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Table 1-1. Mutagenicity of tris (2-butoxyethyl) phosphate in reverse mutation test (I) on bacteria

With (+) or without (-) S9 mix	Test substance dose ( $\mu\text{g}/\text{plate}$ )	Number of revertants (number of colonies/plate, mean $\pm$ S.D.)					
		Base-pair substitution type			Frameshift type		
		TA100	TA1535		TA98	TA1537	
S9 mix (-)	0	110 107 95 (104 $\pm$ 7.9)	10 7 9 ( 9 $\pm$ 1.5)		18 13 19 ( 17 $\pm$ 3.2)	6 10 7 ( 8 $\pm$ 2.1)	
	7.81	122 134 106 (121 $\pm$ 14.0)	ND		ND	5 8 7 ( 7 $\pm$ 1.5)	
	15.6	141 93 139 ( 124 $\pm$ 27.2)	8 14 5 ( 9 $\pm$ 4.6)		21 23 17 ( 20 $\pm$ 3.1)	5 5 7 ( 6 $\pm$ 1.2)	
	31.3	117 116 117 (117 $\pm$ 0.6)	17 11 14 ( 14 $\pm$ 3.0)		15 26 23 ( 21 $\pm$ 5.7)	7 3 6 ( 5 $\pm$ 2.1)	
	62.5	128 122 108 (119 $\pm$ 10.3)	8 8 15 ( 10 $\pm$ 4.0)		15 17 14 ( 15 $\pm$ 1.5)	8 9 7 ( 8 $\pm$ 1)	
	125	127 118 112 (119 $\pm$ 7.5)	11 8 11 ( 10 $\pm$ 1.7)		21 24 14 ( 20 $\pm$ 5.1)	6 3 7 ( 5 $\pm$ 2.1)	
	250	89* 73* 94* ( 85 $\pm$ 11.0)	1* 6* 6* ( 4 $\pm$ 2.9)		18* 17* 20* ( 18 $\pm$ 1.5)	0* 0* 0* ( 0 $\pm$ 0.0)	
	500		2* 2* 4* ( 3 $\pm$ 1.2)		23* 13* 9* ( 15 $\pm$ 7.2)		
S9 mix (+)	0	145 121 138 (135 $\pm$ 12.3)	9 7 18 ( 11 $\pm$ 5.9)			11 9 10 ( 10 $\pm$ 1.0)	
	15.6	163 140 148 (150 $\pm$ 11.7)	11 17 15 ( 14 $\pm$ 3.1)			13 6 15 ( 11 $\pm$ 4.7)	
	31.3	142 150 172 (155 $\pm$ 15.5)	11 15 7 ( 11 $\pm$ 4.0)			9 13 12 ( 11 $\pm$ 2.1)	
	62.5	180 121 172 (158 $\pm$ 32)	10 9 8 ( 9 $\pm$ 1)			12 12 16 ( 13 $\pm$ 2.3)	
	125	153 129 137 (140 $\pm$ 12.2)	7 9 10 ( 9 $\pm$ 1.5)			12 6 12 ( 10 $\pm$ 3.5)	
	250	111 135 151 (132 $\pm$ 20.1)	11 15 6 ( 11 $\pm$ 4.5)			12 14 12 ( 13 $\pm$ 1.2)	
	500	133* 150* 134* (139 $\pm$ 9.5)	10* 5* 12* ( 9 $\pm$ 3.6)			0* 0* 0* ( 0 $\pm$ 0.0)	
Positive control	Chemical	AF2	SA		AF2	9AA	
	Dose ( $\mu\text{g}/\text{plate}$ )	0.01	0.5		0.1	80	
S9 mix (-)	Number of colonies/plate	775 794 860 (810 $\pm$ 44.6)	378 396 365 (380 $\pm$ 15.6)		724 638 737 (700 $\pm$ 53.8)	725 1204 1329 (1086 $\pm$ 318.8)	
Positive control	Chemical	2AA	2AA			2AA	
	Dose ( $\mu\text{g}/\text{plate}$ )	1	2			2	
S9 mix (+)	Number of colonies/plate	632 740 739 (704 $\pm$ 62.1)	301 300 326 (309 $\pm$ 14.7)			315 338 356 (336 $\pm$ 20.6)	

AF2:2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA:Sodium azide, 9AA:9-Aminoacridine, 2AA:2-Aminoanthracene

\*:Inhibition was observed against growth of the bacteria.

Purity was 98.2 %.

ND:Not done

Table 1-2. Mutagenicity of tris (2-butoxyethyl) phosphate in reverse mutation test (I) on bacteria

With (+) or without (-) S9 mix	Test substance dose ( $\mu\text{g}/\text{plate}$ )	Number of revertants (number of colonies/plate, mean $\pm$ S.D.)					
		Base-pair substitution type			Frameshift type		
				WP2 <i>uvrA</i>	TA98		
S9 mix (-)	0			15 20 18 ( 18 $\pm$ 2.5)			
	156			24 19 27 ( 23 $\pm$ 4.0)			
	313			16 24 23 ( 21 $\pm$ 4.4)			
	625			18 22 21 ( 20 $\pm$ 2.1)			
	1250			25 13 18 ( 19 $\pm$ 6.0)			
	2500 c			20 18 25 ( 21 $\pm$ 3.6)			
	5000 c			11 20 17 ( 16 $\pm$ 4.6)			
S9 mix (+)	0			31 14 23 ( 23 $\pm$ 8.5)	26 26 19 ( 24 $\pm$ 4.0)		
	78.1			21 20 20 ( 20 $\pm$ 0.6)	21 31 25 ( 26 $\pm$ 5)		
	156			15 22 22 ( 20 $\pm$ 4.0)	30 28 16 ( 25 $\pm$ 7.6)		
	313			20 19 28 ( 22 $\pm$ 4.9)	25 25 25 ( 25 $\pm$ 0.0)		
	625			17 25 18 ( 20 $\pm$ 4.4)	12 29 25 ( 22 $\pm$ 8.9)		
	1250			18 17 20 ( 18 $\pm$ 1.5)	20 15* 18* ( 18 $\pm$ 2.5)		
	2500			21* 21* 19* ( 20 $\pm$ 1.2)	18* 25* 13* ( 19 $\pm$ 6.0)		
Positive control S9 mix (-)	Chemical			AF2			
	Dose ( $\mu\text{g}/\text{plate}$ )			0.01			
	Number of colonies/plate			314 275 290 (293 $\pm$ 19.7)			
Positive control S9 mix (+)	Chemical			2AA	2AA		
	Dose ( $\mu\text{g}/\text{plate}$ )			10	0.5		
	Number of colonies/plate			688 780 749 (739 $\pm$ 46.8)	282 318 334 (311 $\pm$ 26.6)		

AF2:2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, 2AA:2-Aminoanthracene

\*:Inhibition was observed against growth of the bacteria. c:Precipitate was observed on the surface of agar plates.

Purity was 98.2 %.

Table 2-1. Mutagenicity of tris (2-butoxyethyl) phosphate in reverse mutation test (II) on bacteria

With (+) or without (-) S9 mix	Test substance dose ( $\mu\text{g}/\text{plate}$ )	Number of revertants (number of colonies/plate, mean $\pm$ S.D.)					
		Base-pair substitution type			Frameshift type		
		TA100	TA1535		TA98	TA1537	
S9 mix (-)	0	117 108 101 (109 $\pm$ 8)	11 10 14 ( 12 $\pm$ 2.1)		19 21 14 ( 18 $\pm$ 3.6)	11 16 13 ( 13 $\pm$ 2.5)	
	7.81	112 107 107 (109 $\pm$ 2.9)	ND		ND	6 7 2 ( 5 $\pm$ 2.6)	
	15.6	107 83 93 ( 94 $\pm$ 12.1)	9 8 7 ( 8 $\pm$ 1)		25 20 21 ( 22 $\pm$ 2.6)	8 4 11 ( 8 $\pm$ 3.5)	
	31.3	104 104 94 (101 $\pm$ 5.8)	9 5 11 ( 8 $\pm$ 3.1)		19 23 18 ( 20 $\pm$ 2.6)	9 6 10 ( 8 $\pm$ 2.1)	
	62.5	100 112 100 (104 $\pm$ 6.9)	8 8 8 ( 8 $\pm$ 0)		22 22 16 ( 20 $\pm$ 3.5)	4 4 8 ( 5 $\pm$ 2.3)	
	125	100 104 116 (107 $\pm$ 8.3)	11 10 8 ( 10 $\pm$ 1.5)		10 21 21 ( 17 $\pm$ 6.4)	7 7 6* ( 7 $\pm$ 0.6)	
	250	60* 73* 60* ( 64 $\pm$ 7.5)	5* 5* 5* ( 5 $\pm$ 0)		12* 15* 11 ( 13 $\pm$ 2.1)	0* 0* 0* ( 0 $\pm$ 0.0)	
	500		3* 2* 4* ( 3 $\pm$ 1)		12* 9* 10* ( 10 $\pm$ 1.5)		
S9 mix (+)	0	112 106 107 (108 $\pm$ 3.2)	12 6 8 ( 9 $\pm$ 3.1)			22 8 6 ( 12 $\pm$ 8.7)	
	15.6	97 100 104 (100 $\pm$ 3.5)	7 12 16 ( 12 $\pm$ 4.5)			11 10 5 ( 9 $\pm$ 3.2)	
	31.3	114 100 88 (101 $\pm$ 13)	11 10 10 ( 10 $\pm$ 0.6)			13 8 14 ( 12 $\pm$ 3.2)	
	62.5	132 122 90 (115 $\pm$ 21.9)	8 13 12 ( 11 $\pm$ 2.6)			14 13 12 ( 13 $\pm$ 1.0)	
	125	116 92 125 (111 $\pm$ 17.1)	7 8 12 ( 9 $\pm$ 2.6)			11 6 13 ( 10 $\pm$ 3.6)	
	250	92 90 108 ( 97 $\pm$ 9.9)	9 13 11 ( 11 $\pm$ 2.0)			11 9 9 ( 10 $\pm$ 1.2)	
	500	80* 80* 91* ( 84 $\pm$ 6.4)	7* 7* 9* ( 8 $\pm$ 1.2)			0* 0* 0* ( 0 $\pm$ 0.0)	
Positive control S9 mix (-)	Chemical	AF2	SA		AF2	9AA	
	Dose ( $\mu\text{g}/\text{plate}$ )	0.01	0.5		0.1	80	
	Number of colonies/plate	563 575 567 (568 $\pm$ 6.1)	380 384 351 (372 $\pm$ 18)		531 522 545 (533 $\pm$ 11.6)	1349 1188 1356 (1298 $\pm$ 95.0)	
Positive control S9 mix (+)	Chemical	2AA	2AA			2AA	
	Dose ( $\mu\text{g}/\text{plate}$ )	1	2			2	
	Number of colonies/plate	512 607 650 (590 $\pm$ 70.6)	274 271 345 (297 $\pm$ 41.9)			227 292 311 (277 $\pm$ 44)	

AF2:2-(2-Furyl) 3-(5-nitro-2-furyl) acrylamide, SA: Sodium azide, 9AA:9-Aminoacridine, 2AA:2-Aminoanthracene

\*:Inhibition was observed against growth of the bacteria.

Purity was 98.2 %.

ND: Not done

Table 2-2. Mutagenicity of tris(2-butoxyethyl) phosphate in reverse mutation test (II) on bacteria

With (+) or without (-) S9 mix	Test substance dose ( $\mu\text{g}/\text{plate}$ )	Number of revertants (number of colonies/plate, mean $\pm$ S.D.)					
		Base-pair substitution type			Frameshift type		
				WP2 <i>uvrA</i>	TA98		
S9 mix (-)	0			28 19 24 (24 $\pm$ 4.5)			
	156			28 22 24 (25 $\pm$ 3.1)			
	313			22 22 19 (21 $\pm$ 1.7)			
	625			17 22 30 (23 $\pm$ 6.6)			
	1250			20 17 15 (17 $\pm$ 2.5)			
	2500 c			16 24 20 (20 $\pm$ 4.0)			
	5000 c			16 20 20 (19 $\pm$ 2.3)			
S9 mix (+)	0			25 23 40 (29 $\pm$ 9.3)	30 31 22 (28 $\pm$ 4.9)		
	78.1			24 24 45 (31 $\pm$ 12.1)	25 16 25 (22 $\pm$ 5.2)		
	156			31 28 24 (28 $\pm$ 3.5)	28 18 20 (22 $\pm$ 5.3)		
	313			24 34 27 (28 $\pm$ 5.1)	29 28 31 (29 $\pm$ 1.5)		
	625			23 23 36 (27 $\pm$ 7.5)	23 18 26 (22 $\pm$ 4.0)		
	1250			24 30 24 (26 $\pm$ 3.5)	24* 17* 19* (20 $\pm$ 3.6)		
	2500			19* 22* 20* (20 $\pm$ 1.5)	17* 12* 16* (15 $\pm$ 2.6)		
Positive control	Chemical			AF2			
	Dose ( $\mu\text{g}/\text{plate}$ )			0.01			
S9 mix(-)	Number of colonies/plate			303 312 331 (315 $\pm$ 14.3)			
Positive control	Chemical			2AA	2AA		
	Dose ( $\mu\text{g}/\text{plate}$ )			10	0.5		
S9 mix(+)	Number of colonies/plate			485 499 470 (485 $\pm$ 14.5)	308 348 287 (314 $\pm$ 31.0)		

AF2:2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, 2AA:2-Aminoanthracene

\*:Inhibition was observed against growth of the bacteria. c:Precipitate was observed on the surface of agar plates.

Purity was 98.2 %.

# リン酸トリス(2-ブトキシエチル)エステルの 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

## *In Vitro* Chromosomal Aberration Test of Tris(2-butoxyethyl) phosphate on Cultured Chinese Hamster Cells

### 要約

リン酸トリス(2-ブトキシエチル)エステルの培養細胞に及ぼす細胞遺伝学的影響について、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。

連続処理(24時間)および短時間処理(6時間)における50%細胞増殖抑制濃度は、連続処理(24時間)および短時間処理(6時間)のS9 mix非存在下では0.090 mg/ml、短時間処理(6時間)のS9 mix存在下では0.40 mg/mlであった。各系列での処理濃度は、50%細胞増殖抑制濃度の2倍濃度を最高処理濃度とし、それぞれ公比2で5濃度設定した。連続処理では、S9 mix非存在下で24時間および48時間連続処理後、短時間処理ではS9 mix存在下および非存在下で6時間処理(18時間の回復時間)後、標本を作製し、検鏡することにより染色体異常誘発性を検討した。染色体分析が可能な最高濃度は、24時間連続処理および短時間処理のS9 mix非存在下では0.090 mg/ml、48時間連続処理および短時間処理のS9 mix存在下ではそれぞれ0.045 mg/mlおよび0.20 mg/mlの濃度であったことから、これらの濃度を高濃度群として3濃度群を観察対象とした。

CHL/IU細胞を24時間連続処理した高濃度群(0.090 mg/ml)では、細胞毒性により倍数性細胞の観察細胞が規定に満たなかったが、24時間および48時間連続処理したいずれの処理群においても、染色体の構造異常や倍数性細胞の誘発作用は認められなかった。短時間処理では、S9 mix存在下および非存在下で6時間処理したいずれの処理群においても、染色体の構造異常や倍数性細胞の誘発作用は認められなかった。

以上の結果より、リン酸トリス(2-ブトキシエチル)エステルは、上記の試験条件下で染色体異常を誘発しないと結論した。

### 方法

#### 1. 使用した細胞

リサーチ・リソースバンク(JCRB)から入手(1988年2月、入手時:継代4代、現在12代)したチャイニーズ・ハムスター由来のCHL/IU細胞を、解凍後継代10代以内で試験に用いた。

#### 2. 培養液の調製

培養には、牛胎児血清(FCS:Cansera International)

を10%添加したイーグルMEM(日水製薬(株))培養液を用いた。

#### 3. 培養条件

$2 \times 10^4$ 個のCHL/IU細胞を、培養液5 mlを入れたデイツュ(径6 cm, Corning)に播き、37°CのCO<sub>2</sub>インキュベーター(5% CO<sub>2</sub>)内で培養した。連続処理では、細胞播種3日目に被験物質を加え、24時間および48時間処理した。また、短時間処理では、細胞播種3日目にS9 mix存在下および非存在下で6時間処理し、処理終了後新鮮な培養液でさらに18時間培養した。

#### 4. 被験物質

リン酸トリス(2-ブトキシエチル)エステル(略号:TBEP, CAS No.:78-51-3, ロット番号:K70702, 大八化学工業(株))は、無色透明液体で、水に対しては0.11%(25°C)、DMSOでは1 l/l、アセトンおよびエタノールで1 l/lで溶解し、融点-70°C以下、沸点222°C/5.3 hpaで、分子式C<sub>18</sub>H<sub>35</sub>O<sub>7</sub>P、分子量398.54、純度98.2 wt%(不純物は不明)の物質である。

被験物質原体は、通常の取り扱い条件においては安定であるが、水、熱、アルカリ中では分解する。

#### 5. 被験物質の調製

被験物質の調製は、使用のつど行った。溶媒はDMSO(和光純薬工業(株))を用いた。原体を溶媒に溶解して原液を調製し、ついで原液を溶媒で順次希釈して所定の濃度の被験物質調製液を作製した。被験物質調製液は、すべての試験において培養液の0.5%(v/v)になるように加えた。なお濃度の記載について、純度換算は行わなかった。

#### 6. 細胞増殖抑制試験による処理濃度の決定

染色体異常試験に用いる被験物質の処理濃度を決定するため、被験物質の細胞増殖に及ぼす影響を調べた。被験物質のCHL/IU細胞に対する増殖抑制作用は、単層培養細胞密度計(Monocellater™, オリンパス光学工業(株))を用いて各群の増殖度を計測し、被験物質処理群の溶媒対照群に対する細胞増殖の比をもって指標とした。

その結果、連続処理および短時間処理のS9 mix非存在下における50%の増殖抑制濃度は、0.090 mg/mlであった。また、短時間処理のS9 mix存在下では、0.40 mg/mlであった(Fig. 1, 2)。

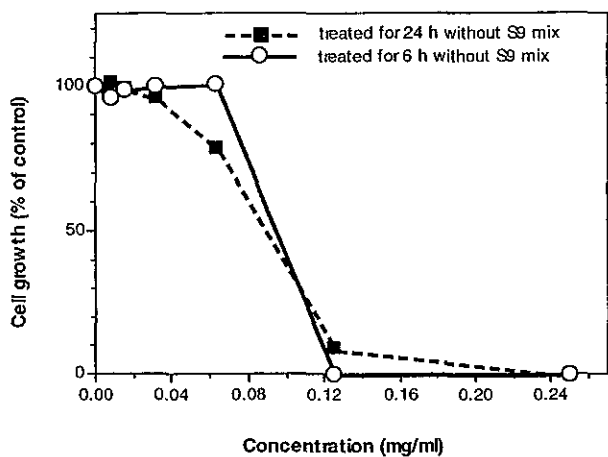


Fig. 1 Growth inhibition of CHL/IU cells treated with tris(2-butoxyethyl) phosphate

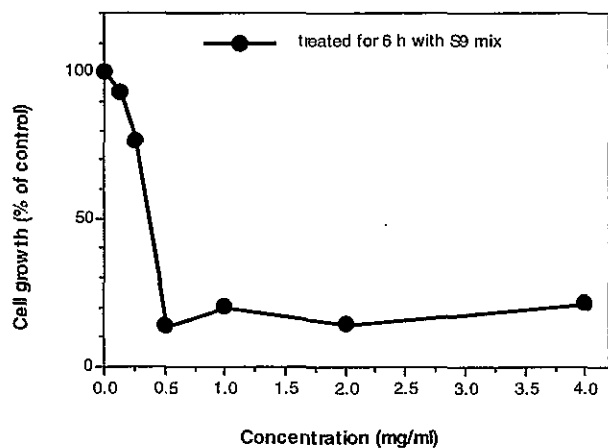


Fig. 2 Growth inhibition of CHL/IU cells treated with tris(2-butoxyethyl) phosphate

7. 実験群の設定

細胞増殖抑制試験の結果より、染色体異常試験において、連続処理および短時間処理のすべての処理群で、50% 増殖抑制濃度の2倍濃度を最高処理濃度とし、公比2で5濃度を設定した(24時間および48時間連続処理および短時間処理の S9 mix 非存在下:0.011, 0.023, 0.045, 0.090, 0.18 mg/ml, 短時間処理の S9 mix 存在下:0.05, 0.10, 0.20, 0.40, 0.80 mg/ml). 陽性対照物質として用いたマイトマイシンC(MC, 協和醗酵工業(株))およびシクロホスファミド(CPA, Sigma Chemical Co.)は、注射用水(株大塚製薬工場)に溶解して調製した。それぞれ染色体異常を誘発することが知られている濃度を適用した。

染色体異常試験においては1濃度あたり4枚ディッシュを用い、そのうちの2枚は染色体標本作製し、別の2枚については単層培養細胞密度計により細胞増殖率を測定した。

8. 染色体標本作製法

培養終了の2時間前に、コルセミドを最終濃度が約

0.1 μg/ml になるように培養液に加えた。染色体標本の作製は常法に従って行った。スライド標本は各ディッシュにつき6枚作製した。作製した標本を3% ギムザ溶液で染色した。

9. 染色体分析

細胞増殖率測定の結果と分裂指数により、20% 以上の相対増殖率で、かつ2ディッシュともに0.5% 以上の分裂指数を示した最も高い濃度を観察対象の最高濃度群とし、観察対象の3濃度群を決定した。その結果(Table 1, 2), 24時間連続処理および短時間処理の S9 mix 非存在下では0.090 mg/ml, 48時間連続処理および短時間処理の S9 mix 存在下では、それぞれ0.045 mg/ml および0.20 mg/ml が、染色体分析の可能な最高濃度であったことから、これらの濃度を含む3濃度群を観察対象とした。

作製したスライド標本のうち、1つのディッシュから得られた異なるスライドを、4名の観察者がそれぞれ処理条件が分からないようにコード化した状態で分析した。染色体の分析は、日本環境変異原学会、哺乳動物試験(MMS)研究会<sup>1)</sup>による分類法に基づいて行い、染色体型あるいは染色分体型のギャップ、切断、交換などの構造異常の有無と倍数性細胞(polyploid)の有無について観察した。また構造異常については1群200個、倍数性細胞については1群800個の分裂中期細胞を分析した。

10. 記録と判定

無処理対照、溶媒および陽性対照群と被験物質処理群についての分析結果は、観察した細胞数、構造異常の種類と数、倍数性細胞の数について集計し、各群の値を記録用紙に記入した。

染色体異常を有する細胞の出現頻度について、溶媒の背景データと被験物質処理群間でフィッシャーの直接確率法<sup>2)</sup>(多重性を考慮して familywise の有意水準を5%とした)により、有意差検定を実施した。また、フィッシャーの直接確率法で有意差が認められた場合には、用量依存性に関してコクラン・アーミテッジの傾向性検定<sup>3)</sup>( $p < 0.05$ )を行った。最終的な判定は、統計学および生物学的な評価に基づいて行った。

結果および考察

連続処理による染色体分析の結果を Table 1 に示した。リン酸トリス(2-ブトキシエチル)エステルを加えて24時間連続処理した高濃度群(0.090 mg/ml)では、細胞毒性により倍数性細胞の観察細胞が規定に満たなかったが、24時間および48時間連続処理したいずれの処理群においても、染色体の構造異常および倍数性細胞の誘発作用は認められなかった。

短時間処理による染色体分析の結果を Table 2 に示した。リン酸トリス(2-ブトキシエチル)エステルを加えて S9 mix 存在下および非存在下で6時間処理したいずれの処理群においても、染色体の構造異常および倍数性

細胞の誘発作用は認められなかった。

従って、リン酸トリス(2-ブトキシエチル)エステルは、上記の試験条件下で、試験管内の CHL/IU 細胞に染色体異常を誘発しないと結論した。

### 文献

- 1) 日本環境変異原学会・哺乳動物試験分科会編, "化学物質による染色体異常アトラス," 朝倉書店, 東京, 1988.
- 2) 吉村 功編, "毒性・薬効データの統計解析, 事例研究によるアプローチ," サイエンティスト社, 東京, 1987.
- 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, pp.218-223.

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Table 1 Chromosome analysis of Chinese hamster cells(CHL/IU) continuously treated with tris(2- butoxyethyl) phosphate(TBEP)\* without S9 mix

Group	Concentration (mg/ml)	Time of exposure (h)	No. of cells analysed	No. of structural aberrations								No. of cells			Concurrent <sup>6)</sup> cytotoxicity			
												Others <sup>3)</sup>	with aberrations		Polyploid <sup>4)</sup> (%)	Trend test <sup>5)</sup>		cytotoxicity (%)
				gap	ctb	cte	csb	cse	mul <sup>2)</sup>	total	TAG (%)		TA (%)	SA		NA		
Control			200	0	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.50			-	
Solvent <sup>1)</sup>	0	24	200	0	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.25			100.0	
TBEP	0.023	24	200	1	1	0	0	0	10	12	0	3 ( 1.5)	2 ( 1.0)	0.13			85.5	
TBEP	0.045	24	200	0	1	0	2	0	0	3	0	2 ( 1.0)	2 ( 1.0)	0.38	NT	NT	79.0	
TBEP	0.090	24	200	0	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.40 <sup>7)</sup>			45.0	
TBEP	0.18 **	24	-											-			0.0	
MC	0.00005	24	200	3	61	96	5	1	10	176	0	93 (46.5)	91 (45.5)	0.00			-	
Solvent <sup>1)</sup>	0	48	200	0	0	0	0	1	0	1	0	1 ( 0.5)	1 ( 0.5)	0.63			100.0	
TBEP	0.011	48	200	0	1	0	0	0	0	1	0	1 ( 0.5)	1 ( 0.5)	0.13			107.0	
TBEP	0.023	48	200	0	1	0	3	0	0	4	0	3 ( 1.5)	3 ( 1.5)	0.25	NT	NT	101.5	
TBEP	0.045	48	200	0	1	0	0	0	0	1	0	1 ( 0.5)	1 ( 0.5)	0.00			86.0	
TBEP	0.090 **	48	-											-			18.5	
TBEP	0.18 **	48	-											-			0.0	
MC	0.00005	48	200	1	47	124	3	3	0	178	9	95 (47.5)	95 (47.5)	0.50			-	

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 nine 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™. 7)Seven hundred and fifty-seven cells were analysed. \*:Purity was 98.2 %. \*\*:Chromosome analysis was not performed because of severe cytotoxicity.

Table 2 Chromosome analysis of Chinese hamster cells(CHL/IU) treated with tris(2- butoxyethyl) phosphate(TBEP)\* with and without S9 mix

Group	Concentration (mg/ml)	S9 mix	Time of exposure (h)	No. of cells analysed	No. of structural aberrations								No. of cells			Concurrent <sup>6)</sup> cytotoxicity			
													Others <sup>3)</sup>	with aberrations		Polyploid <sup>4)</sup> (%)	Trend test <sup>5)</sup>		cytotoxicity (%)
					gap	ctb	cte	csb	cse	mul <sup>2)</sup>	total	TAG (%)		TA (%)	SA		NA		
Control				200	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.13			-		
Solvent <sup>1)</sup>	0	-	6-(18)	200	0	1	0	0	0	0	1	1 ( 0.5)	1 ( 0.5)	0.13			100.0		
TBEP	0.023	-	6-(18)	200	0	0	0	0	0	0	1	0 ( 0.0)	0 ( 0.0)	0.13			99.5		
TBEP	0.045	-	6-(18)	200	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.50	NT	NT	105.0		
TBEP	0.090	-	6-(18)	200	0	1	0	2	0	3	0	2 ( 1.0)	2 ( 1.0)	0.25			80.5		
TBEP	1.8 **	-	6-(18)	-										-			0.0		
CPA	0.005	-	6-(18)	200	0	2	0	0	0	2	1	2 ( 1.0)	2 ( 1.0)	0.50			-		
Solvent <sup>1)</sup>	0	+	6-(18)	200	0	0	0	0	0	0	1	0 ( 0.0)	0 ( 0.0)	0.13			100.0		
TBEP	0.050	+	6-(18)	200	1	1	0	0	0	2	2	1 ( 0.5)	1 ( 0.5)	0.13			99.0		
TBEP	0.10	+	6-(18)	200	1	1	0	4	0	6	0	5 ( 2.5)	4 ( 2.0)	0.38	NT	NT	91.5		
TBEP	0.20	+	6-(18)	200	0	0	0	0	0	0	0	0 ( 0.0)	0 ( 0.0)	0.13			87.0		
TBEP	0.40 **	+	6-(18)	-										-			7.5		
TBEP	0.80 **	+	6-(18)	-										-			16.5		
CPA	0.005	+	6-(18)	200	0	102	226	7	1	50	386	0	134 (67.0)	134 (67.0)	0.00			-	

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, 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 98.2 %. \*\*:Chromosome analysis was not performed because of severe cytotoxicity.



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UNITED NATIONS ENVIRONMENT PROGRAMME  
INTERNATIONAL LABOUR ORGANISATION  
WORLD HEALTH ORGANIZATION

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Environmental Health Criteria 218

FLAME RETARDANTS: TRIS(2-BUTOXYETHYL)  
PHOSPHATE, TRIS(2-ETHYLHEXYL)  
PHOSPHATE AND TETRAKIS(HYDROXYMETHYL)  
PHOSPHONIUM SALTS

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

First draft prepared by Dr G.J. van Esch, Bilthoven, the Netherlands

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World Health Organization  
Geneva, 2000

The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

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Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

\* \* \*

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