WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

LABORATORY DECONTAMINATION AND DESTRUCTION OF CARCINOGENS IN LABORATORY WASTES: SOME AROMATIC AMINES AND 4-NITROBIPHENYL

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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 authorative information on different aspects of cancer research.

Publications in this series:

Laboratory Decontamination and Destruction of Aflatoxins B_1, B_2, G_1, G_2 in Laboratory Wastes (IARC Scientific Publications No. 37), 1980

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some *N*-Nitrosamines (IARC Scientific Publications No. 43), 1982

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Polycyclic Aromatic Hydrocarbons (IARC Scientific Publications No. 49), 1983

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Hydrazines (IARC Scientific Publications No. 54), 1983

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some *N*-Nitrosamides (IARC Scientific Publications No. 55), 1983

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Haloethers (IARC Scientific Publications No. 61), 1984

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Aromatic Amines and 4-Nitrobiphenyl (IARC Scientific Publications No. 64), 1985

CONTENTS

FOREWORD

As early as 1895, Rehn, in Germany described four cases of bladder cancer in workers employed in the manufacture of magenta, a dye from aniline. Since this time, an enormous amount of work has been devoted to study the biological effects of these compounds, their mode of action, and to determine their presence not only in the industrial environment but also in our general environment. Biochemical research involving these compounds inevitably results in the production of contaminated laboratory wastes and may also lead to the contamination of equipment and work surfaces for which emergency strategies must be available.

In this series, *'Laboratory Decontamination and Destruction of Laboratory Wastes'*, IARC has dealt with the problem of treatment of small quantities of aromatic amines of the laboratory scale; however some of the methods can easily be adapted to the treatment of larger scale contaminations.

The Agency is very grateful for the sustained support of the Division of Safety of the National Institute of Health, USA, in this programme which, it is hoped, will contribute to increase the safety in and around laboratories involved in work with chemical carcinogens.

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 seventh of a series that has thus far included disposal methods for aflatoxins, *N*-nitrosamines, polycyclic aromatic hydrocarbons, hydrazines, *N*-nitrosamides, and haloethers.

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

AROMATIC AMINES CONSIDERED

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

Aromatic amine	Chemical Abstracts Services Registry No.	Chemical Abstracts Name	Abbreviation used
4-Aminobiphenyl	92-67-1	1,1'-Biphenyl-4-amine	4-ABP
Benzidine	92-87-5	(1,1'-Biphenyl)-4,4'-diamine	Bz
3,3'-Dichlorobenzidine	91-94-1	(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-	DCIB
3,3'-Dimethoxybenzidine	119-90-4	(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dimethoxy-	DMoB
3,3'-Dimethylbenzidine	119-93-7	(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dimethyl-	DMB
4,4'-Methylene bis-(2-chloroaniline)	101-14-4	Benzenamine, 4,4'-methylene bis(2-chloro-	MOCA
1-Naphthylamine	134-32-7	1-Naphthalenamine	1-NAP
2-Naphthylamine	91-59-8	2-Naphthalenamine	2-NAP
2,4-Diaminotoluene	95-80-7	1,3-Benzenediamine, 4-methyl-	TOL
*4-Nitrobiphenyl	92-93-3	1,1'-Biphenyl, 4-nitro-	4-NBP

*Although this substance is not an aromatic amine, it has been included for convenience as it was evaluated with the aromatic amines in the IARC *Monograph* series. It is the industrial precursor of 4-aminobiphenyl and its principal metabolite is 4-aminobiphenyl.

INTRODUCTION

Carcinogenicity

Aromatic amines were among the earliest groups of compounds found to be occupationally associated with the occurrence of cancer. The first report was from Germany when bladder cancer was observed in workers employed in the manufacture of fuchsin (magenta) from aniline in dye factories (Rehn, 1895). Although the disease was described as 'aniline cancer', subsequent animal and epidemiological studies have produced only limited evidence for this assumption (IARC, 1982a), and it now seems that impurities such as naphthylamines which might have been present in industrial aniline are more likely to have been the responsible carcinogen.

The vital breakthrough in making the epidemiological link between benzidine and naphthylamine exposure and bladder cancer was made in the study by Case *et al.* (1954) of the British chemical industry. They demonstrated a relatively high risk for workers exposed to these agents. Subsequently, 4-aminobiphenyl, benzidine, *N,N*-bis(2-chloroethyl)-2-naphthylamine and 2-naphthylamine have also been identified as human carcinogens. However, exposure in industry generally involves a number of possibly toxic compounds and it is thus extremely difficult to ascribe excess cancer risks to individual compounds. A pertinent example of this is the rubber industry (IARC, 1982b).

Many aromatic amines have been investigated in animal studies, and about 20 have so far been shown to be carcinogenic, including those shown to be carcinogenic to humans. (IARC, 1972, 1974, 1975, 1978, 1979, 1982a,b,c). Uehleke and Nestel (1967) demonstrated that 4-nitrobiphenyl was reduced to 4-aminobiphenyl; 4-nitrobiphenyl has been shown to induce bladder cancer in dogs although only limited animal experiments have been performed on this compound.

Recent biochemical studies have shown that many carcinogens require metabolic activation. In the case of amines, it has been demonstrated that the first step in this process is *N*-hydroxylation, which is then followed by an esterification to produce the proximate carcinogen, although esterification is not always necessary. The subject has been reviewed by several authors, including Miller and Miller (1969), Weisburger and Weisburger (1973) and Bartsch (1981).

A corollary from these metabolic studies is that, in planning a programme for disposal of laboratory waste involving aromatic amines, the products from biochemical and biological reactions will contain not only the aromatic amines but also the highly carcinogenic and often relatively unstable hydroxylated metabolites. Therefore, both analytical and degradation techniques for these metabolites will be required.

Analysis

The analysis of aromatic amines and some of their metabolites has been reviewed in depth in a recent IARC manual of selected methods of analysis for environmental carcinogens (Egan *et al.*, 1981). This volume also gives a number of methods in complete detail. While the methods selected mainly involve gas chromatography (GC) or high-performance liquid chromatography (HPLC) in the determinative stage, methods employing thin-layer chromatography (TLC), spectrophotometry and spectrofluorimetry are also described. GC may be carried out directly, using flame ionization detection or thermal conductivity. Sensitivity may be increased more than 100 fold by converting the amine to a suitable derivative and using electron capture detection. After reversed-phase HPLC separation, the amines may be determined by ultraviolet (UV), spectrophotometric, spectrofluorimetric or electrochemical detectors. The latter offers somewhat greater sensitivity and selectivity. TLC, although less frequently used, does offer a sensitive method when spectrofluorimetric detection is employed. A range of colorimetric methods is also available. Those most commonly used are based on diazotization of the amine and coupling with a phenol. However, while reasonably sensitive, these methods tend to suffer interference and are less frequently employed, particularly for analysis of biological media.

A number of titrimetric methods have been described in the literature for macro-determination of amines (reference is made to several of those on p. 9), but they mainly involve oxidation to a quinoneimine form of the amines. As these compounds may pose as great a carcinogenic risk as the amines themselves (Brill & Radomski, 1965), such methods are best avoided.

Techniques for the analysis of some metabolites have been discussed in an IARC analytical manual (Egan *et al.*, 1981), in which selected methods are also detailed for hydroxymetabolites of some amines.

Analysis of the hydroxylamine has also been reviewed by Weisburger and Weisburger (1973). Methods include colorimetric determination after reaction with sodium pentacyanoamino ferrate, *para*-dimethylaminobenzaldehyde or salicylaldehyde. Of these, the first appeared to be the most sensitive and the latter two less subject to interference. The *N*-hydroxy compounds can also be analysed on GC after conversion to the volatile silyl derivatives, using bis(trimethylsilyl)acetamide.

DEGRADATION TECHNIQUES

Methods that have been investigated

(a) Chemical methods

Schmitt and Cagle (1975) showed that an aqueous solution of 1% sulfamic acid and 0.5% surfactant could be used to decontaminate surfaces contaminated with MOCA to levels below the detectability limit of 10 μg per 25 in^2, as determined by standard wipe tests and GC analysis. However, the carcinogenicity or lack of carcinogenicity of the product, amine sulfamate, was not demonstrated. Hackman and Rust (1981), in a similar technique for 3,3'- dichlorobenzidine, achieved decontamination of the workplace by spraying an area with a 1:10 mixture of 5% tetrapotassium pyrophosphate and 10% sodium ethyl hexyl sulfate. The washings were collected and then diazotized by addition of ice, sulfuric acid (to pH 1.5) and an excess of sodium nitrite. The tetrazonium compound formed was then allowed to dissociate in water to nitrogen and 3,3'-dichloro 4,4'-dihydroxybiphenyl. Genin (1973) also used diazotization as a method for decontamination of industrial effluents containing benzidine.

The effect of ozonation was investigated for a variety of compounds found as trace constituents in water supplies, including benzidine, and its efficiency was followed using mutagenicity testing. Benzidine was found to give a transient increase in mutagenicity, which disappeared completely after 4 min treatment (Caulfield *et al.* 1979). Burleson *et al.* (1979) found that short periods of ozonation rendered 2-naphthylamine inactive in mutagenicity assays. Klibanov and Morris (1981) developed an oxidation method for the removal of carcinogenic aromatic amines from industrial aqueous effluents. Water was treated with horseradish peroxidase and hydrogen peroxide, producing virtually complete precipitation of the amines in 3 h as cross-linked highly polymerized azo dyes. Mutagenic activity was removed from the water. The only polymeric product tested (that from 5-nitro-1-naphthylamine) was not mutagenic. Similarly, a polymeric product was obtained by water chlorination (Jenkins *et al.*, 1978). By this method, Saunders and Wodak (1966) found that the two main products in the oxidation of 2-naphthylamine were dibenzo[*a,h*]phenazine and 5-(2-naphthylamino)dibenzo[*a,h*]phenazine. Saeed and Warren (1973) have found that the horseradish peroxidase-hydrogen peroxide-catalysed oxidation of 3,3'-dimethoxybenzidine was inhibited by certain anti-inflammatory drugs, probably by competition for oxidant.

(b) Biodegradation methods

Biodegradation techniques have received fairly wide consideration for several aromatic amines. Baird *et al.* (1977) confirmed earlier investigations (Malaney, 1960) which showed that an activated sludge could be acclimatized to aniline, and, with

analytical data from GC/mass spectrometry (MS), showed that, for one selected sludge, benzidine could be 85-93% degraded and 3,3'-dimethoxybenzidine could be 95-100% degraded. A purple-coloured compound formed as an intermediate disappeared after a few days. GC/MS analysis indicated that carcinogenic metabolites did not survive the activated sludge treatment. A second sludge obtained from a different water treatment site was not as effective as the first. *N,N*-Methylated amines were somewhat resistant to degradation.

Pitter (1976) found that 1-naphthylamine was not biodegraded at all by an adapted activated sludge. Tabak and Barth (1978) found that acclimatized, extended aeration sludges could completely oxidize doses of up to 1 mg/L of benzidine without accumulation of degradation products. Joel and Grady (1977), who studied the effect of nitrification on effluent from an activated sludge acclimatized to an aniline concentration of 250 mg/L, found that 100% nitrification could be obtained. If, however, a 'shock' load of aniline produced an increase of as little as 1 mg/L of aniline in the effluent, nitrification was totally inhibited. The operation could be performed sequentially or in a single stage with a sludge mixed with the nitrifying bacteria.

In studies related to biodegradation, Jenkins *et al.* (1978), who examined chlorination of benzidine, aniline and *N,N*-dimethylaniline in aqueous environments, found that amine depletion was optimum at a 1:1 molar ratio of chlorine:amine and was somewhat higher in distilled water than in activated sludge effluent with combined chlorine. Thus, amine depletion in distilled water was about 95% for aniline and 90% for benzidine compared with 80% amine depletion for each in secondary effluent. The method was unsatisfactory for monophenylamines. The product of the chlorination of benzidine was a polymerized quinoneimine, which was not tested for carcinogenicity.

In other biologically related studies, Sikka *et al.* (1978) found that 3,3'-dichlorobenzidine was recalcitrant to naturally occurring microbial communities, but rapidly degraded under natural and artificial light in aqueous solution; it was, however, more stable in organic solvents. Monochlorobenzidine and benzidine were intermediate by-products so that irradiation led to destruction, but not necessarily to detoxification.

Some methods that may warrant investigation for decontamination of laboratory wastes

Using 3,3'-dichlorobenzidine, Pliss (1975) studied the effect of bromination on carcinogenic activity, his thesis being that substituents in the 3 position could tend to hinder hydroxylation of the amino group. He found that 3,3'-dichloro-5,5'-dibromobenzidine did not induce malignant tumours in rats. As bromination may be carried out directly in aqueous or organic solvents, it could offer a simple disposal procedure, as excess bromine can be removed as bromide by a number of reagents. A novel method of bromination of amines was described by Fletcher *et al.* (1957), using *tert*-butyl bromide in dimethyl sulfoxide. The method was claimed to give higher yields than direct bromination and gave no *N*-alkylation. Gutenmann and Lisk (1963) used direct bromination of diphenylamine in an acetone/hexane extract using iodine as a catalyst in a rapid determination of diphenylamine in apples.

One factor that would require further investigation concerns the fate of the brominated amines in the environment. An alternative would be to use a procedure which results in bromination with deamination (Fuchs, 1915; Doyle *et al.*, 1980).

The most common method of deamination involves diazotization and subsequent degradation of the diazo compound by standing, or heating, with water or ethanol, but a number of other diazo reactions may be used. The reactions of diazonium salts are treated extensively in a number of text books of organic chemistry, including an extensive review (Patai, 1978). Methods of oxidation should be treated with caution, as the immediate products of oxidation are frequently quinoneimines (Orr *et al.*, 1956; Berka *et al.*, 1976; Barek & Berka, 1976, 1977), which, as already noted (Brill & Radomski, 1965), may be carcinogenic. Furthermore, the quinoneimines are readily reduced back to the amine by ascorbic acid (Dohnal & Zýka, 1975; Berka *et al.*, 1976). Oxidation with persulfate results in formation of the sulfate ester and the aminophenol (a proximate carcinogen, Sims, 1958). Oxidation may also lead to the formation of nitroso and nitro derivatives (Aksnes & Sandberg, 1957; Gutmann, 1964); and oxidation of benzidine with lead peroxide in chloroform gives the azo compound, 4,4'-diaminoazobiphenyl and diphenylamine (Cheesemann & Praill, 1974). Therefore, although oxidation is comparatively easy to carry out, the products should be carefully tested for carcinogenicity. A potentially useful waste disposal technique is oxidation with neutral or alkaline permanganate which, in primary aromatic amines, results in ring cleavage and then complete breakdown of the molecule (Stewart, 1965; Berka *et al.*, 1976). This does not appear to apply to secondary amines. Diphenylamine is oxidized by alkaline permanganate, and two main products have been identified (Degering, 1950).

Aromatic amines undergo a number of reactions which may temporarily block the amine function. Reactions of amines have been summarized by a number of authors, including Degering (1950), Hickinbottom (1957), Smith (1965) and Sidgwick (1966). Some reactions must be viewed with caution as they may be reversed by hydrolysis.

Oxidation of the hydroxylamine metabolites leads to the formation of nitroso derivatives (Baumgarten *et al.*, 1965) and reduction, to amines (Weisburger & Weisburger, 1973; Becker & Sternson, 1981).

Summary

Three general routes of chemical degradation appear to justify investigation:

(1) Oxidation by alkaline permanganate, ozonation or horseradish peroxidase-hydrogen peroxide

(2) Diazotization and hydrolysis in aqueous or ethanolic solution (the latter may be preferable, as phenolic ethers may be less toxic than the parent hydrocarbons)

(3) Bromination

There are, however, a number of additional reactions which could be considered if 1, 2 or 3 fails to meet the requirements. These are tabulated in Table 1 and in Appendix B.

4-Nitrobiphenyl can be reduced quantitatively by a number of strong reducing agents to 4-aminobiphenyl and then treated accordingly.

In principle, biodegradation appears to be a potentially successful method for decontaminating laboratory waste involving aromatic amines, but the process requires access to suitable activated sludges and an expertise that may be a problem for many laboratories. Thus, it has not been included in Table 1 at this stage.

Table 1. Summary of possible degradation techniques for particular types of waste

Medium	Chemical methods suitable for investigation (in order of preference)
Solid compounds	1[a], 2 or 3
Aqueous solutions	1 or 2
Solutions in organic solvent	3, or 1 or 2 after acid extraction
Glassware	Decontamination (Schmitt & Cagle, 1975; Hackman & Rust, 1981), then 1 or 2
Hoods and fixed equipment	As for glassware
Biological media	1 or 2
Litter	3 or incineration[b]
Animal carcasses	Incineration[b]

1, Oxidation
2, Diazotization
3, Bromination

[a]The enzymatic method must not be used with solid compounds.

[b]It is important that a suitable high-temperature incinerator be employed, as the products of pyrolysis may be carcinogenic.