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㈱)培養液を用いた．

3. 培養条件

2×10^4個のCHL/IU細胞を，培養液5 mlを入れたディッシュ(径6 cm，Corning)に播き，37℃のCO_2インキュベーター(5% CO_2)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㈱)は，無色透明液体で，水に対しては0.11%(25℃)，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℃以下，沸点222℃/5.3 hpaで，分子式$C_{18}H_{39}O_7P$，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㈱)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㈱)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)．

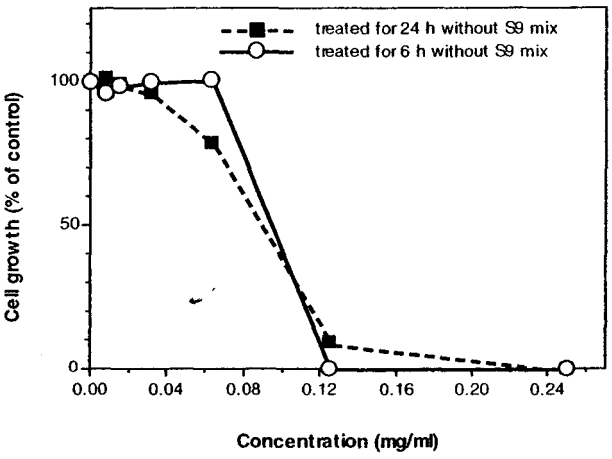

Fig. 1 Growth inhibition of CHL/IU cells treated with tris(2-butoxyethyl) phosphate

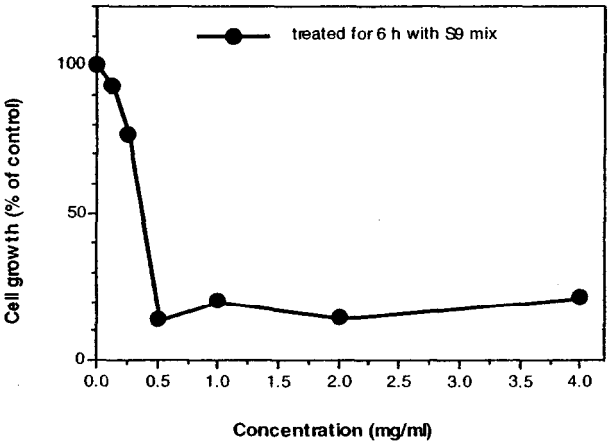

Fig. 2 Growth inhibition of CHL/IU cells treated with tris(2-butoxyethyl) phosphate

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㈱)およびシクロホスファミド(CPA，Sigma Chemical Co.)は，注射用水(㈱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枚は染色体標本を作製し，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[1]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[2](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[3] ($p<0.05$) を行った．最終的な判定は，統計学的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.

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連絡先
試験責任者：田中憲穂
試験担当者：山影康次，佐々木澄志，
中川ゆづき，若栗　忍，日下部博一，
古畑紀久子，渡辺美香，出石由紀，
橋本恵子
㈶食品薬品安全センター秦野研究所
〒257　神奈川県秦野市落合729-5
Tel 0463-82-4751　Fax 0463-82-9627

Correspondence
Authors: Noriho Tanaka (Study director)
Kohji Yamakage, Sasaki Kiyoshi,
Yuzuki Nakagawa, Shinobu Wakuri,
Hirokazu Kusakabe, Kikuko Furuhata
Mika Watanabe, Yuki Izushi,
Keiko Hashimoto
Hatano Research Institute, Food and Drug Safety Center
729-5 Ochiai, Hadano, Kanagawa, 257, Japan
Tel +81-463-82-4751　Fax +81-463-82-9627

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							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			200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.50			-
Solvent[1]	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[7]			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[1]	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							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				200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.13			-
Solvent[1]	0	-	6-(18)	200	0	1	0	0	0	0	1	0	1 (0.5)	1 (0.5)	0.13			100.0
TBEP	0.023	-	6-(18)	200	0	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)	0.50	NT	NT	105.0
TBEP	0.090	-	6-(18)	200	0	1	0	2	0	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	0	2	1	2 (1.0)	2 (1.0)	0.50			-
Solvent[1]	0	+	6-(18)	200	0	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	0	2	2	1 (0.5)	1 (0.5)	0.13			99.0
TBEP	0.10	+	6-(18)	200	1	1	0	4	0	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)	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.

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

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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.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and

Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

WHO Library Cataloguing-in-Publication Data

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(Environmental health criteria ; 218)

1.Organophosphorus compounds - toxicity 2.Phosphoric acid esters - toxicity 3.Flame retardants - toxicity
4.No-observed-adverse-effect level 5.Environmental exposure
6.Occupational exposure I.Series

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A7. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

A7.1 Single exposure

A7.1.1 Oral and dermal
A7.1.2 Inhalation

A7.2 Short-term repeated exposure

A7.2.1 Oral
A7.2.2 Dermal

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

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.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (telephone no. + 41 22 - 9799111, fax no. + 41 22 - 7973460, E-mail irptc@unep.ch).

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Environmental Health Criteria

PREAMBLE

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for

the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are only used when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

* Summary -- a review of the salient facts and the risk evaluation of the chemical
* Identity -- physical and chemical properties, analytical methods
* Sources of exposure
* Environmental transport, distribution and transformation
* Environmental levels and human exposure
* Kinetics and metabolism in laboratory animals and humans
* Effects on laboratory mammals and *in vitro* test systems
* Effects on humans
* Effects on other organisms in the laboratory and field
* Evaluation of human health risks and effects on the environment
* Conclusions and recommendations for protection of human health and the environment

* Further research
* Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in: Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e. the substance is of major interest to several countries; adequate data

on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. While observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson. Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

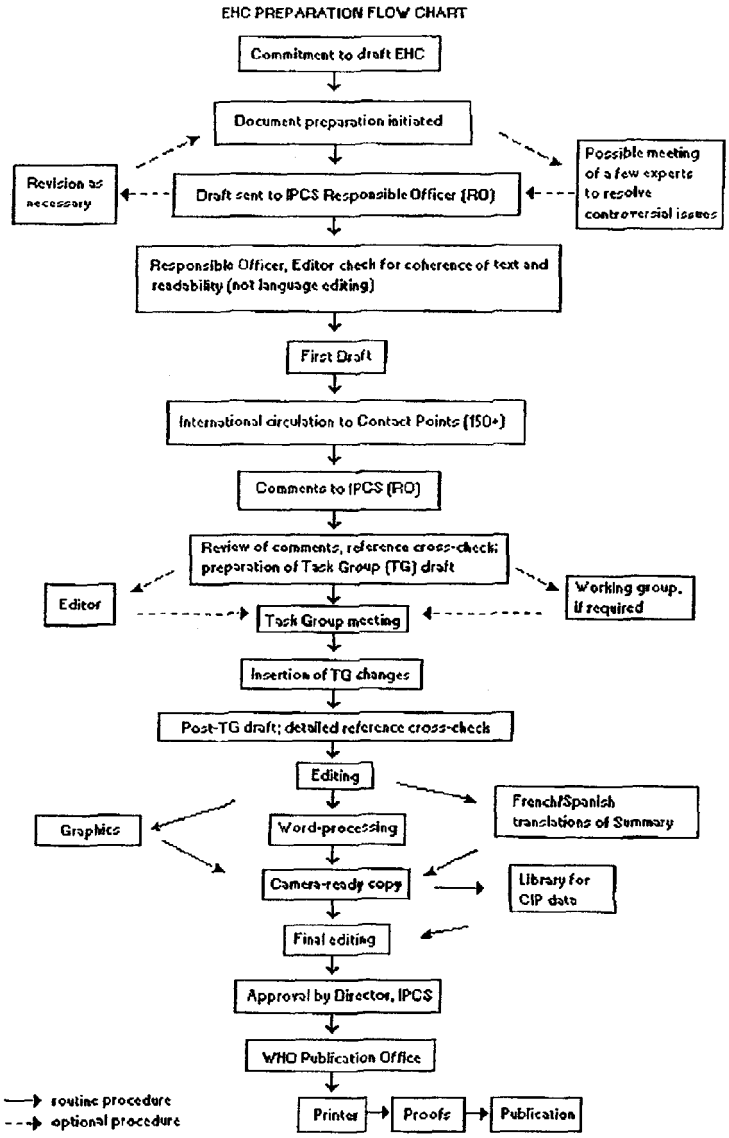
All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the

document, it then goes for language editing, reference checking, and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.



WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR FLAME RETARDANTS: TRIS(2-BUTOXYETHYL) PHOSPHATE, TRIS(2-ETHYLHEXYL) PHOSPHATE AND TETRAKIS(HYDROXYMETHYL) PHOSPHONIUM SALTS

Members

Dr R. Benson, US Environmental Protection Agency, Denver, Colorado, USA

Dr P. Brantom, British Industry Biological Research Association (BIBRA) International, Carshalton, Surrey, United Kingdom

Dr S. Dobson, Institute of Terrestrial Ecology, Monks Wood, Huntingdon, Cambridgeshire, United Kingdom *(Chairman)*

Professor J. Liesivuori, Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland

Mr D. Renshaw, Department of Health, Elephant and Castle, London, United Kingdom

Dr E. Söderlund, National Institute of Public Health, Department of Environmental Medicine, Oslo, Norway *(Rapporteur)*

Observers

Dr L. Kotkoskic, FMC Corporation, Princetown, New Jersey, USA

Dr P. Martin, Albright and Wilson UK Limited, European Business Services - Product Stewardship, Oldbury, West Midlands, United Kingdom

Secretariat

Dr M. Baril, International Programme on Chemical Safety, Montreal, Quebec, Canada

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

A WHO Task Group on Environmental Health Criteria for Flame retardants: tris(2-butoxyethyl) phosphate, tris(2-ethylhexyl) phosphate and tetrakis(hydroxymethyl) phosphonium salts met at the British Industrial Biological Research Association, Carshalton, United Kingdom from 18 to 22 January 1999. Dr P. Brantom opened the meeting and welcome the participants on behalf of the host institute. Dr M. Baril, IPCS, welcomed the participants on behalf of IPCS and the three cooperating organizations (UNEP/ILO/WHO). The Task Group reviewed and revised the draft criteria monograph and made an evaluation of the risk to human health and the environment from exposure to these flame retardants.

Financial support for this Task Group was provided by the United Kingdom Department of Health as part of its contribution to the IPCS.

The first draft of this monograph was prepared by Dr G. J. van Esch, Bilthoven, the Netherlands. The second draft prepared by Dr M. Baril incorporated the comments received following circulation of the first draft to the IPCS contact points for Environmental Health Criteria.

Dr P.G. Jenkins (IPCS Central Unit, Geneva) and Dr M. Baril (IPCS technical advisor, Montreal) were responsible for the overall technical editing and scientific content, respectively.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

* * *

ABBREVIATIONS

AChE	acetylcholinesterase
ALAT	alanine aminotransferase
ASAT	aspartate aminotransferase
BCME	bis(chloromethyl) ether
BEHP	bis(2-ethylhexyl) phosphate
BMPA	bishydroxymethyl phosphonic acid
BuCHE	butyrylcholinesterase
CHO	Chinese hamster ovary
DMSO	dimethyl sulfoxide
EC_{50}	median effective concentration
FDA	Food and Drug Administration (USA)
GC	gas chromatography
HPLC	high performance liquid chromatography
IC_{50}	median inhibitory concentration
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MS	mass spectrometry
nd	not detected
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NPD	nitrogen-phosphorus sensitive detector
NTE	neuropathy target esterase
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
PVC	polyvinyl chloride
SCE	sister-chromatid exchange
TBEP	tris(2-butoxyethyl) phosphate
TEHP	tris(2-ethylhexyl) phosphate
THP	tetrakis(hydroxymethyl) phosphonium
THPC	tetrakis(hydroxymethyl) phosphonium chloride
THPO	trihydroxymethyl phosphine oxide
THPS	tetrakis(hydroxymethyl) phosphonium sulfate
TOCP	tri- *ortho*-cresyl phosphate

PART A

Tris(2-butoxyethyl) phosphate

(TBEP)

A. SUMMARY, EVALUATION AND RECOMMENDATIONS

A1. Tris(2-butoxyethyl) phosphate (TBEP)

A1.1 Summary

Tris(2-butoxyethyl) phosphate (TBEP) is used in floor polishes and as a plasticizer in rubber and plastics. The worldwide production volume is not available but is estimated to be in the range of 5000-6000 tonnes.

TBEP occurs in the environment only as a result of human