As additional test, the MEST was performed with 10 mice (17). The concentration of the purified TETA (purity: 99.5%) for the induction procedure was 10 % and the challenge concentration was 2.5 %. Oxazolone served as positive control. In 4/10 mice positive reactions were seen.
Cross reactions between triethylene tetramine, ethylenediamine and diethylenetriamine were also observed in guinea pigs (18).
Numerous reports concern the sensitizing potential of triethylene tetramine in humans (18).
In Poland, 20 - 51.2 % out of 20 - 447 examined workers exposed to epoxy resins reacted positive to triethylene tetramine (19). At another factory dermatitis was observed in 126 out of 422 workers. Skin tests were carried out on 99 patients. A positive reaction was observed in 55.1 % of these cases (18). In an examination of 20 workers exposed to casting resins and triethylene tetramine 5 showed positive reaction to triethylene tetramine whereas in another group of 23 epoxy resin-workers, suffering from dermatitis, none

reacted positive on a patch test with triethylene tetramine (18). In a control group of 112 persons 2 persons (1.5 %) gave positive patch test results (18).

Cross reactions between triethylene tetramine, diethylenetriamine and ethylenediamine were also reported (18).

Conclusion:
Triethylene tetramine induces skin sensitization in guinea pigs, mice and man. According to EC Directive 67/584/EEC triethelyene tetramine is labelled: R 43 = may cause sensitization by skin contact.

3.2 Ecotoxicity

3.2.1 Aquatic organisms

a) Toxicity to fish

Poecilia reticulata	96h-LC_{50}	570 mg/l

Other test results with *Leuciscus idus* and *Pimephales promelas*, which could not be validated, are in the same order of magnitude.

b) Toxicity to invertebrates

Daphnia magna	48h-EC_{50}	31.1 - 33.9 mg/l
(several tests)		
Effect: immobilisation	21d-EC_{50}	> 3.2 - < 10 mg/l
	21d-NOEC	1 mg/l

(immobilisation of parental organisms was the most sensitive effect parameter)

Furthermore, concentrations of 293 - 7313 mg/l had no teratogenic effects on sea-urchin (*Paracentrotus lividus*) eggs. The larvae were most sensitive and showed delay of development at 293 mg/l

c) Toxicity to algae

Scenedesmus subspicatus	72h-E_BC_{50}	2.5 mg/l
	72h-E_BC_{10}	0.67 mg/l
	72h-$E_\mu C_{50}$	>= 100 mg/l
	72h-$E_\mu C_{10}$	0.95 mg/l

Effect: growth inhibition (B = biomass; μ = growth rate)

Due to the intensive growth of the algae the pH in the control and in the concentrations up to 1 mg/l increased within 72 h to 10.2 - 10.3.

Selenastrum capricornutum	72h-EC_{50}	20 mg/l
Effect: growth inhibition (biomass)	72h-NOEC	< 2.5 mg/l

Selenastrum capricornutum	96h-EC_{50}	3,7 mg/l

Effect: growth inhibition (biomass)

A further test with *Chlorella pyrenoidosa* was considered to be non valid.

d) Toxicity to microorganisms

Pseudomonas fluorescens	24h-EC_0	500 mg/l

Effect: growth inhibition (biomass)

e) Derivation of PNEC

Algae are clearly the most sensitive species to TETA. According to the EU-Technical Guidance Document (3), the value of the safety factor is **F = 50** (long term tests have been performed for two trophic levels and with the organisms which were the most sensitive in the acute tests).
With the lowest aquatic effect concentration of 0.67 mg/l:

$$\textbf{PNEC} = \frac{670}{50} = \textbf{13.4 µg/l}$$

3.2.2 Terrestrial organisms

Acute oral toxicity to the redwinged blackbird (*Agelaius phoeniceus*) was determined to be 18h-LD_{50} > 101 mg/kg bw.

4. Initial Assessment

4.1 Human toxicity

4.1.1 Identification of critical toxic effects

Triethylene tetramine is a severe irritant to skin and eyes and induces skin sensitizations. Triethylene tetramine is of moderate acute toxicity: LD50(oral, rat) > 2000 mg/kg bw, LD50(dermal, rabbit) = 550 - 805 mg/kg bw. Acute exposure to saturated vapour via inhalation was tolerated without impairment.
Following repeated oral dosing via drinking water only in mice but not in rats at concentration of 3000 ppm there were signs of impairment. The NOAEL is 600 ppm [92 mg/kg bw (oral, 90 days)]. Lifelong dermal application to mice (1.2 mg/mouse) did not result in tumour formation.

There are differing results of the genetic toxicity for triethylene tetramine. The positive results of the in vitro tests may be the result of a direct genetic action as well as a result of an interference with essential metal ions. Due to this uncertainty of the in vitro tests, the genetic toxicity of triethylene tetramine has to be assessed on the basis of in vivo tests. The in vivo micronucleus tests (i.p. and oral) and the SLRL test showed negative results.

There are no data on reproductive toxicity (fertility assessment). The analogue diethylene triamine had no effects on reproduction. Triethylene tetramine shows developmental toxicity in animal studies if the chelating property of the substance is effective. The NOEL is 830 mg/kg bw (oral).
Experience with female patients suffering from Wilson's disease demonstrated that no miscarriages and no fetal abnormalities occur during treatment with triethylene tetramine.

4.1.2 Comparison of Exposure and Critical effects

Workplace

There are no measurements of the concentration of triethylene tetramine in the air at the workplace. To estimate the exposition at the workplace adequately the results of the concentration measurements of the product with the lowest boiling point has to be applied: ethylene diamine (see chapter 4.2). All results of these measurements are below 1 mg/m^3 (TLV: 25 mg/m^3). Because of the higher boiling point and the lower vapour pressure of triethylene tetramine it can be assumed that the concentration in the air at the workplace is below or equal than 0.1 mg/m^3.

The EHE (Estimated Human Exposure) can be calculated according to the following equitation:

$$EHE = \frac{\text{respiratory rate (10 m}^3\text{) * exposition (mg/m}^3\text{)}}{\text{body weight (70 kg)}}$$

exposition < 1 mg/m^3	EHE < 0.143 mg/kg bw
exposition < 0.1 mg/m^3	EHE < 0.0143 mg/kg bw

Thus the estimated human exposure is far below the NOAEL described in animal experiments of 92 mg/kg bw for subacute toxicity and a NOAEL of 850 mg/kg bw for teratogenicity. The safety margin based on the lowest NOAEL is between:

$$\frac{92 \text{ mg/kg bw}}{< 0.143 \text{ mg/kg bw}} > 643.4 \quad \text{and} \quad \frac{92 \text{ mg/kg bw}}{< 0.0143 \text{ mg/kg bw}} > 6434$$

and thus does not suggest a particular risk.

Isolated cases of exposure through skin contact cannot be ruled out. However, the risk is to be assumed very low.

Consumer area

Data on consumer exposure are not available. However, it cannot be excluded that products containing triethylene tetramine give off small amounts of the substance. Due to the low toxicity in animal experiments it can be assumed that the probability of acute poisoning is very low. In addition, the application of triethylene tetramine as drug excluded high toxicity to humans. Also multiple administration of TETA to animals did cause neither significant systemic effects nor the formation of tumours.

Exposure via the environment

Data are not available on exposure of the general population. Exposure of the population via the hydrosphere is considered to be minimal, even assuming the concentration in drinking water to be equal to the regional predicted concentration in surface waters (0.18 µg/l). With 2 l drinking water/person/day, the daily dose would be 0.005 µg/kg bw/day. Compared to the exposure at the working place the exposure through the environment is negligible.

4.2 Assessment of environmental hazards

In the following table, the PEC/PNEC ratios for the different exposure scenarios are presented:

Scenario	$PEC_{local} + PEC_{regional}$ [µg/l]	PEC/PNEC
production (site)	1 + 0.18	0.08
processing (site)	4.5 + 0.18	0.35

A PEC/PNEC < 1 in all scenarios, a low potential risk to the aquatic compartment is at present to be expected.

A significant exposure to the **terrestrial** compartment could not be identified. Further work is presently not necessary for an assessment of risks to this compartment.

5. Conclusions and Recommendations

An environmental hazard assessment of triethylenetetramine was possible with the available data and showed that the compound was presently of low concern to the environment. No further work is recommended.

On the basis of the known facts and properties, triethylene tetramine may represent a hazard for human health. The chemical is a severe irritant to skin and eyes and induces skin sensitization. The substance is classified and labelled accordingly within the EU: R 34 = causes burns; R 43 = may cause sensitization by skin contact.

From experience with humans (substance given as a drug) there is no reason to assume that the substance reveals further toxic effects. Besides appropriate classification and labelling no further work is recommended.

References

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I U C L I D Data Set

Existing Chemical	ID: 112-24-3
CAS No.	112-24-3
EINECS Name	trientine
EC No.	203-950-6
TSCA Name	1,2-Ethanediamine, N,N'-bis(2-aminoethyl)-
Molecular Formula	C6H18N4
Producer Related Part	
Company:	Bayer AG
Creation date:	15-MAR-1993
Substance Related Part	
Company:	Bayer AG
Creation date:	15-MAR-1993
Memo:	AKTUELL OECD-SIDS
Printing date:	24-JUL-2002
Revision date:	17-MAY-1993
Date of last Update:	**27-JAN-1998**
Number of Pages:	**56**
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags:without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: cooperating company
Name: Bayer AG
Town: 51368 Leverkusen 1
Country: Germany

10-MAY-1994

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

1.1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: 60 - 70 % w/w

Remark: technical mixture

1.1.2 Spectra

1.2 Synonyms and Tradenames

1,2-Bis-(2-aminoethylamino)-ethan

1,2-Di-(aminoethylamino)-ethan

1,4,7,10-Tetraazadecan

1,8-Diamino-3,6-diaza-octan

2,2'-(1.2-Ethylenbis-amino-)bis-ethanamin

3,6-Diazaoctan-1,8-diamin

N,N'-Bis-(2-aminoethyl)-1,2-ethanediamine

N,N'-Bis-(2-aminoethyl)-ethylendiamin

N,N'-Di-(2-aminoethyl)-1.2-ethandiamin

N,N'-Di-(2-aminoethyl)-1.2-ethylendiamin

TETA

Tetramin

Trien

Triethylentetramin

1.3 Impurities

EINECS-Name: N,N¦-Bis-(2-aminoethyl)piperazin
Contents: 11 - 13 % w/w

EINECS-Name: N-(Piperazin-1-ethyl)-ethan-1,2-diamin
Contents: 10 - 13 % w/w

EINECS-Name: Tris-(2-aminoethyl)-amin
Contents: 4 - 6 % w/w

CAS-No: 111-40-0
EC-No: 203-865-4
EINECS-Name: 2,2'-iminodi(ethylamine)
Contents: <= 3 - % w/w

EINECS-Name: Water
Contents: <= ,5 - % w/w

1.4 Additives

1.5 Total Quantity

Quantity: 1000 - 5000 tonnes produced

Remark: in 1989-1991 (BRD)
29-NOV-1994 (1)

Remark: Netherland: ca. 6000 t/a
USA: ca. 1100 t/a
Japan: ca. 1800 t/a
29-NOV-1994 (1)

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: (C) corrosive
R-Phrases: (21) Harmful in contact with skin
(34) Causes burns
(43) May cause sensitization by skin contact

S-Phrases: (26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(36/37/39) Wear suitable protective clothing, gloves and eye/face protection

Country: Germany

1.6.2 Classification

Classified: as in Directive 67/548/EEC
Class of danger: corrosive
R-Phrases: (21) Harmful in contact with skin
(34) Causes burns
(43) May cause sensitization by skin contact

Country: Germany

1.6.3 Packaging

1.7 Use Pattern

Type: industrial

Category: Chemical industry: used in synthesis

Remark: Intermediate for - hardeners for epoxy resins > 80 %
- agents used in glues, paper industry and textile industry > 15 %

Type: use

Remark: TETA can also be used directly as hardener in epoxy resins (approx. 8 % of total production)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: Bayer AG
Labelled by: other: Bayer AG
Class of danger: 2 (water polluting)
Country: Germany

1.8.4 Major Accident Hazards

Substance listed: no

1.8.5 Air Pollution

Classified by: TA-Luft (DE)
Labelled by: TA-Luft (DE)
Number: 3.1.7 (organic substances)
Class of danger: III

1.8.6 Listings e.g. Chemical Inventories

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

Country: Germany

Remark: air: 6 kg/a at one processing site;
no release into the atmosphere at all other production and processing sites
water: 4,4 kg/a at all production and processing sites
waste treatment:
water: biological waste water treatment plant
air: incineration
There is no solid waste from production and processing.
Possible emissionof very small amounts through migration out of epoxy resins (residual concentration of TETA in hardeners: at max. approx. 7.9 %)

29-NOV-1994 (1)

1.11 Additional Remarks

1.12 Last Literature Search

1.13 Reviews

2.1 Melting Point

Value: = 12 degree C
(2)

Remark: Solidification point: approx. -35 degree C (technical product)
26-APR-1994 (3)

2.2 Boiling Point

Value: 266 - 267 degree C
(4)

Value: = 277,5 degree C
Decomposition: yes

Remark: 93 - 96 % purity
(5)

Value: = 277,9 degree C
(6)

Value: = 278 degree C
Decomposition: yes
(7)

Value: ca. 280 degree C

Remark: technical product
26-APR-1994 (3)

2.3 Density

Type: density
Value: = ,9739 g/cm³ at 20 degree C
(8)

Type: density
Value: ca. ,98 g/cm³ at 20 degree C

Remark: technical product
26-APR-1994 (3)

Type: density
Value: = ,9818 g/cm³ at 20 degree C
(5)

Type: density
Value: = ,9839 g/cm³ at 20 degree C
(6)

Type: density
Value: = ,977 g/cm³ at 25 degree C
(9)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = ,013 hPa at 20 degree C

(6)

Value: < ,1 hPa at 20 degree C

Remark: technical product
26-APR-1994 (3)

2.5 Partition Coefficient

log Pow: = -1,66

Remark: calculated (no further information)

(10)

log Pow: = -1,41

Remark: calculated (no further information)

(11)

log Pow: = -1,4

Method: other (calculated): Leo, Hansch: A. Leo, CLOGP-3.63 (1991) Daylight, Chemical Information Systems, Inc. Irvine, CA, USA

Remark: undissociated form

(12)

2.6.1 Solubility in different media

Remark: completely miscible

(7)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 118 degree C

(13)

Value: = 125 degree C

(6)

Value: ca. 129 degree C
Method: other: DIN 51758
Remark: technical product
26-APR-1994 (3)

Value: = 135 degree C

(5)

2.8 Auto Flammability

2.9 Flammability

Remark: LFL: 1.0 % v/v (180 deg. C)
UFL: 3.6 % v/v (180 deg. C)
Source: DOW Europe S.A., Switzerland
24-MAY-1994 (14)

2.10 Explosive Properties

2.11 Oxidizing Properties

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Remark: Henry-constant : 6.7x10E-11 Pa.m3/mol (at 25 degree C, calculated)
29-NOV-1994 (1)

Remark: Ignition-temperature : 335 Grad C (DIN 51794)
26-APR-1994 (3)

Remark: Ignition-temperature : 338 Grad C
(5)

Remark: UV-Spectrum in water : epsilon < 10 e/molxcm at lamda > 240 nm
(15)

3.1.1 Photodegradation

Type: other: photochemical degradation in atmosphere
INDIRECT PHOTOLYSIS
Sensitizer: OH
Rate constant: ,000000000225 cm³/(molecule * sec)
Degradation: 50 % after 1,7 hour(s)

Method: other (calculated): according to Atkinson

29-NOV-1994 (16) (1)

3.1.2 Stability in Water

Type: abiotic

Year: 1985
Test substance: other TS: technical grade (purity > 70 %)

Remark: No hydrolysis in water during the experiment of 36 days. Tested concentrations: 1, 100 and 200 mg/l

(17)

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Remark: Based on the physico-chemical properties transport from water to air is not to be expected (Henry-constant: H = 6.7 x 10E-11 Pa.m3/mol, 25 degree C, calculated)

29-NOV-1994 (1)

3.3.2 Distribution

Remark: Based on the physical-chemical data, the preferred environmental compartment of TETA is the hydrosphere

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 100 mg/l related to DOC (Dissolved Organic Carbon)
Degradation: 0 % after 28 day(s)
Result: under test conditions no biodegradation observed

Method: OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"

Year: 1989
GLP: no data
Remark: technical product

(18)

Type: aerobic
Inoculum: predominantly domestic sewage, adapted
Concentration: related to Test substance
Degradation: 0 % after 20 day(s)
Result: under test conditions no biodegradation observed

Method: other: in accordance with OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year: 1977
GLP: no data

Remark: technical product;
Substance concentrations: 2.6, 8.5, 25.5, 85 mg/l

(18)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Remark: Bioaccumulation is not to be expected (logPow = -1,4; -1.66 calculated)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: semistatic
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: 180 -
LC50: 570 -
LC100: 1800 -

Method: Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year: 1989
GLP: yes
Test substance: other TS: Triethylenetetramine, purity: 97.5%

Remark: 48h-LC50 = 1140 mg/l
10-MAY-1994 (19)

Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
LC0: 200 -

Method: other: Bestimmung der akuten Wirkung von Stoffen auf Fische. Arbeitskreis "Fischtest" im Hauptausschuss "Detergentien" (15.10.73)
GLP: no

Remark: open system;
at 500 mg/l, all test organisms had died after 27 h;
no further information on test conditions
(18)

Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: 495 -

Remark: validation not possible
Source: DOW Europe S.A., Switzerland
26-APR-1995 (20)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: 18 -
EC50: 31,1 -
EC100: 56 -

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1989
GLP: yes
Test substance: other TS: Triethylentetramine, purity: 97.5%

Remark: static test
24h-EC50: 75 mg/l
10-MAY-1994 (21)

Species: Daphnia magna (Crustacea)
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:**
NOEC: 1 -

Method: OECD Guide-line 202

Remark: EC50: > 3.2 - < 10 (Immobilization of parental organisms); a NOEC for the inhibition of the reproduction rate could not be determined
26-APR-1995 (18)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: 22 -
EC50: 92,4 -
EC100: 354 -

Method: other: Daphnien-Schwimmunfaehigkeits-Test, UBA-Verfahrensvorschlag Mai 1984, Bestimmung der Schwimmunfaehigkeit beim Wasserfloh Daphnia magna, EC0, EC50, EC100 24h, statisches System
Year: 1989
GLP: yes

Remark: Distillate of technical product
(18)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 33,9 -

Method: other: EEC, 1989, Methods for the determination of ecotoxicity. C.2 Acute toxicitty for Daphnia (Updated Version 11/89). EEC Directive 79(831, Annex V, Part C. Brussels, Belgium (static)
Year: 1994
GLP: no data
Test substance: other TS: purity > 99 %

Remark: Arithmetic mean of 3 test results (standard deviation was 5.3 mg/l).
26-APR-1995 (22)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : 12 -

Remark: validation not possible
Source: DOW Europe S.A., Switzerland
26-APR-1995 (20)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella pyrenoidosa (Algae)
Endpoint: growth rate
Exposure period: 5 day(s)
Unit: mg/l **Analytical monitoring:**
EC100 : >= 146 -

Remark: Validity uncertain. Slow growth of the control culture.
Test condition: 25 degree C, pH 7

Species: Scenedesmus subspicatus (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: ,67 -
EC50: 2,5 -

Method: other: Scenedesmus-Zellvermehrungs-Hemmtest, DIN 38412 Teil 9, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen
Year: 1989
GLP: yes
Test substance: other TS: purity 98.04 %

Remark: Due to the high growth rate, the pH rose to 10.2 - 10.3 after 72 hours in the control and for concentrations of TETA up to 1 mg/l

(18)

Species: Scenedesmus subspicatus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: ,95 -
EC50: >= 100 -

Method: other: Scenedesmus-Zellvermehrungs-Hemmtest, DIN 38412 Teil 9, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen
Year: 1989
GLP: yes
Test substance: other TS: purity 98.04 %

Remark: Due to the high growth rate, the pH rose to 10.2 - 10.3 after 72 hours in the control and for concentrations of TETA up to 1 mg/l

(18)

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: < 2,5 -
EC50: 20 -

Method: Directive 87/302/EEC, part C, p. 89 "Algal inhibition test"
Year: 1990
GLP: yes

Test substance: other TS: Triethylenetetramine, purity 97.5%

Remark: For the enspoint ¦growth rate¦, the same results were obtained

10-MAY-1994 (24)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: 3,7 -

Method: other: EEC, 1988, Methods for the determination of ecotoxicity. Algal inhibition test. Off J. Eur. Comm. L 133 1988-0530
Year: 1994
GLP: no data
Test substance: other TS: purity > 99 %

Remark: Arithmetic mean of 5 test results (standard deviation: 1.5 mg/l). The culture medium was modified by increasing the KH2PO4 conc. from 1.6 to 160 mg/l and the NaHCO3 conc. from 50 to 100 mg/l, to improve the growth of algae and the buffer capacity of the medium.

26-APR-1995 (22)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas fluorescens (Bacteria)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC0: 500 -

Method: other: Bestimmung der biologischen Schadwirkung toxischer Abwaesser gegen Bakterien. DEV, L 8 (1968) modifiziert

Remark: technical product;
no further information on test conditions

(18)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Remark: no validated information

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Species: other avian: Agelaius Phoenicus (redwinged blackbird)
Endpoint: mortality
Unit: mg/kg bw
LD50 : > 101 -

Method: other: no data
GLP: no data
Test substance: other TS: TETA (no information about purity)

Remark: Estimated LD50 based on food consumption data over a 18 h period

29-NOV-1994 (25)

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Remark: Sea-urchin: Inhibition of development
Eggs of the species Paracentrotus lividus were incubated in sea-water 30 min after impregnation (concentration TETA: 293 - 7313 mg/l). No teratogenic effects observed.
Depending on the developmental stage there was an effect on larvae (293 mg/l), gastrula (731 mg/l), blastula (2925 mg/l), cleavage stage (7313 mg/l).
(26)

Remark: Application of 1460 mg/l TETA (alcoholic solution) to 1-2 days old larval stages and 2 days old egg-stages of the species Dysdercus koenigii F. had no acute toxic effects and no effects on the eggs as well as no sterilizing effects.
(27)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Value: = 2780 mg/kg bw

Method: other: male rats, undiluted testsubstance (no further information)
GLP: no data
Test substance: no data

29-JUL-1996 (28)

Type: LD50
Species: rat
Value: ca. 3750 mg/kg bw

Method: other: 3 animals per group; doses: 1000, 2500, 3750, 5000 mg/kg; test substance diluted in water
GLP: no data
Test substance: no data

17-OCT-1994 (29)

Type: LD50
Species: rat
Value: = 4340 mg/kg bw

Method: other: 5 animals per group, test substance diluted in water
GLP: no data
Test substance: no data

(30)

Type: LD50
Species: rat
Value: = 2500 mg/kg bw

GLP: no data
Test substance: no data

Remark: method: no data

(13)

Type: LD50
Species: rat
Value: = 4300 mg/kg bw

GLP: no data
Test substance: no data

Remark: method: no data
17-OCT-1994 (31)

Type: LD50
Species: mouse
Value: = 1600 mg/kg bw

GLP: no data
Test substance: no data

Remark: method: no data
17-OCT-1994 (31)

Type: LD50
Species: rabbit
Value: = 5500 mg/kg bw

GLP: no data
Test substance: no data

Remark: method: no data
17-OCT-1994 (31)

5.1.2 Acute Inhalation Toxicity

Type: other: see method
Species: rat

Method: other: saturated vapor at 21 degree C, 8 h exposure, 6 animals
GLP: no data
Test substance: no data

Remark: no symptoms
17-OCT-1994 (28)

Type: other: see method
Species: rat

Method: other: saturated vapor inhalation up to 8 h
GLP: no data
Test substance: no data

Remark: maximal time for no deaths 4 h
(30)

Type: other: see method
Species: other: see method

Method: other: 2 rats, 1 rabbit, 1 guinea pig, and 4 mice were exposed together to aerosol (10 ml of 40 % (v/v) ethanol solution, 400 l chamber) for 1 h
GLP: no data
Test substance: no data

Remark: effects: slight irratation of the mucous membranes and impeded respiration, effects reversible
17-OCT-1994 (29)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Value: = 550 mg/kg bw

Method: other: 4 animals per dose, undiluted test substance
GLP: no data
Test substance: no data

Remark: no further information available
17-OCT-1994 (28)

Type: LD50
Species: rabbit
Value: = 805 mg/kg bw

Method: other: occlusive application of undiluted test substance
GLP: no data
Test substance: no data

Remark: no further information available
(30)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Route of admin.: i.p.
Value: = 200 mg/kg bw

Method: 3-5 animals per group, test substance as aqueous solution
GLP: no data
Test substance: no data

Remark: impeded respiration
17-OCT-1994 (29)

Type: LD50
Species: rat
Route of admin.: i.p.
Value: = 78,4 mg/kg bw

Method: no data
GLP: no data
Test substance: no data

Remark: symptoms like hyperemia, extravasations; regressive changes in liver and kidneys; abstract
(32)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 604 mg/kg bw

Method: test substance neutralized with HCl, 10 mice per group
GLP: no data
Test substance: no data

Remark: convulsions for max. 20 min, hyperemia of inner organs in the dead animals

(33)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit

Method: other: non occlusive appl.;
a) 0.01 ml undiluted
b) 10% in water
GLP: no data
Test substance: no data

Remark: effects: a) 2 out of 2 animals with necrosis
b) no effects
no further information available
17-OCT-1994 (28)

Species: rabbit

Method: other: 20 mg applied to skin
GLP: no data
Test substance: no data

Remark: effects: necrotic foci and extravasations
no further information available, abstract

(32)

Species: rabbit

Method: other: undiluted drug applied to the skin of 5 animals; no further information available
GLP: no data
Test substance: no data

Remark: effects: erythema, edema, necrosis

(30)

Species: guinea pig

Method: other: intracutaneous injection of 0.1 ml 0.5-1% solution in water (non neutralized) or 2-3% solution in neutralized form
GLP: no data
Test substance: no data

Remark: effects: slight necrosis
no further information available

(34)

Species: rat

Method: other: a) 1000 mg/kg undiluted; b) 50 mg/kg (25% in water); application on the shaved ventral skin; exposure time: 2 h
GLP: no data
Test substance: no data

Remark: effects: strong irritations in both cases
17-OCT-1994 (29)

5.2.2 Eye Irritation

Species: rabbit

Method: other: instillation of a) 0.005 ml undiluted or b) 0.5 ml of a 40% watery solution
GLP: no data
Test substance: no data

Remark: effects: a) severe damage of the cornea b) 15% of the cornea damaged
17-OCT-1994 (28)

Species: rabbit

Method: other: 20 mg applied to the conjunctival sac
GLP: no data
Test substance: no data

Remark: effects: inflammation and lymphatic exudation
no further information available, abstract
(32)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Result: sensitizing

Method: other: 10 animals tested; induction concentration 0.5% intradermal and topical, challenge 2%
GLP: no data
Test substance: other TS: purity 99.5 %

Remark: 90% positive
(35)

Type: Guinea pig maximization test
Species: guinea pig
Result: sensitizing

Method: other: 15 animals tested; induction concentration 0.5% intradermal and topical, challenge 2% (in water)
GLP: no data
Test substance: other TS: technical grade (no specification)

Remark: 80% of guinea pigs with positive reaction
(36)

Type: Mouse ear swelling test
Species: mouse
Result: sensitizing

GLP: no data
Test substance: other TS: purity 99.5 %

Remark: 4/10 positive (significant), induction conc. 10%, challenge 2.5%.
(35)

Type: Open epicutaneous test
Species: human

Remark: 10 out of 22 workers exposed to araldite D and hardener TETA showed slight dermatosis, one worker serious allergic eczema. One of the 11 (the one with serious allergic eczema) showed allergic hypersensitivity in epicutaneous testing to TETA.
(37)

Type: Patch-Test
Species: guinea pig
Result: not sensitizing

Method: other: no data
GLP: no data
Test substance: no data

Remark: no further information available, abstract
(32)

Type: Patch-Test
Species: human

Test substance: no data

Remark: 4 out of 10 patients with dermatitis due to oil-based, amine containing drilling mud, showed allergic response to a 0.5% solution in the patch test.
(38)

Type: Patch-Test
Species: human

Remark: In 23 out of 135 (18%) workers exposed to epoxy resins, a work-related dermatosis on the hands and/or forearms had been presented during the past 3 years. In all workers patch tests were performed and in 2 positive reactions to TETA were observed (2 out of 112 without dermatosis).
(39)

Type: Patch-Test
Species: human

Remark: 422 employees of 8 factories had contact to epoxy resins and hardener TETA. In the course of 7 years there were 126 cases of dermatitis, 99 of whom were patch tested. 55.1% were positive to 1% TETA in water. The mean period between starting work and occurrence of dermatitis was 18.5 months.
(40)

Type: Patch-Test
Species: human

Remark: 1544 patients(dermatitis) without exposure to epoxy resin systems and 137 patients in occupational contact with epoxy resins were patch tested. 28 out of the 1544 patients were

positive to ethylenediamine; 12 of these were tested with TETA, 2 were positive. 400 out of the 1544 patients were also tested with TETA and re- sults were negative. Tests with 137 patients in occupational contact to resins resulted in coexistence of positive reactions to TETA and ethylenediamine and TETA and diethylenetriamine.

(41)

Type: Patch-Test
Species: human

Remark: A 58 years old woman with dermatitis due to exposure with epoxy resins showed positive reaction in the patch test to epoxy resin and TETA as well as to ethylenediamine.

(42)

Type: Patch-Test
Species: human

Remark: 12 out of 32 ethylenediamine-sensitive patients showed cross-sensitivity reaction to TETA in the patch test.

(43)

Type: Patch-Test
Species: human

Remark: 19 out of 71 patients with allergic epoxy resin dermatitis were also allergic to different hardeners. 3 of them showed positive reactions to TETA in epicutaneous testing.

(44)

Type: Patch-Test
Species: human

Remark: A shipwright''s yard worker complained a chronic dermatitis of the fingertips and palms. Beside other material he used epoxy resin SP 106. In the patch test a positive reaction to TETA was demonstrated after 48 and 96 h.

(45)

Type: Patch-Test
Species: human

Test substance: no data

Remark: 31 students and instructors at the same dental school were patch tested to contactants in dental components including TETA. None had any history of allergy. No positive allergic reactions were found.

(46)

Type: Patch-Test
Species: human

Test substance: no data

Remark: 2 out of 7 patients with airborn contact dermatitis of hands and face due to epoxy resins showed positive reactions in the patch test to TETA.

(47)

Type: Patch-Test
Species: human

Remark: 14 young female patients (12 of them were seborrhean) in occupational contact with araldite D and hardener 951 (mainly TETA) suffering from eczema were patch tested. 1 of the 14 women was positiv to 3% of the hardener in ethanol (48 h).

(48)

Type: other
Species: human

Remark: 20 workers (6 without, 8 with slight and 6 with severe dermatosis) were patch tested with technical TETA (1% in water). 5 of the 6 workers with severe dermatosis showed a positive reaction.

(34)

Type: other: see remarks
Species: human

Remark: 164 out of 328 workers from 11 factories producing electrical equipment showed slight dermatosis (21%, erytamotous itching patches) or severe eczemas (22%) caused by direct contact to araldite resin D or hardener TETA. TETA concentration in air was below analytic limits of 0.00015 mg/l.

(49) (50)

Type: other: see remarks
Species: human

Remark: 6 workers with diagnoses of occupational asthma were examined for sensitivity to epoxy resin systems and their components. In one worker asthma followed exposure to TETA fume in inhalation challenge testing. Skin sensitivity test was negative.

(51)

Type: other: see remarks
Species: human

Remark: 447 patients suffering from eczema, occupationally exposed to epoxy resins, have been tested with Epidian 5 (resin) and five concentrations of the hardener TETA. In Poland these health damages were characterized by a considerable percentage of those sensitized to TETA. The calculation of eczema incubation period and testing the allergen by several allergen concentrations demonstrated that the sensitivity to TETA was sometimes very enhanced.

(52)

5.4 Repeated Dose Toxicity

Species: rat **Sex:** male/female
Strain: other: Harlan-Wistar
Route of administration: oral feed
Exposure period: 7 days
Frequency of treatment: daily ad libitum
Post exposure period: no data
Doses: m: 0.5, 1.23, 2.98 g/kg b.w.; f: 0.47, 1.38, 2.63 g/kg b.w.
Control Group: no data specified
NOAEL: ,5

Method: other: 5 rats per dose and sex
GLP: no data

Test substance: no data

Remark: LOEL: 1.23 (m) and 1.38 (f) mg/kg b.w./day
remarks: no deaths occurred
Result: highest dose:
depression of body weight gain, decrease of relative and absolute liver weights, increase of relative kidney weights.
medium dose:
increase of relative kidney weights.

17-OCT-1994 (28)

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 90 d
Frequency of treatment: daily
Post exposure period: no
Doses: 0, 120, 600, 3000 ppm (see remarks)
Control Group: other: concurrent no treatment (diet: cereal based NIH-31, purified AIN-76A, Cu-deficient AIN-76A)
NOAEL: = 3000 ppm

Method: other: 18 rats/sex and dose group, different diets: cereal based (NIH-31) or purified (AIN-76A) diet; hematology and plasma chemistry; necropsy and histopathology; statistical analyses
Year: 1996
GLP: no data
Test substance: other TS: trientine-2HCl: purity: > 99 %

Remark: test substance consumption:
NIH-31 diet: f:14, 70, 352 mg/kg bw; m:10, 55, 276 mg/kg bw
AIN-76A diet: f:13, 60, 323 mg/kg bw; m:10, 53, 270 mg/kg bw
Result: no death occurred; pobabely attributed to dosing with trien-2HCL: females: a significant trend toward an increased prevalence of uterine dilatation; no other findings

23-JUN-1997 (53)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: dermal
Exposure period: 17 days

Frequency of treatment: once daily (3rd - 19th day of gestation)
Post exposure period: no
Doses: ca. 4 mg/rat and day
Control Group: yes

Method: other: 10 rats per group. One drop of the test substance was rubbed into the shaved skin

GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: pregnant and nonpregnant rats: reduced weight gain, progressive emaciation, apathy, lack of appetite, local inflammatory symptoms such as erythema, edema and superficial erosions. pregnant rats: increase of plasma sialic acid; increased activity of lactate dehydrogenase, aspertate aminotransferase and acid phosphatase in the serum; decreased plasma activity of alkaline phosphatase; reduced haptaglobin concentration; increased acti- vity of leucylnaphthylamidase in amniotic fluid. nonpregnant rats: decreased total plasma protein and elevated concentrations of seromucoid a. haptaglobin; in the serum increa- sed activity of lactate dehydrogenase, leucylnaphthylamidase and alkaline phosphatase; inhibited activity of aspartate and alanine aminotransferase.

(54)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: dermal
Exposure period: 17 days
Frequency of treatment: once daily
Post exposure period: no
Doses: ca 4 mg/rat and day
Control Group: yes

Method: other: 10 rats per group. No data about stage of pregnancy in pregnant rats. One drop of test substance was rubbed into the shaved skin.
GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: pregnant and nonpregnant rats:
weight loss, hyperemia of liver and kidneys, dermis and subcutaneous tissue with inflammatory infiltrates.
pregnant rats: aspartate aminotransferase activity in the liver inhibited.
nonpregnant rats: increased activity of gammaglutamyltranspeptidase in the kidney and aspartate and alanine aminotransferases in the liver.

(55)

Species: rat **Sex:** no data
Strain: no data
Route of administration: oral unspecified
Exposure period: a) 4 months b) 10 months
Frequency of treatment: a) no data b) daily
Post exposure period: no data

Doses: a) 215 or 430 mg/kg b) 0.8 or 4 mg/kg
Control Group: no data specified

Method: other: no data
GLP: no data
Test substance: no data

Remark: LOEL: a) 215 mg/kg b.w. b) 0.8 mg/kg b.w./day, 10 months no dose effect relation; abstract, no further information available.
Result: 4 months both doses:
Excitability of the central nervous system decreased. Plasma levels of hippuric acid, protein and hemaglobin were decreased. Inhibited activities of catalase and peroxidase.
10 months both doses:
Increased excitability, stimulated tactile reflexes. Antitoxic, carbohydrate and protein function of the liver disturbed. Transient inhibition of nicotinamide coenzymes and stimulation of cytochrome oxidase.

17-OCT-1994 (31)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 90 d
Frequency of treatment: daily
Post exposure period: no
Doses: 0, 120, 600, 3000 ppm (see remarks)
Control Group: other: concurrent no treatment, (diet: cereal based NIH-31, purified AIN-76 A, Cu-deficient AIN-76A)
NOAEL: = 600 ppm

Method: other: 20 mice/sex and dose group; different diets: cereal based (NIH-31) or purified (AIN-76A); hematology and plasma chemistry; necropsy, histopathology, statistical analyses
Year: 1996
GLP: no data
Test substance: other TS: trientine-2HCl; purity: > 99 %

Remark: test substance consumption:
NIH-31 diet: f:22,107, 551 mg/kg bw; m:22,107, 487 mg/kg bw
AIN-76A diet: f:19, 99, 483 mg/kg bw; m:17, 92, 443 mg/kg bw
Result: diet AIN-76A, 3000 ppm: chronic interstititial inflammation and alveolar histocytic infiltration of the lung, spleen hemapoetic cell proliferation, liver periportal fatty change, kidney weight reduction, reduced renal cytoplasmatic vacuolization, body weight gain reduction

27-JAN-1998 (53)

Species: guinea pig **Sex:** female
Strain: no data
Route of administration: dermal
Exposure period: 55 days
Frequency of treatment: once daily
Post exposure period: no
Doses: ca.4 mg/animal and day
Control Group: yes

Method: other: starting exposition in pregnant guinea pigs on day 10 of gestation. One drop of the test substance was rubbed into the shaved skin.
GLP: no data
Test substance: no data

Remark: LOEL: no data
remarks: 6 out of 10 nonpregnant and 2 out of 9 pregnant exposed guinea pigs died before end of experiment. No further information about toxic effects available.

Result: pregnant guinea pigs:
activity of gammaglutamyltranspeptidase significantly elevated in kidney and blood.
nonpregnant guinea pigs:
significantly increased activity of liver aspartate aminotransferase.

(56)

Species: guinea pig **Sex:** female
Strain: no data
Route of administration: dermal
Exposure period: once daily for 10 days, then every second day for 45 days
Post exposure period: no
Doses: ca.4 mg/animal and day
Control Group: yes

Method: other: 11 animals/group; exposure started on day 10 of gestation; one drop of the test substance was rubbed into the shaved skin
GLP: no data
Test substance: no data

Remark: LOEL: no data
Result: 7 out of 11 pregnant and 7 out of 11 nonpregnant guinea pigs died within the first 10 days. Surviving pregnant and nonpregnant animals showed weight loss with advanced emaciation; skin revealed inflammatory alterations indicated by erythema, edema and erosion. Surviving and nonsurviving ainmals showed all fatty degeneration of the liver, congestion of the kidney and brain, and brain edema. Pregnant animals showed necrotic changes in the placenta and miscarriage or mortification of fetuses.

(57)

Species: other: see remarks **Sex:** no data
Strain: no data
Route of administration: inhalation
Exposure period: 1 h/d for 2 weeks, 5 d a week
Post exposure period: no data
Doses: 0.4 ml in 5 ml ethanol as aerosol in a 400 l chamber
Control Group: no data specified

Method: other: 1 guinea pig, 1 rabbit, 2 rats, 4 mice were exposed together in one chamber.
GLP: no data
Test substance: no data

Remark: LOEL: no data
no further information available
Result: no effects
17-OCT-1994 (29)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium, TA 100, TA 1535
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data

Remark: abstract, no further information available
(58)

Type: Ames test
System of testing: Salmonella typhimurium, TA 100,
Metabolic activation: no data
Result: positive

Method: other: no data
GLP: no data
Test substance: no data

Remark: 0.07 revertants per nmole;
abstract, no further information available
(59)

Type: Bacterial gene mutation assay
System of testing: Escherichia coli
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data
(60)

Type: Ames test
System of testing: Salmonella typhimurium, TA 92, 98, 100
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: no data
(60)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537, 1538
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data

Test substance: other TS: purified TETA-2Hydrochloride

(61)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537
Metabolic activation: with and without
Result: positive

Method: other: preincubation assay
GLP: no data
Test substance: other TS: technical grade (68.1%)

(62)

Type: Ames test
System of testing: Salmonella typhimurium, TA 98, 100, 1535, 1537, 1538
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: yes
Test substance: other TS: techn. grade; 2 samples: 56.4 and 68.5% purity

(63) (64)

Type: Mammalian cell gene mutation assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

Remark: no clear dose-response relationship

(65)

Type: Mammalian cell gene mutation assay
System of testing: CHO cells
Metabolic activation: with and without
Result: negative

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Unscheduled DNA synthesis
System of testing: rat hepatocytes
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 99.42%

(66)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

(65)

Type: Unscheduled DNA synthesis
System of testing: rat hepatocytes
Metabolic activation: without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 79.15%

(65)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

Method: other: no data
GLP: no data
Test substance: other TS: purity 56.4%, technical grade

Remark: with metab. activation only at the lowest concentration (0.5 g/l) significant increase of SCEs/chromosome; no increase at 0.6 and 0.8 g/l.

(67)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster **Sex:** no data
Route of admin.: unspecified
Exposure period: no data
Doses: no data

Method: other: no data
GLP: no data
Test substance: no data

Result: no effects

(68)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Route of admin.: i.p.
Exposure period: single injection
Doses: 185, 370, 600 mg/kg

Method: other: Bushy Run Research Center standard protocol
GLP: yes
Test substance: other TS: purity 68.5%, technical grade

Result: not clastogenic

(69)

Type: Micronucleus assay
Species: mouse **Sex:** no data
Route of admin.: i.p.
Exposure period: single injection
Doses: 130, 190, 250 mg/kg

Method: other: according to Schmid, W., Mitt. III der Komm. fuer Mutagenitaetsfragen, 53 (1975)
GLP: no data
Test substance: other TS: purified TETA-Dihydrochloride

Result: not clastogenic

(61)

Type: Micronucleus assay
Species: mouse **Sex:** no data
Route of admin.: oral unspecified
Exposure period: single application
Doses: 1500, 3000, 6000 mg/kg

Method: other: according to several published methods
GLP: no data
Test substance: other TS: purified TETA-2Hydrochloride

Result: not clastogenic

(61)

5.7 Carcinogenicity

Species: mouse **Sex:** male
Strain: other: C3H/HeJ
Route of administration: dermal
Exposure period: life-time
Frequency of treatment: 3 times a week
Post exposure period: no
Doses: ca. 1.2 mg/mouse and application
Control Group: other: deionized water

Method: other: see remarks
GLP: no data
Test substance: other TS: purity 79.15% (analytic)

Remark: method: no further data available
remarks: 50 animals per group; 0.025 ml of 5% aqueous solution applied; dose highest one that resulted in neither skin irratation nor reduced weight gain. No increased mortality. Dosage very low compared to LD50.
Result: No treatment related skin tumors, no evidence of increased incidence of any other tumor.

(70)

Species: mouse **Sex:** male
Strain: other: C3H/HeJ
Route of administration: dermal
Exposure period: 2 years
Frequency of treatment: 3 times/week
Doses: 0, 0.2 or 2.0 % in ethanol

Remark: 50 animals/group
Result: No effects were observed on any parameter, including mortality, body weights and incidence of tumorous or non-tumorous lesions.
Source: DOW Europe S.A., Switzerland
24-MAY-1994 (71)

5.8.1 Toxicity to Fertility

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: day 6-15 of gestation
Frequency of treatment: once daily
Doses: 75, 325, 750 mg/kg
Control Group: yes

Method: other: test substance diluted in water
GLP: no data
Test substance: other TS: purity > 98%

Remark: no further information available
Result: No substance related effects on dams or fetuses, except increased fetal body weight at 750 mg/kg (no data about significance).
(72)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: day 0-21 of gestation
Frequency of treatment: daily ad libitum
Doses: 0.17, 0.83, 1.66% in the diet (170, 830, 1660 mg/kg b.w. and day)
Control Group: yes

GLP: no data
Test substance: other TS: purity > 99%, TETA-4Hydrochloride

Remark: litter size unchanged, all described effects significant and dose related. Authors comment: teratogenicity of the drug in part due to induced Cu deficiency and Zn toxicity.

Result: Controls (n=7): no resorbed or abnormal fetuses.
0.17%
dams(n=5): no effects except reduced liver copper and increased kidney zinc concentration. Fetuses: 5.8% resorbed (3/52), whole fetus and liver Zn conc. elevated, Cu liver conc. reduced.

0.83%
dams (n=9): reduced weight gain, decreased Cu conc. in liver and plasma, Zn conc. increased in kidney and muscle.
Fetuses: 8.7% resorbed (7/93), 25,6% abnormalities (22/86) like hemorrhage and edema, Cu decreased in whole body, liver and placenta, Zn concentration elevated in whole body and liver.
1.66%
dams (n=5): reduced food consumption;
highly signif. reduced weight gain and copper concentration in liver and plasma. Zn conc. in kidney and muscle, manganese conc. in muscle and iron conc. in liver increased.
Fetuses: 18.8% resorbed (9/48); 100% abnormalities (39/39) like hemorrhages, edema, reduced ossification of caudal vertebrae and phalanges; fetal weight and length reduced.
Trace elements same results as in medium dose.
(73) (74) (75) (76)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: day 0-21 of gestation
Frequency of treatment: daily ad libitum
Doses: 0, 0.83 or 1.67% in diet combined with 0.05 or 0.5 mg Cu/kg diet
Control Group: yes

Method: other: 4 rats per group
GLP: no data
Test substance: other TS: purity > 99%

Remark: litter size not altered by test substance or Cu administration.
Authors comment: teratogenicity of the test substance in part due to induced Cu deficiency. Doses used here correspond to 830 or 1670 mg per kg b.w. and day.
Result: Maternal weight gain and fetal weight and length were significantly decreased at 1.67% without improvement by copper supplement. Frequency of resorption not different in any group. Significant incidence of fetal abnormalities (69%, 27 out of 39 fetuses) due to 1.67% in combination with the low Cu concentraion was lowered to 6.5% (3/46) by high Cu concentration. Types of abnormalities: hemorrhage, edema, hydronephrotic kidneys, micrognathia and domed skulls. The lowered teratogenetic effect of 1.67% was correlated with an increase in maternal and fetal tissue copper levels by Cu supplement.
Increased maternal and
fetal zinc levels due to the test substance were not altered by Cu coadministration.
(77) (78) (79)

Species: rabbit **Sex:** female
Strain: other: New Zealand
Route of administration: dermal
Exposure period: day 6-18 of gestation
Frequency of treatment: 6 h each day

Doses: 5, 50, 125 mg/kg dissolved in 2 ml distilled water

Control Group: yes
NOAEL Teratogenicity: 125 mg/kg bw

Method: other: 22 rabbits per group; application occlusive
GLP: no data
Test substance: other TS: purity 95%

Result: No embryotoxic or teratogenic drug related effects at any dose.
Maternal toxicity:
125 mg/kg induced delayed weight gain and death of 2 out of 22 rabbits. Strong local irritations of the skin at 50 and 125 mg/kg and slight reversible irratations at 5 mg/kg. No reduction of copper concentrations in urine and plasma.
(80)

Species: other: chicken **Sex:** no data
Strain: other: White Leghorn
Route of administration: other
Exposure period: once in 3 days old embryos
Doses: 0.051, 0.102, 0.204 or 0.408 mg per egg dissolved in 5 ul acetone
Control Group: other: solvent

Method: other: injection on the inner shell membrane
GLP: no data
Test substance: other TS: technical grade

Result:

	deaths of embryos	malformed survivors
0.051 mg	1 out of 30	2 out of 29
0.102 mg	3/30	3/27
0.204 mg	10/30	4/20
0.408 mg	20/20	----
acetone	1/100	0/100

Malformations occurred in the eyes, wings and abdominal wall. Oedema, enlarged lymph sacs and stunting and twisting of the backbone. ED50 for embryotoxicity: 0.155 mg per egg.
(81)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: TETA-2Hydrochloride is used in the therapy of Wilson''s disease (inherited metabolic desease characterised by copper accumulation predominantly in liver, cornea, brain, and kidney) when the drug of choice (Penicillamine) is not tolerated. All authors reported no serious side effects.
(82) (83) (84) (85) (86) (87) (88) (89) (90) (91)

Remark: In primary biliary cirrhosis treatment TETA is an unsuitable drug due to gastrointestinal side effects, skin rash and rhabdomyolysis (one out of 4 patients 48 h after 1. dose)

(92)

Remark: There was no evidence of teratogenicity in 4 patients who became pregnant while taking TETA-2Hydrochoride against Wilson''s disease (6 pregnancies).

(89)

Remark: 6 out of 20 employees working with ethoxylin cast resin and the hardener TETA suffered from work related eczematous dematosis. 8/20 showed slight skin irratations like erythemaand itching. In epicutaneous skin test 5 out of 6 workers with strong dermatosis were sensitized to TETA (technical grade).

(93)

Remark: Serum monoamine oxidase activity in 15 workers handling with epoxy resin and hardener TETA was significantly elevated compared to a control group. Increased activity reflect possibly increased amine metabolism in the connective tissue.

(94)

Remark: 12 workers exposed to araldite and hardener TETA were examined 2 to 4 times at intervals of 6 months. After 1 year there was a decrease in the relative percentage of lymphocytes and a corresponding increase in neutrophils. 5 workers reported subjective symptomes like drowsiness, headache, gastric pain, fatigue, weakness and decreased appetite. 7 showed dermatosis.

(95)

Remark: No significant improvement occurred in hand eczema of 23 nickel-sensitive patients treated with 300 mg TETA/d in a double blind study.

(96)

Remark: Plasma levels were measured in 4 male and 4 female patients receiving treatment for excess copper. Maximal plasma levels of 0.3- 15 mg/l (male) and 1.0- 2.2 mg/l (female) were seen 3 h after oral administration of 8.3 mg/kg b.w..

The free form of the drug was not detected, indicating chelation with metal ions (predominantly copper).
test substance: TETA-2Hydrochloride

(97)

Remark: Using the oral copper loading test and the 24 h urine excretion test on patients with Wilson''s disease it could be
shown, that longterm therapy with 1.2 g/d TETA (more than 3 months) led to a decreased intestinal copper absorption and to an increased urine copper excretion.
test substance: TETA-2Hydrochloride

(98)

5.11 Additional Remarks

Type: Biochemical or cellular interactions

Remark: Female F-344 rats received i.m. 0.75 mmol/kg TETA prior to 0.068 or 0.10 mmol/kg nickeldichloride (i.p. or i.m.). In rats killed 6 h after injection of TETA and nickelchloride, Ni concentration in liver, kidney, spleen, lung and heart averaged 3.4, 0.72, 0.27, 0.22, and 0.12 times corresponding Ni concentrations in contol rats that received only nickelchlorid. Ni-induced hyperglycemia and hyperglucagonemia were not prevented. TETA markedly reduced plasma Ni conc. and increased urine Ni excretion during 6 h after injection. Test substance: purified TETA-4Hydrochloride

(99)

Type: Biochemical or cellular interactions

Remark: Norwegian hooded rats received 100 mg TETA per rat with the diet for 3 days and the urine copper concentration was determined. The basal copper excretion of 65.1 nmol/24 h rose after drug application to 305.9 nmol/24 h. Test substance: TETA-2Hydrochloride

(100)

Type: Biochemical or cellular interactions

Remark: Female mixed-breed dogs were administered 150 mg TETA orally in gelantine capsules twice daily for 23 days and serum and 24 h urine were analysed on day 0, 9, 15, and 23. Cu concentration in serum was unchanged but increased in urine from 0.119 to 0.663 mg/24 h. Zn and Fe concentration in plasma and urine were not changed. Predictive value reduced by low number of animals (n=3). Test substance: TETA-4Hydrochloride

(101)

Type: Biochemical or cellular interactions

Remark: Nickel-poisened rats survived at a nickel:TETA ratio of 1:1. Urinary and biliary excretion of nickel was significantly enhanced.

(102)

Type: Biochemical or cellular interactions

Remark: Sodium diethyldithiocarbamate and D- pencillamine are significantly more effective upon acute toxicity of nickel carbonyl in rats than TETA.

(103)

Type: Biochemical or cellular interactions

Remark: The distribution of radioactive nickel, iron, manganese, and tin in plasma was studied in rats which received i.p. injections of their salts with or without i.m. injection of TETA. TETA was most effective in reducing nickel, followed by iron, manganese and tin.

test substance: no data

(104)

Type: Biochemical or cellular interactions

Remark: A single i.p. application of TETA decreased significantly the total body burden of zinc 24 h after i.v. injection of Zn chloride (0.14 mg/kg). Simultaneous peroral administration of TETA with Zn increased whole body burden of Zn, indicating possibly enhanced absorption of zinc.
test substance: TETA-2Hydrochloride

(105)

Type: Biochemical or cellular interactions

Remark: In a comparative study on the effects of 7 chelating drugs on trace metal and biochem. alteration in the rat TETA is one of the drugs producing least effects on the levels of trace metals and biochem. parameters.
test substance: no data

(106)

Type: Biochemical or cellular interactions

Remark: TETA is an effective antidote to acute nickel carbonyl poisoning (4.35 mg/l for 15 min) when it is administered 10 min after and not 10 min before exposure in rats.
test substance: no data

(107)

Type: Biochemical or cellular interactions

Remark: In a comparative study with 16 chelating agents TETA has been shown to be one of the most effective drugs enhancing urinary excretion of copper in the rat.
test substance: no data

(108)

Type: Biochemical or cellular interactions

Remark: 6 daily i.p. injections of 146 mg/kg TETA enhanced significantly excretion of all essential trace metals in rats. In serum levels there were no significant changes indicating redistribution.
test substance: no data

(109)

Type: Biochemical or cellular interactions

Remark: In cadmium preexposed rats 500 mg/kg TETA reduced the hepatic Cd burden but did not elicit any influence on other tissues except pancreas.
test substance: TETA-hydrochloride

(110)

Type: Toxicokinetics

Remark: The maximal plasma concentration 2 h after a single oral administration of 25 mg/kg was 8 microg/ml in fasted, 3 in nonfasted rats(max after 1h) and 24 microg/ml after

intraduodenal application. Bioavailability 4 h after administration was 6.6, 2.3,and 17.6%, respectively. Plasma levels after i.v. administration of 0.1 mg per rat were 0.0013 mg/ml 10 min. after injection and 0.00045 mg/ml after 4 h. The urinary excretion of unchanged TETA during 24 h was 3.1% of the oral dose and total urinary excretion including not identified metabolites amounted to 35.7% of the dose. Main absorption by permeation across the plasma membrane of intestinal epithelial cells. Binding to the brush border membran was totally inhibited by 0.05 mmol copper.
test substance: TETA-2Hydrochloride

(111)

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