LABORATORY DECONTAMINATION AND DESTRUCTION OF CARCINOGENS IN LABORATORY WASTES: SOME HALOETHERS

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WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly, as an independently financed organization within the framework of the World Health Organization. The headquarters of the agency are at Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon, and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency are intended to contribute to the dissemination of authoritative information on different aspects of cancer research.

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FOREWORD

Although the production of bis(chloromethyl)ether (BCME) has decreased greatly during the past decade, chloromethylmethylether (CMME), very often contaminated with BCME, is still in use. Since BCME and technical-grade CMME are considered to be carcinogenic to humans, it was considered essential to develop methods for the degradation of these compounds, both in waste generated during their handling and in the spills that might occur during handling.

Some of the methods described in this volume, although devised for laboratory-scale treatment of small quantities, could be adapted for more widespread decontamination.

This volume, the sixth in the series *Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes*, has been published with the support of the Division of Safety of the National Institutes of Health, USA. The Agency is very grateful for this continued support; we also wish to thank all of the laboratories that took part in the validation of these methods.

L. TOMATIS, M.D.
Director, IARC

PREAMBLE

Biomedical research involving toxic chemicals, including carcinogens, inevitably results in the production of waste products containing these potentially hazardous materials. Such waste products range from those minimally contaminated, such as spent fluids from cell culture applications and carcasses of animals used in bioassay projects, to unused pure compounds no longer required after the completion of a research programme. The safe and environmentally sound disposal of these wastes has become an ever-increasing concern of governments, research institutions, investigators and private citizens, and has resulted in a significant number of enquiries for recommended disposal methods. During our investigation of this area, two significant problems became apparent: (1) there is a paucity of published information on the destruction and disposal of carcinogenic wastes, and (2), of the published methods available, few have been thoroughly evaluated or rigorously tested to ensure that the destruction of the parent compound is complete and that the reaction by-products are relatively innocuous.

In September 1978, the Division of Safety, National Institutes of Health, established with the International Agency for Research on Cancer a special programme to develop an authoritative series of monographs on methods for the destruction and disposal of carcinogenic waste from biomedical research laboratories. The word 'authoritative' has specific meaning and was, indeed, the basis for seeking the involvement of the Agency in this project. We wanted to draw upon the experience of the Agency in bringing together internationally recognized scientific experts to review critically data applicable to the destruction and disposal of carcinogenic waste, to recommend destruction strategies, to develop new methods where necessary, and to subject the designated methods to interlaboratory collaborative verification to confirm their efficacy. The current volume is the sixth of a series that has thus far included disposal methods for aflatoxins, *N*-nitrosamines, polycyclic aromatic hydrocarbons, hydrazines, and *N*-nitrosamides.

Throughout the period of this programme, the Agency and the Division of Safety have encouraged individual scientists and laboratories in the international community to contribute individually to the development of methods and to participate in validation studies. It is our hope that this programme serves as a catalyst for stimulating research in this area and for sharing the results of such investigations. However, to ensure that effective methods are available for application to the vast array of chemical carcinogens in use in laboratories, the pool of available resources and talents must be increased. Therefore, we continue to encourage researchers to become involved in this effort in order to meet our common responsibility for the safe disposal of potentially hazardous laboratory waste.

W. Emmett Barkley, Ph.D.
Director,
Division of Safety,
National Institutes of Health

HALOETHERS CONSIDERED

The following haloethers were considered in this volume. The methods described for their destruction may be applicable to other haloethers; however, when dealing with compounds other than those listed below, the efficiency of the method should first be verified.

Haloether	Chemical Abstract Services Registry Number	Abbreviation used
Chloromethylmethylether	107-30-02	CMME
Bis(chloromethyl)ether	542-88-1	BCME

INTRODUCTION

Carcinogenicity

As part of a long-term investigation of the relationship of chemical structure and reactivity with carcinogenicity, Van Duuren and his colleagues (1968, 1969) showed chloromethylmethylether (CMME) and bis(chloromethyl)ether (BCME) to be carcinogenic to the skin and subcutaneous tissue of rodents. In view of the volatility of these compounds and the importance of CMME (which is normally contaminated with a few percent of BCME) as a chemical intermediate in the production of ion-exchange resins, these compounds were also tested by inhalation in mice at levels of 1 ppm BCME and 2 ppm CMME; both were found to cause an increase in the incidence of pulmonary adenomas (Leong *et al.*, 1971).

In the light of the striking carcinogenicity of BCME in Van Duuren's studies, Laskin *et al.* (1971) and Kuschner et al. (1975) studied the effect in rats of inhalation of 0.1 ppm and found a high incidence of squamous-cell carcinomas of the lungs and olfactory esthesioneuroepithe-liomas. When tested at a level of 1 ppm (Laskin *et al.*, 1975), CMME was found to produce similar effects, but at a lower level of incidence, which could have been consistent with contamination by BCME. The authors noted that, as hydrolysis of CMME leads to formation of hydrogen chloride and formaldehyde, themselves the precursors of BCME, CMME must be regarded as a potential carcinogen from the operational point of view.

Since BCME can be formed by reaction of acid chloride with formaldehyde, there has been speculation and concern that BCME might be formed in air which contains formaldehyde and hydrogen chloride as contaminants.

In view of the potential health implications, several independent groups of workers have carefully investigated the formation of BCME in air (Kallos & Solomon, 1973; Davies, 1974; Frankel *et al.*, 1974) and in water (Tou & Kallos, 1974b, 1976). Their findings demonstrate that concentrations as high as 500 to 3000 ppm of the reactants are required to form ppb levels of BCME in dry air under experimental conditions. At reactant concentrations below 200 ppm, BCME levels were less than about 0.1 ppb and hence undetectable. Similar results were found in studies in the aqueous phase in which BCME is rapidly hydrolysed. Although less effective than BCME, a purified sample of CMME was carcinogenic by subcutaneous injections (Van Duuren *et al.*, 1972).

In 1973, an expert working group met at the IARC to evaluate the data available at that time and concluded that these two haloethers produced an elevated lung cancer risk in exposed workers (International Agency for Research on Cancer, 1974). Of particular significance for the present document was the report by Thiess *et al.* (1973) of a high incidence of lung cancer in a small group of BCME workers employed in a testing laboratory.

Since the IARC meeting, subsequent laboratory and epidemiological studies have confirmed that these two compounds present a human carcinogenic hazard. Nelson (1976), summarizing the evidence available at that time, reported that for a series of chloroethers, mutagenic activity parallels carcinogenic activity. He concluded that despite the incompleteness of the epidemiological evidence there was a clear trend showing increasing incidence of lung cancer with intensity of exposure, longer periods of exposure and greater interval since exposure occurred.

More recently, Weiss *et al.* (1979), reviewing earlier epidemiological studies of a cohort of 1446 male CMME production employees followed from 1960 to 1975, found that the relative risk of lung cancer was significantly increased and there was a dose-response relationship. A review of the pathological evidence suggested that small-cell (oat-cell) carcinoma was a specific response to inhalation of chloro-methylethers.

Analytical Methods

Both the laboratory and epidemiological studies reported above have indicated the need for reliable analytical techniques for the measurement of CMME and, in particular, of BCME in the working environment, and in water supplies contaminated by factory effluents. In this field, a main priority has been to develop methods which allow the continuous monitoring of the workplace atmosphere. High-resolution mass spectrometry was among the first techniques to be used to measure air concentrations of these haloethers (Collier, 1972). BCME was adsorbed from a volume of air onto Porapak and then thermally eluted into the reservoir of the mass spectrometer; the intensity of the ions at m/e 78.9950, $ClCH_2OCH_2^+$ was measured with a resolution of $1/3500$. The method was claimed to be capable of measuring 0.1-ppb levels in air in the presence of other organic chemicals. Tou *et al.* (1974, 1975) used high-resolution mass spectrometry in conjunction with a semi-permeable hollow fibre probe to study the hydrolysis of BCME and to demonstrate the absence of BCME in water-soluble anion resin.

Subsequently, low-resolution mass spectrometry (MS) coupled with gas chromato-graphy (GC) has been used more generally to monitor the 79^+ and 81^+ ions due to the chlorine isotopes. This combination, coupled with the precise GC retention data, was considered to provide adequate specificity. The only difference in the techniques employed was the adsorbing materials employed to concentrate the BCME from the air sample. Thus, Shadoff *et al.* (1973) and Sawicki *et al.* (1975) used Chromosorb 101 in the trapping tube, Ellgehausen (1975) found Porapak Q, Tenax-GC and Chromosorb 1 to be satisfactory and Bruner *et al.* (1978) used Tenax-GC and Carbopak C. In the official method of the US National Institute for Occupational Safety & Health (1977), Chromosorb 101 is specified . Limits of detection are generally of the order of 1 ppb, using a 5-L sample of air. In principle, this limit can be reduced by taking a larger

volume sample. As positive detection of the BCME depends upon obtaining the correct ratio for the $79^+ : 81^+$ ions, which may be affected by the presence of other organic constituents eluted simultaneously, Pellizzari *et al.* (1975, 1976) studied the performance of several adsorbants for analysing a number of hazardous vapour-phase organics, and found Tenax-GC to be superior in most cases. Evans *et al.* (1975) utilized GC coupled with high-resolution MS, monitoring at m/e 78.9950. The method was applicable down to levels of 0.1 ppb v/v on a 1-L sample of air or to 0.01 ppb using a 10-L air sample, and was not subject to the uncertainty resulting from interferences which alter the expected $79^+ : 81^+$ ratio.

A disadvantage of using MS is its unsuitability for on-line monitoring, in that samples must be taken on site and transported to the laboratory. As a result, attention has been directed to the development of suitable GC methods. These have employed direct GC of air samples containing CMME and BCME, which are then detected by flame ionization (FID). The haloethers are concentrated from the sample either by adsorption on Chromosorb 101 or Tenax-GC from which the samples are thermally eluted onto the GC column, or by trapping in a solution containing sodium methoxide or phenoxide which reacts with the CMME and BCME to form bi- and tri-alkoxo ethers, respectively (Frankel & Black, 1976; Parkes *et al.*, 1976; Ault & Solomon, 1977a,b, 1978). These ethers are then extracted, usually in hexane, and the extract is concentrated by evaporation and then analysed by gas chromatography. An alternative approach is to use 2,4,6-trichlorophenol in the presence of sodium methoxide to form the corresponding phenoxy ether, which is sensitive to electron capture detection (Solomon & Kallos, 1975; Sawicki *et al.,* 1976; Solomon, 1976; Kallos, 1977; Ault & Solomon, 1978; Langhorst *et al.*, 1981). A thiolate may similarly be used, with detection by flame photometry (Ault & Solomon, 1977a; Baba & Tanaka, 1978). Both the latter methods are considerably more sensitive than FID.

Problems in the use of trichlorophenol are that more than one derivative can be formed and that, in principle, the large excess of sodium methoxide must contribute to the extensive decomposition of the sample in the scrubbers used to collect the haloether (Yao *et al.*, 1979). It is argued, nevertheless, that the method is sensitive and reproducible and therefore the analytical data are reliable. In a more recent modification of the technique, 'on-column reaction', the potassium salt of 2,4,6-trichlorophenol has been employed (Kallos *et al.*, 1977). A somewhat similar technique in which BCME is converted to a phenoxymethyl derivative on a Tenax-GC adsorption trap has been used by Ven and Venema (1979). A number of these GC methods have been adapted to permit automated analysis of CMME and BCME in air samples (Wilkins & Frankel, 1974; Frankel & Black, 1976; Solomon, 1976; Ault & Solomon, 1976, 1977a,b, 1978; Kallos, 1977).

A personal monitoring badge for the detection of a number of alkylating agents is based on the colour developed with *p*-nitrobenzyl pyridine (Segal *et al.*, 1978). This reagent was also used to monitor the atmosphere of animals in an inhalation study on toxicity of CMME and BCME (Drew *et al.*, 1975).

DEGRADATION TECHNIQUES

Methods that have been proposed for the degradation of BCME and CMME in various media are presented in Tables 1 and 2.

Other relevant chemical information, which might be useful for the treatment of wastes contaminated with CMME or BCME, is reported in Appendix B. This Apppendix includes reactions of BCME and CMME with alkali salts of alcohols, phenols and substituted phenols which have been used as derivatization methods in analytical techniques.

Table 1. Methods used to degrade BCME

Medium	Product	Degradation method	Efficiency	Reference
Solution	Hexamethylene-tetramine and ammonium chloride	Treatment with 1% aqueous ammonia or ammonia containing 50% methanol to assist miscibility with water	100% in 5 min	Alvarez & Rosen (1976)
Solution	Formaldehyde and sodium chloride	1% solutions of sodium hydroxide or sodium carbonate containing 50% methanol	100% in 5 min	Alvarez & Rosen (1976)
Air	Formaldehyde and sodium chloride	Contaminated air may be scrubbed using a 1% solution of sodium carbonate without addition of methanol	100%	Alvarez & Rosen (1976)
Air	Hydrochloric acid, methylal, methanol and formaldehyde	Passage through beds or columns of silica gel (280 mm long) or activated alumina (150 mm long) at temperatures $\simeq 70°C$	Generally 99% or better	Black et al. (1976)

Table 1 (contd)

Medium	Product	Degradation method	Efficiency	Reference
Air		Contaminated air from process of alkylating dihydro-safrole is scrubbed, using an aqueous solution of alkali metal hy-droxide, a lower dialkanol-amine and a lower alkylene glycol operating at temperature range -20° to +40°C	Emissions generally reduced from levels in the order of 10^2 ppb to less than 1 ppb	Velenovsky & Alvarez (1978)
Chloro-acetyl chloride		Contact with hydrogen chloride at a temperature between 30°C in and 160°C in the presence of a catalytic amount of a Lewis acid or strong protic acid such as aluminium chloride or oleum	Content reduced to < 0.3 ppm in 1 h	Dhingra (1977)

Table 2. Methods used to degrade CMME

Medium	Product	Degradation method	Efficiency	Reference
Air	Hydrochloric acid, methylal, methanol and formaldehyde	Passage through beds or columns of silica gel or activated alumina at temperatures ≃ 70°C		Black *et al.* (1976)

RECOMMENDED METHODS OF DEGRADATION