WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS
ON THE
EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Some Industrial Chemicals and Dyestuffs

VOLUME 29

IARC, LYON, FRANCE

May 1982

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This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans which met in Lyon,

13-20 October 1981

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IARC MONOGRAPHS

In 1971, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. In 1980, the programme was expanded to include the evaluation of the carcinogenic risk associated with employment in specific occupations.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for chemicals and complex mixtures to which humans are known to be exposed, and on specific occupational exposures, to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields, and to indicate where additional research efforts are needed.

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Lyon, 13-20 October 1981

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IARC WORKING GROUP ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS: SOME AROMATIC AMINES, ANTHRAQUINONES AND NITROSO COMPOUNDS, FORMALDEHYDE AND INORGANIC FLUORIDES USED IN DRINKING-WATER AND DENTAL PREPARATIONS

Lyon, 10-17 February 1981

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NOTE TO THE READER

The term "carcinogenic risk" in the *IARC Monograph* series is taken to mean the probability that exposure to the chemical will lead to cancer in humans.

Inclusion of a chemical in the monographs does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that a chemical has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of a chemical for humans is encouraged to make this information available to the Division of Chemical and Biological Carcinogenesis, International Agency for Research on Cancer, Lyon, France, in order that the chemical may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Division of Chemical and Biological Carcinogenesis, so that corrections can be reported in future volumes.

IARC MONOGRAPH PROGRAMME ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

PREAMBLE

1. BACKGROUND

In 1971, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans with the object of producing monographs on individual chemicals[1]. The criteria established at that time to evaluate carcinogenic risk to humans were adopted by all the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monograph* series. In October 1977, a joint IARC/WHO *ad hoc* Working Group met to re-evaluate these guiding criteria; this preamble reflects the results of their deliberations(1) and those of a subsequent IARC *ad hoc* Working Group which met in April 1978(2).

A further *ad hoc* Working Group, which met in Lyon in April 1979 to prepare criteria to select chemicals for *IARC Monographs*(3), recommended that the *Monograph* programme be expanded to include consideration of human exposures to complex mixtures which occur, for example in selected occupations. The Working Group which met in June 1980 therefore considered occupational exposures in the wood, leather and some associated industries; their deliberations resulted in Volume 25 of the *Monograph* series. A further Working Group which met in June 1981 evaluated the carcinogenic risks associated with occupations in the rubber manufacturing industry, and their conclusions were published as Volume 28 of the *Monograph* series.

2. OBJECTIVE AND SCOPE

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for groups of chemicals to which humans are known to be exposed, to evaluate the data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields, and to indicate where additional research efforts are needed.

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[1] Since 1972, the programme has undergone considerable expansion, primarily with the scientific collaboration and financial support of the US National Cancer Institute, Bethesda, MD.

The critical analyses of the data are intended to assist national and international authorities in formulating decisions concerning preventive measures. No recommendations are given concerning legislation, since this depends on risk-benefit evaluations, which seem best made by individual governments and/or other international agencies. In this connection, WHO recommendations on food additives(4), drugs(5), pesticides and contaminants(6) and occupational carcinogens(7) are particularly informative.

Up to November 1981, 29 volumes of the *Monographs* had been published or were in press(8). A total of 585 compounds, industrial processes or occupational exposures had been evaluated or re-evaluated. For 44 chemicals, groups of chemicals, industrial processes or industrial exposures, a positive association or a suspicion of an association with human cancer has been found. For 24 of the individual chemicals or groups of chemicals, exposures are predominantly in occupational settings, although the general population may be exposed through environmental contamination. For 13 chemicals, human exposure was related to therapeutic uses; for one compound, exposure occurs *via* the diet. The preponderance of experimental data over epidemiological data on the 585 compounds or processes is striking; however, for 147 of them, there was *sufficient evidence* of carcinogenicity in experimental animals.

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of environmental chemicals. The first users' survey, made in 1976, indicates that the monographs are consulted routinely by various agencies in 24 countries. Each volume is printed in 4000 copies and distributed *via* the WHO publications service. (See last page for a listing of IARC publications and back outside cover for distribution and sales services.)

3. SELECTION OF CHEMICALS FOR MONOGRAPHS

The chemicals (natural and synthetic, including those which occur as mixtures and in manufacturing processes) are selected for evaluation on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some experimental evidence of carcinogenicity and/or there is some evidence or suspicion of a risk to humans. In certain instances, chemical analogues are also considered. The scientific literature is surveyed for published data relevant to the monograph programme. In addition, the IARC *Survey of Chemicals Being Tested for Carcinogenicity*(9) often indicates those chemicals that may be scheduled for future meetings.

Inclusion of a chemical in a volume does not imply that it is carcinogenic, only that the published data have been examined. The evaluations must be consulted to ascertain the conclusions of the Working Group. Equally, the fact that a chemical has not appeared in a monograph does not mean that it is without carcinogenic hazard.

As new data on chemicals for which monographs have already been prepared and new principles for evaluating carcinogenic risk receive acceptance, re-evaluations will be made at subsequent meetings, and revised monographs will be published as necessary.

4. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, a list of the substances to be considered is prepared by IARC staff in consultation with other experts. Subsequently, all relevant biological data are collected by IARC; in addition to the published literature, US Public Health Service Publication No. 149(10) has been particularly valuable and has been used in conjunction with other recognized sources of information on chemical carcinogenesis and systems such as CANCERLINE, MEDLINE and TOXLINE. The major collection of data and the preparation of first drafts for the sections on chemical and physical properties, on production, use, occurrence and on analysis are carried out by SRI International, Stanford, CA, USA under a separate contract with the US National Cancer Institute. Most of the data so obtained on production, use and occurrence refer to the United States and Japan; SRI International and IARC supplement this information with that from other sources in Europe. Bibliographical sources for data on mutagenicity and teratogenicity are the Environmental Mutagen Information Center and the Environmental Teratology Information Center, both located at the Oak Ridge National Laboratory, TN, USA.

Six to nine months before the meeting, reprints of articles containing relevant biological data are sent to an expert(s), or are used by the IARC staff, for the preparation of first draft monographs. These drafts are edited by IARC staff and are sent prior to the meeting to all participants of the Working Group for their comments. The Working Group then meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, then edited by a professional editor and prepared for reproduction. The monographs are usually published within six months after the Working Group meeting.

5. DATA FOR EVALUATIONS

With regard to biological data, only reports that have been published or accepted for publication are reviewed by the working groups, although a few exceptions have been made; often, reports from government agencies that have undergone peer review and are widely available are considered. The monographs do not cite all of the literature on a particular chemical: only those data considered by the Working Group to be relevant to the evaluation of the carcinogenic risk of the chemical to humans are included.

Anyone who is aware of data that have been published or are in press which are relevant to the evaluations of the carcinogenic risk to humans of chemicals for which monographs have appeared is urged to make them available to the Division of Environmental Carcinogenesis, International Agency for Research on Cancer, Lyon, France.

6. THE WORKING GROUP

The tasks of the Working Group are five-fold: (a) to ascertain that all data have been collected; (b) to select the data relevant for the evaluation; (c) to ensure that the summaries of the data enable the reader to follow the reasoning of the committee; (d) to judge the significance of the results of experimental and epidemiological studies; and (e) to make an evaluation of the carcinogenic risk of the chemical.

Working Group participants who contributed to the consideration and evaluation of chemicals within a particular volume are listed, with their addresses, at the beginning of each publication (see p. 5). Each member serves as an individual scientist and not as a representative of any organization or government. In addition, observers are often invited from national and international agencies, organizations and industrial associations.

7. GENERAL PRINCIPLES FOR EVALUATING THE CARCINOGENIC RISK OF CHEMICALS

The widely accepted meaning of the term 'chemical carcinogenesis', and that used in these monographs, is the induction by chemicals of neoplasms that are not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, and/or the induction by chemicals of more neoplasms than are usually found - although fundamentally different mechanisms may be involved in these three situations. Etymologically, the term 'carcinogenesis' means the induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign tumours. In the monographs, the words 'tumour' and 'neoplasm' are used interchangeably. (In scientific literature the terms 'tumourigen', 'oncogen' and 'blastomogen' have all been used synonymously with 'carcinogen', although occasionally 'tumourigen' has been used specifically to denote a substance that induces benign tumours.)

(*a*) *Experimental Evidence*

(i) *Qualitative aspects*

Both the interpretation and evaluation of a particular study as well as the overall assessment of the carcinogenic activity of a chemical involve several qualitatively important considerations, including: (a) the experimental parameters under which the chemical was tested, including route of administration and exposure, species, strain, sex, age, etc.; (b) the consistency with which the chemical has been shown to be carcinogenic, e.g., in how many species and at which target organ(s); (c) the spectrum of neoplastic response, from benign neoplasm to multiple malignant tumours; (d) the stage of tumour formation in which a chemical may be involved: some chemicals act as complete carcinogens and have initiating and promoting activity, while others are promoters only; and (e) the possible role of modifying factors.

There are problems not only of differential survival but of differential toxicity, which may be manifested by unequal growth and weight gain in treated and control animals. These complexities are also considered in the interpretation of data.

Many chemicals induce both benign and malignant tumours. Few instances are recorded in which only benign neoplasms are induced by chemicals that have been studied extensively. Benign tumours may represent a stage in the evolution of a malignant neoplasm or they may be 'end-points' that do not readily undergo transition to malignancy. If a substance is found to induce only benign tumours in experimental animals, it should be suspected of being a carcinogen and requires further investigation.

(ii) *Hormonal carcinogenesis*

Hormonal carcinogenesis presents certain distinctive features: the chemicals involved occur both endogenously and exogenously; in many instances, long exposure is required; tumours occur in the target tissue in association with a stimulation of non-neoplastic growth, but in some cases hormones promote the proliferation of tumour cells in a target organ. Hormones that occur in excessive amounts, hormone-mimetic agents and agents that cause hyperactivity or imbalance in the endocrine system may require evaluative methods comparable with those used to identify chemical carcinogens; particular emphasis must be laid on quantitative aspects and duration of exposure. Some chemical carcinogens have significant side effects on the endocrine system, which may also result in hormonal carcinogenesis. Synthetic hormones and anti- hormones can be expected to possess other pharmacological and toxicological actions in addition to those on the endocrine system, and in this respect they must be treated like any other chemical with regard to intrinsic carcinogenic potential.

(iii) *Quantitative aspects*

Dose-response studies are important in the evaluation of carcinogenesis: the confidence with which a carcinogenic effect can be established is strengthened by the observation of an increasing incidence of neoplasms with increasing exposure.

The assessment of carcinogenicity in animals is frequently complicated by recognized differences among the test animals (species, strain, sex, age), route(s) of administration and in dose/duration of exposure; often, target organs at which a cancer occurs and its histological type may vary with these parameters. Nevertheless, indices of carcinogenic potency in particular experimental systems [for instance, the dose-rate required under continuous exposure to halve the probability of the animals remaining tumourless(11)] have been formulated in the hope that, at least among categories of fairly similar agents, such indices may be of some predictive value in other systems, including humans.

Chemical carcinogens differ widely in the dose required to produce a given level of tumour induction, although many of them share common biological properties, which include metabolism to reactive [electrophilic(12-14)] intermediates capable of interacting with DNA. The reason for this variation in dose-response is not understood, but it may be due either to differences within a common metabolic process or to the operation of qualitatively distinct mechanisms.

(iv) *Statistical analysis of animal studies*

Tumours which would have arisen had an animal lived longer may not be observed because of the death of the animal from unrelated causes, and this possibility must be allowed for. Various analytical techniques have been developed which use the assumption of independence of competing risks to allow for the effects of intercurrent mortality on the final numbers of tumour-bearing animals in particular treatment groups.

For externally visible tumours and for neoplasms that cause death, methods such as Kaplan-Meier (i.e., 'life-table', 'product-limit' or 'actuarial') estimates(11), with associated significance tests(15,16), have been recommended.

For internal neoplasms which are discovered 'incidentally'(15) at autopsy but which did not cause the death of the host, different estimates(17) and significance tests(15,16) may be necessary for the unbiased study of the numbers of tumour-bearing animals.

All of these methods(11,15-17) can be used to analyse the numbers of animals bearing particular tumour types, but they do not distinguish between animals with one or many such tumours. In experiments which end at a particular fixed time, with the simultaneous sacrifice of many animals, analysis of the total numbers of internal neoplasms per animal found at autopsy at the end of the experiment is straightforward. However, there are no adequate statistical methods for analysing the numbers of particular neoplasms that kill an animal. The design and statistical analysis of long-term carcinogenicity experiments were recently reviewed, in Supplement 2 to the *Monograph* series(18).

(*b*) *Evidence of Carcinogenicity in Humans*

Evidence of carcinogenicity in humans can be derived from three types of study, the first two of which usually provide only suggestive evidence: (1) reports concerning individual cancer patients (case reports), including a history of exposure to the supposed carcinogenic agent; (2) descriptive epidemiological studies in which the incidence of cancer in human populations is found to vary (spatially or temporally) with exposure to the agent; and (3) analytical epidemiological studies (e.g., case-control or cohort studies) in which individual exposure to the agent is found to be associated with an increased risk of cancer.

An analytical study that shows a positive association between an agent and a cancer may be interpreted as implying causality to a greater or lesser extent, on the basis of the following criteria: (a) There is no identifiable positive bias. (By 'positive bias' is meant the operation of factors in study design or execution which lead erroneously to a more strongly positive association between an agent and disease than in fact exists. Examples of positive bias include, in case-control studies, better documentation of exposure to the agent for cases than for controls, and, in cohort studies, the use of better means of detecting cancer in individuals exposed to the agent than in individuals not exposed.) (b) The possibility of positive confounding has been considered. (By 'positive confounding' is meant a situation in which the relationship between an agent and a disease is rendered more strongly positive than it truly is as a result of an association between that agent and another agent which either causes or prevents the disease. An example of positive confounding is the association between coffee consumption and lung cancer, which results from their joint association with cigarette smoking.) (c) The association is unlikely to be due to chance alone. (d) The association is strong. (e) There is a dose- response relationship.

In some instances, a single epidemiological study may be strongly indicative of a cause-effect relationship; however, the most convincing evidence of causality comes when several independent studies done under different circumstances result in 'positive' findings.

Analytical epidemiological studies that show no association between an agent and a cancer ('negative' studies) should be interpreted according to criteria analogous to those listed above: (a) there is no identifiable negative bias; (b) the possibility of negative confounding has been considered; and (c) the possible effects of misclassification of exposure or outcome have been weighed. In addition, it must be recognized that in any study there are confidence limits around the estimate of association or relative risk. In a study regarded as 'negative', the upper confidence limit may indicate a relative risk substantially greater than unity; in that case, the study excludes only relative risks that are above the upper limit. This usually means that a 'negative' study must be large to be convincing. Confidence in a 'negative' result is increased when several independent studies carried out under different circumstances are in agreement. Finally, a 'negative' study may be considered to be relevant only to dose levels within or below the range of those observed in the study and is pertinent only if sufficient time has elapsed since first human exposure to the agent. Experience with human cancers of known etiology suggests that the period from first exposure to a chemical carcinogen to development of clinically observed cancer is usually measured in decades and may be in excess of 30 years.

The Working Group whose deliberations resulted in Supplement 1 to the *Monographs* (20) defined *sufficient evidence* of the carcinogenicity of a chemical to humans as that which provides a causal association between exposure and cancer; *limited evidence* was defined as that which indicates a possible carcinogenic effect in humans.

(c) *Relevance of Experimental Data to the Evaluation of Carcinogenic Risk to Humans*

No adequate criteria are presently available to interpret experimental carcinogenicity data directly in terms of carcinogenic potential for humans. Nonetheless, utilizing data collected from appropriate tests in animals, positive extrapolations to possible human risk can be approximated.

Information compiled from the first 29 volumes of the *IARC Monographs*(19-21) shows that of the 44 chemicals, groups of chemicals, manufacturing processes or occupational exposures now generally accepted to cause or probably to cause cancer in humans, all (with the possible exception of arsenic) of those which have been tested appropriately produce cancer in at least one animal species. For several of the chemicals that are carcinogenic for humans (aflatoxins, 4-aminobiphenyl, diethylstilboestrol, melphalan, mustard gas and vinyl chloride), evidence of carcinogenicity in experimental animals preceded evidence obtained from epidemiological studies or case reports.

In general, the evidence that a chemical produces tumours in experimental animals is of two degrees: (a) *sufficient evidence* of carcinogenicity is provided by the production of malignant tumours; and (b) *limited evidence* of carcinogenicity reflects qualitative and/or quantitative limitations of the experimental results.

Sufficient evidence of carcinogenicity is provided by experimental studies that show an increased incidence of malignant tumours: (i) in multiple species or strains, and/or

(ii) in multiple experiments (routes and/or doses), and/or (iii) to an unusual degree (with regard to incidence, site, type and/or precocity of onset). Additional evidence may be provided by data concerning dose-response, mutagenicity or structure.

For many of the chemicals evaluated in the first 29 volumes of the *IARC Monographs* for which there is *sufficient evidence* of carcinogenicity in animals, data relating to carcinogenicity for humans are either insufficient or nonexistent. *In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans.* The use of the expressions 'for practical purposes' and 'as if they presented a carcinogenic risk' indicates that at the present time a correlation between carcinogenicity in animals and possible human risk cannot be made on a purely scientific basis, but only pragmatically. Such a pragmatical correlation may be useful to regulatory agencies in making decisions related to the primary prevention of cancer.

In the present state of knowledge, it would be difficult to define a predictable relationship between the dose (mg/kg bw/day) of a particular chemical required to produce cancer in test animals and the dose which would produce a similar incidence of cancer in humans. The available data suggest, however, that such a relationship may exist(22,23), at least for certain classes of carcinogenic chemicals. Data that provide *sufficient evidence* of carcinogenicity in test animals may therefore be used in an approximate quantitative evaluation of the human risk at some given exposure level, provided that the nature of the chemical concerned and the physiological, pharmacological and toxicological differences between the test animals and humans are taken into account. However, no acceptable methods are currently available for quantifying the possible errors in such a procedure, whether it is used to generalize among species or to extrapolate from high to low doses. The methodology for such quantitative extrapolation to humans requires further development.

Evidence for the carcinogenicity of some chemicals in experimental animals may be *limited* for two reasons. Firstly, experimental data may be restricted to such a point that it is not possible to determine a causal relationship between administration of a chemical and the development of a particular lesion in the animals. Secondly, there are certain neoplasms, including lung tumours and hepatomas in mice, which may occur spontaneously in high incidence in certain strains, and their malignancy has often been difficult to establish in the past. An evaluation of the significance of these tumours following administration of a chemical is the responsibility of particular working groups preparing individual monographs, and it has not been possible to set down rigid guidelines; the relevance of these tumours must be determined by considerations which include experimental design, completeness of reporting and consideration of all available information on the chemical being tested.

Some chemicals for which there is *limited evidence* of carcinogenicity in animals have also been studied in humans with, in general, inconclusive results. While such chemicals may indeed be carcinogenic to humans, more experimental and epidemiological investigation is required.

Hence, *'sufficient evidence'* of carcinogenicity and *'limited evidence'* of carcinogenicity do not indicate categories of chemicals: the inherent definitions of those terms indicate varying degrees of experimental evidence, which may change if and when new data on the chemicals become available. The main drawback to any rigid classification

of chemicals with regard to their carcinogenic capacity is the as yet incomplete knowledge of the mechanism(s) of carcinogenesis.

In recent years, several short-term tests for the detection of potential carcinogens have been developed. When only inadequate experimental data are available, positive results in validated short- term tests (see section 8(*c*)(ii)) are an indication that the compound is a potential carcinogen and that it should be tested in animals for an assessment of its carcinogenicity. Negative results from short-term tests cannot be considered as evidence to rule out carcinogenicity. Whether short-term tests will eventually be as reliable as long-term tests in predicting carcinogenicity in humans will depend on further demonstrations of consistency with long-term experiments and with data from humans. Available screening assays are evaluated in Supplement 2 to the *Monographs*(18).

8. EXPLANATORY NOTES ON THE MONOGRAPH CONTENTS

(a) Chemical and Physical Data (Section 1)

The Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name (9th Collective Index)(24) and the IUPAC Systematic Name(25) are recorded in section 1. Other synonyms and trade names are given, but no comprehensive list is provided. Further, some of the trade names are those of mixtures in which the compound being evaluated is only one of the ingredients.

The structural and molecular formulae, molecular weight and chemical and physical properties are given. The properties listed refer to the pure substance, unless otherwise specified, and include, in particular, data that might be relevant to carcinogenicity (e.g., lipid solubility) and those that concern identification.

A separate description of the composition of technical products includes available information on impurities and formulated products.

(b) Production, Use, Occurrence and Analysis (Section 2)

The purpose of section 2 is to provide indications of the extent of past and present human exposure to the chemical.

(i) *Synthesis*

Since cancer is a delayed toxic effect, the dates of first synthesis and of first commercial production of the chemical are provided. In addition, methods of synthesis used in past and present commercial production are described. This information allows a reasonable estimate to be made of the date before which no human exposure could have occurred.

(ii) *Production*

Since Europe, Japan and the United States are reasonably representative industrialized areas of the world, most data on production, foreign trade and uses are obtained from those countries. It should not, however, be inferred that those nations are the sole or even the major sources or users of any individual chemical.

Production and foreign trade data are obtained from both governmental and trade publications by chemical economists in the three geographical areas. In some cases, separate production data on organic chemicals manufactured in the United States are not available because their publication could disclose confidential information. In such cases, an indication of the minimum quantity produced can be inferred from the number of companies reporting commercial production. Each company is required to report on individual chemicals if the sales value or the weight of the annual production exceeds a specified minimum level. These levels vary for chemicals classified for different uses, e.g., medicinals and plastics; in fact, the minimal annual sales value is between $1000 and $50 000, and the minimal annual weight of production is between 450 and 22 700 kg. Data on production in some European countries are obtained by means of general questionnaires sent to companies thought to produce the compounds being evaluated. Information from the completed questionnaires is compiled by country, and the resulting estimates of production are included in the individual monographs.

(iii) *Use*

Information on uses is meant to serve as a guide only and is not complete. It is usually obtained from published data but is often complemented by direct contact with manufacturers of the chemical. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their clinical efficacy.

Statements concerning regulations and standards (e.g., pesticide registrations, maximum levels permitted in foods, occupational standards and allowable limits) in specific countries are mentioned as examples only. They may not reflect the most recent situation, since such legislation is in a constant state of change; nor should it be taken to imply that other countries do not have similar regulations.

(iv) *Occurrence*

Information on the occurrence of a chemical in the environment is obtained from published data, including that derived from the monitoring and surveillance of levels of the chemical in occupational environments, air, water, soil, foods and tissues of animals and humans. When available, data on the generation, persistence and bioaccumulation of a chemical are also included.

(v) *Analysis*

The purpose of the section on analysis is to give the reader an indication, rather than a complete review, of methods cited in the literature. No attempt is made to evaluate critically or to recommend any of the methods.

(c) Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans (Section 3)

In general, the data recorded in section 3 are summarized as given by the author; however, comments made by the Working Group on certain shortcomings of reporting, of statistical analysis or of experimental design are given in square brackets. The nature and extent of impurities/contaminants in the chemicals being tested are given when available.

(i) *Carcinogenicity studies in animals*

The monographs are not intended to cover all reported studies. Some studies are purposely omitted (a) because they are inadequate, as judged from previously described criteria(26-29) (e.g., too short a duration, too few animals, poor survival); (b) because they only confirm findings that have already been fully described; or (c) because they are judged irrelevant for the purpose of the evaluation. In certain cases, however, such studies are mentioned briefly, particularly when the information is considered to be a useful supplement to other reports or when it is the only data available. Their inclusion does not, however, imply acceptance of the adequacy of their experimental design or of the analysis and interpretation of their results.

Mention is made of all routes of administration by which the compound has been adequately tested and of all species in which relevant tests have been done(6,28). In most cases, animal strains are given. [General characteristics of mouse strains have been reviewed(30).] Quantitative data are given to indicate the order of magnitude of the effective carcinogenic doses. In general, the doses and schedules are indicated as they appear in the paper; sometimes units have been converted for easier comparison. Experiments in which the compound was administered in conjunction with known carcinogens and experiments on factors that modify the carcinogenic effect are also reported. Experiments on the carcinogenicity of known metabolites, chemical precursors, analogues and derivatives are also included.

(ii) *Other relevant biological data*

Lethality data are given when available, and other data on toxicity are included when considered relevant. The metabolic data are restricted to studies that show the metabolic fate of the chemical in animals and humans, and comparisons of data from animals and humans are made when possible. Information is also given on absorption, distribution, excretion and placental transfer.

Prenatal toxicity. Data on effects on reproduction, teratogenicity and feto- and embryotoxicity from studies in experimental animals and from observations in humans are also included. There appears to be no causal relationship between teratogenicity(31) and carcinogenicity, but chemicals often have both properties. Evidence of prenatal toxicity suggests transplacental transfer, which is a prerequisite for transplacental carcinogenesis.

Indirect tests (mutagenicity and other short-term tests). Data from indirect tests are also included. Since most of these tests have the advantage of taking less time and being less expensive than mammalian carcinogenicity studies, they are generally known as 'short-term' tests. They comprise assay procedures which rely on the induction of biological and biochemical effects in *in vivo* and/or *in vitro* systems. The end-point of

the majority of these tests is the production not of neoplasms in animals but of changes at the molecular, cellular or multicellular level: these include the induction of DNA damage and repair, mutagenesis in bacteria and other organisms, transformation of mammalian cells in culture, and other systems.

The short-term tests may be useful (a) in predicting potential carcinogenicity in the absence of carcinogenicity data in animals, (b) as a contribution in deciding which chemicals should be tested in animals, (c) in identifying active fractions of complex mixtures containing carcinogens, (d) for recognizing active metabolites of known carcinogens in human and/or animal body fluids and (e) in helping to elucidate mechanisms of carcinogenesis. [See Supplement 2 to the *Monographs*(18) and references 48-56.]

Although the theory that cancer is induced as a result of somatic mutation suggests that agents which damage DNA *in vivo* may be carcinogens, the precise relevance of short-term tests to the mechanism by which cancer is induced is not known. Predictions of potential carcinogenicity are currently based on correlations between responses in short-term tests and data from animal carcinogenicity and/or human epidemiological studies. This approach is limited because the number of chemicals known to be carcinogenic in humans is insufficient to provide a basis for validation, and most validation studies involve chemicals that have been evaluated for carcinogenicity only in animals. The selection of chemicals is in turn limited to those classes for which data on carcinogenicity are available. The results of validation studies could be strongly influenced by such selection of chemicals and by the proportion of carcinogens in the series of chemicals tested; this should be kept in mind when evaluating the predictivity of a particular test. The usefulness of any test is reflected by its ability to classify carcinogens and noncarcinogens, using the animal data as a standard; however, animal tests may not always provide a perfect standard. The attainable level of correlation between short-term tests and animal bioassays is still under investigation.

Since many chemicals require metabolism to an active form, tests that do not take this into account may fail to detect certain potential carcinogens. The metabolic activation systems used in short-term tests (e.g., the cell-free systems used in bacterial tests) are meant to approximate the metabolic capacity of the whole organism. Each test has its advantages and limitations; thus, more confidence can be placed in the conclusions when negative or positive results for a chemical are confirmed in several such test systems. Deficiencies in metabolic competence may lead to misclassification of chemicals, which means that not all tests are suitable for assessing the potential carcinogenicity of all classes of compounds.

The present state of knowledge does not permit the selection of a specific test(s) as the most appropriate for identifying potential carcinogenicity. Before the results of a particular test can be considered to be fully acceptable for predicting potential carcinogenicity, certain criteria should be met: (a) the test should have been validated with respect to known animal carcinogens and found to have a high capacity for discriminating between carcinogens and noncarcinogens, and (b), when possible, a structurally related carcinogen(s) and noncarcinogen(s) should have been tested simultaneously with the chemical in question. The results should have been reproduced in different laboratories, and a prediction of carcinogenicity should have been confirmed in additional test systems. Confidence in positive results is increased if a mechanism of action can be deduced and if appropriate dose-response data are available. For optimum usefulness, data on purity must be given.

The short-term tests in current use that have been the most extensively validated are the *Salmonella typhimurium* plate-incorporation assay(32-36), the X-linked recessive lethal test in *Drosophila melano gaster*(37), unscheduled DNA synthesis(38) and *in vitro* transformation (36,39). Each is compatible with current concepts of the possible mechanism(s) of carcinogenesis.

An adequate assessment of the genetic activity of a chemical depends on data from a wide range of test systems. The monographs include, therefore, data not only from those already mentioned, but also on the induction of point mutations in other systems(40-45), on structural(46) and numerical chromosomal aberrations, including dominant lethal effects(47), on mitotic recombination in fungi(40) and on sister chromatid exchanges (48-50).

The existence of a correlation between quantitative aspects of mutagenic and carcinogenic activity has been suggested(6,47-53), but it is not sufficiently well established to allow general use.

Further information about mutagenicity and other short-term tests is given in references 48-56.

(iii) *Case reports and epidemiological studies*

Observations in humans are summarized in this section. The criteria for including a study in this section are described above (section 7(*b*)).

(d) Summary of Data Reported and Evaluation (Section 4)

Section 4 summarizes the relevant data from animals and humans and gives the critical views of the Working Group on those data.

(i) *Experimental data*

Data relevant to the evaluation of the carcinogenicity of the chemical in animals are summarized in this section. The animal species mentioned are those in which the carcinogenicity of the substance was clearly demonstrated. Tumour sites are also indicated. If the substance has produced tumours after prenatal exposure or in single-dose experiments, this is indicated. Dose-response data are given when available.

Results from validated mutagenicity and other short-term tests and from tests for prenatal toxicity are reported if the Working Group considered the data to be relevant.

(ii) *Human data*

Human exposure to the chemical is summarized on the basis of data on production, use and occurrence. Case reports and epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are described. Other biological data which are considered to be relevant are also mentioned.

(iii) *Evaluation*

This section comprises the overall evaluation by the Working Group of the carcinogenic risk of the chemical, complex mixture or occupational exposure to humans. All of the data in the monograph, and particularly the summarized experimental and human data, are considered in order to make this evaluation.

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GENERAL REMARKS ON THE SUBSTANCES CONSIDERED

This twenty-ninth volume of *IARC Monographs* covers a number of miscellaneous industrial chemicals, including benzene, benzidine and three benzidine-based dyes. Since new data had become available on some of these compounds that had been evaluated previously - benzyl chloride, benzene, benzidine and its salts, diaminodiphenyl ether, *ortho-* and *para*-dichlorobenzene and 3,3′-dichlorobenzidine and its salts (IARC, 1972, 1974a,b, 1976, 1978) - these monographs were updated and new evaluations made.

In February 1981, an IARC Working Group considered, among other chemicals, formaldehyde. That Group was aware of experiments in progress at New York University and the Chemical Industries Institute of Toxicology; but, since the final results of those studies had not yet been published, they could not be taken into account in making an evaluation of the possible carcinogenicity of formaldehyde. Since the beginning of the *Monograph* programme, the IARC has followed the rule of considering published data only in making evaluations. It was decided, therefore, to postpone the publication of the monograph on formaldehyde until a further group would have considered the additional data. The present Working Group therefore reviewed the monograph prepared in February, added summaries of the new data that had become available and made its own evaluation of the carcinogenicity of formaldehyde. The Working Group was aware, however, that a number of studies were still in progress on the mechanism of action of formaldehyde and on epidemiological aspects of exposure to this compound.

Of the other compounds considered, benzidine is an important azo dye intermediate, and 3,3′-dichlorobenzidine is the starting material for a series of organic pigments. Also included are monographs on three benzidine-based dues (Direct Black 38, Direct Blue 6 and Direct Brown 95) that have been tested for carcinogenicity. Direct Black 38 is produced in the US in larger quantities than any other benzidine-based dye, and the other two dyes are important commercially; however, another 14 benzidine-based dyes were still being produced commercially in the US in 1979. Eight organic pigments derived from 3,3′-dichlorobenzidine were also being produced in the US and Japan. Available data on Japanese production of the eight pigments in 1979 and 1980, on US production (or sales) and imports of the 14 dyes and eight pigments during the period 1971-1979 and listings of their principal uses are given in an Appendix to these Remarks.

Toxicity or carcinogenesis attributed to a chemical is often due to one or more of its metabolites, acting by several mechanisms which may include covalent binding of chemically reactive intermediates to cellular macromolecules (Jollow *et al.*, 1977). Thus, there is evidence that some of the compounds discussed in this volume exert their adverse effects only after metabolic activation: these include the dyes Direct Black 38, Direct Blue 6 and Direct Brown 95, each of which is metabolically degraded to form benzidine as one of its metabolites (Rinde and Troll, 1975; National Institute for Occupational Safety and Health, 1980). The carcinogenicity of these dyes is thought to result from the activation of benzidine to ultimate carcinogenic forms rather than from

the dyes themselves. By the same token, the hepatotoxicity of *ortho*-dichlorobenzene has been reported to be related to its metabolic activation and covalent binding (Reid and Krishna, 1973); and the toxicity of benzene to bone marrow also appears to be the result of metabolic activation (Snyder *et al.*, 1981). It is expected that similar mechanisms apply to other compounds reviewed in this volume.

In the statistical analysis of the data on carcinogenicity in animals, in experiments in which several doses were tested, statistical significance was based on a test of positive trend with dose, as recommended in the Annex to Supplement 2 of this *Monograph* series (IARC, 1980). Pairwise comparisons between groups were sometimes included for illustrative purposes. On many occasions, the reported data were insufficient for a full life-table analysis, and crude comparisons were made, unadjusted for different survival in the experimental groups. If survival was shorter in the groups with greater exposure, such crude comparisons were conservative estimates.

Epidemiological assessment of the carcinogenicity of the chemical compounds evaluated in this volume has involved several features not previously considered in the *IARC Monograph* series. First, there has been formal mathematical evaluation of the statistical power of the studies considered, particularly of studies that present apparently negative conclusions (Beaumont and Breslow, 1981). Power is defined as the probability that a study will detect in a population an increase in disease or death over that expected, if such an increase has in fact occurred. Alternatively, power may be defined as the probability that a particular study will not provide a false negative result (a type II statistical error). The power of a study increases with increase in size of the exposed population under study and as the ratio of observed to expected events (risk ratio) rises.

The concept of power is related to the fact that in any study there are limits of confidence around the estimates of relative risk (see preamble, section 7(*b*)). However, specific calculation of the power of a particular study to detect a doubling, trebling or other increase in the relative risk of disease or death provides a more direct statement of the adequacy of that study for evaluating the carcinogenicity of an exposure.

Another new aspect is that, for the first time in the *IARC Monograph* series, and in response to insistent demands addressed to the Agency, this Working Group has attempted to make quantitative estimates of carcinogenic risk. These estimates were based entirely on data from human epidemiological studies and did not incorporate the results of studies in animals. The reasoning used to arrive at such statements is described in the introduction to the Annex at the end of this volume, which includes the estimates made for two substances, benzene and benzidine. Those two compounds were chosen since it was considered that some of the available studies contained enough information to do so; however, it should be emphasized that, even in these cases, exposures solely to the compound in question were rare.

Assessment of most of the epidemiological studies evaluated in these monographs had to take into consideration the fact that people had had mixed exposures. This problem is a perennial one in epidemiology, particularly in studies of workplace exposures. Among the compounds considered in this volume, mixed exposures were seen particularly with regard to 3,3'-dichlorobenzidine, which may be produced in plants where benzidine is made, with regard to the benzidine-based dyes, and with regard to the production of benzoyl chloride. In such cases, it is sometimes necessary to evaluate total exposure to the production process rather than that to any one compound alone. That practice was followed in certain of the monographs in this volume.

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Appendix. Additional Commercial Dyes and Pigments Derived from Benzidine and 3,3′-Dichlorobenzidine

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1000 kg (year)	US Imports[b] 1000 kg (year)	Use[c]
Acid Red 85	3567-65-5	74 (1971)	4 (1971)	Colourant for dyeing and printing textiles and for casein plastics and synthetic resins Leather dye
		57 (1972)	< 1 (1972)	
C.I. 22245	1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-((4′-((4-(((4-methylphenyl)sulfonyl)-oxy)phenyl)azo) (1,1′-biphenyl)-4-yl)azo)-, disodium salt	77 (1973)	7 (1973)	
		> 1 (1974)	3 (1974)	
		30 (1975)	1 (1975)	
		33 (1976)	1 (1976)	
		> 4 (1977)		
		18 (1978)	< 1 (1978)	
		> 2 (1979)	1 (1979)	
		> 2 (1980)	7 (1980)	
Direct Black 4	2429-83-6	39 (1971)		Colourant for dyeing and printing textiles Leather dye Beater dye for paper
		45 (1972)		
C.I. 30245	2,7-Naphthalenedisulfonic acid, 4-amino-3-((4′-((2,4-diamino-5-methylphenyl)azo)-(1,1′-biphenyl)-4-yl)azo)-5-hydroxy-6-(phenylazo)-, disodium salt	75 (1973)		
		5 (1974) (sales)		
		> 1 (1975)		
		> 4 (1976)		
		> 4 (1977)		
		12 (1978)		
		> 2 (1979)		
Direct Blue 2	2429-73-4	580 (1971)		Colourant for dyeing cellulose and leather Beater dye for paper
		465 (1972)		
C.I. 22590	2,7-Naphthalenedisulfonic acid, 5-amino-3-((4′-((7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo) (1,1′-biphenyl)-4-yl)azo)-4-hydroxy-, trisodium salt	539 (1973)		
		> 1 (1974)		
		110 (1975) (sales)	5 (1975)	
		350 (1976)	17 1976)	
		> 4 (1977)		
		99 (1978)	14 (1978)	
		> 2 (1979)	32 (1979)	
		> 2 (1980)	25 (1980)	

Direct Brown 2	2429-82-5	119 (1971)		Colourant for dyeing and printing textiles
		117 (1972)		
C.I. 22311	Benzoic acid, 5-((4′-((7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo) (1,1′-biphenyl)-4-yl)azo)-2-hydroxy, disodium salt	67 (1973)		Leather dye
		> 1 (1974)		Beater dye for paper
		57 (1975)	1 (1975)	Heavy metal salts used as pigments
		85 (1976)	9 (1976)	
		> 4 (1977)	1 (1977)	
		13 (1978)		
		> 2 (1979)	6 (1979)	
		> 2 (1980)	4 (1980)	
Direct Brown 6	2893-80-3	> 1 (1971)		Colourant for dyeing textiles and leather
		> 0.5 (1972)		
C.I. 30140	Benzoic acid, 5-((4′-((2,4-dihydroxy-3-((4-sulfophenyl)-azo)phenyl)azo) (1,1′-biphenyl)-4-yl)azo)-2-hydroxy-, disodium salt	> 0.5 (1973)		Beater dye for paper
		> 0.5 (1975)		
		> 2 (1976)	3 (1976)	
		> 2 (1977)		
		4 (1978)		
		> 2 (1979)		
Direct Brown 31	2429-81-4	75 (1971)		Colourant for dyeing and printing cellulose
		55 (1972)		
C.I. 35660	Benzoic acid, 5-((4′-((2,6-diamino-3-((8-hydroxy-3,6-disulfo-7-((4-sulfo-1-naphthalenyl)azo)-2-naphthalenyl)azo)-5-methylphenyl)azo)-(1,1′-biphenyl)-4-yl)azo)-2-hydroxy-, tetrasodium salt	39 (1973)		Leather dye
		15 (1974) (sales)		Beater dye for paper
		33 (1975)		Heavy metal salts used as pigments
		21 (1976)		
		> 4 (1977)		
		17 (1978)		
		> 4 (1979)		
		> 2 (1980)		

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1000 kg (year)	US Imports[b] 1000 kg (year)	Use[c]
Direct Brown 74	8014-91-3	31 (1971) 30 (1972)	< 1 (1972)	Colourant for textiles Leather dye
C.I. 36300	Benzoic acid, 3,3′-((3,7-disulfo-1,5-naphthalenediyl)bis-(azo(6-hydroxy-3,1-phenylene)-azo(6(or 7)sulfo-4,1-naphthalenediyl)azo(1,1′-biphenyl)-4,4′-diylazo))bis(6-hydroxy-, hexasodium salt	> 0.5 (1973) > 0.5 (1974) > 0.5 (1975) > 4 (1976) > 2 (1977) 15 (1978) > 2 (1979) > 2 (1980)		
Direct Brown 154	6360-54-9	201 (1971) 178 (1972)		Colourant for dyeing and printing textiles and for dyeing paper and leather
C.I. 30120	Benzoic acid, 5-((4′-((2,6-diamino-3-methyl-5-((4-sulfophenyl)azo)phenyl)azo) (1,1′-biphenyl)-4-yl)azo)-2-hydroxy-3-methyl, disodium salt	> 1 (1973) > 1 (1974) > 1 (1975) > 4 (1976) > 2 (1977) 29 (1978) > 2 (1979) > 2 (1980)		
Direct Green 1	3626-28-6	109 (1971) 106 (1972)		Colourant for dyeing and printing textiles, for aqueous inks and for regenerated cellulose film Leather dye Beater dye for paper
C.I. 30280	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3-((4′-((4-hydroxyphenyl)-azo) (1,1′-biphenyl)-4-yl)-azo)-6-(phenylazo)-, disodium salt	86 (1973) 26 (1974) 60 (1975) 77 (1976) > 4 (1977) 6 (1978) > 2 (1979) > 2 (1980)	< 1 (1974) < 1 (1975)	

Direct Green 6	4335-09-5	235 (1971)		Colourant for dyeing and printing textiles, for aqueous inks, for regenerated cellulose film and for dyeing soap Leather dye Beater dye for paper Heavy metal salts used as pigments
		183 (1972)		
C.I. 30295	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-6-((4′-((4-hydroxyphenyl)azo)-(1,1′-biphenyl)-4-yl)azo)-3-((4-nitrophenyl)azo)-, disodium salt	177 (1973) (sales)		
		65 (1974)		
		> 1 (1975)	< 1 (1975)	
		> 6 (1976)		
		> 4 (1977)		
		49 (1978)	2 (1978)	
		> 2 (1979)		
		> 2 (1980)		
Direct Orange 8	2429-79-0	28 (1971)		Colourant for dyeing cellulose Beater dye for paper
		49 (1972)		
C.I. 22130 and 22140	Benzoic acid, 5-((4′-((1-amino-4-sulfo-2-naphthalenyl)azo)-(1,1′-biphenyl)-4-yl)azo)-2-hydroxy-, disodium salt	60 (1973)	1 (1973)	
		39 (1974)		
		16 (1975) (sales)		
		38 (1976) (sales)	2 (1976)	
		> 2 (1977)		
		12 (1978)		
		> 2 (1979)		
		> 2 (1980)		
Direct Red 1	2429-84-7	56 (1971)	10 (1971)	Colourant for dyeing and printing textiles Leather dye Beater dye for paper Heavy metal salts used as pigments
		58 (1972)	5 (1972)	
C.I. 22310	Benzoic acid, 5-((4′-((2-amino-8-hydroxy-6-sulfo-1-naphthalenyl)azo) (1,1′-biphenyl)-4-yl)azo)-2-hydroxy-, disodium salt	91 (1973)	1 (1973)	
		44 (1974)		
		60 (1975)		
		28 (1976)		
		> 4 (1977)	2 (1977)	
		12 (1978)		
		> 2 (1979)	2 (1979)	
		> 2 (1980)		

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1 000 kg (year)	US Imports[b] 1 000 kg (year)	Use[c]
Direct Red 28	573-58-0	48 (1971)	10 (1971)	Colourant for dyeing
		79 (1972)	2 (1972)	textiles
C.I. 22120	1-Naphthalenesulfonic acid,	136 (1973)	12 (1973)	Biological stain
	3,3′-((1,1′-biphenyl)-4,4′-	> 1 (1974)	5 (1974)	pH indicator
	diylbis (azo))bis(4-amino-,	> 1 (1975)		Beater dye for paper
	disodium salt	> 4 (1976)		
		> 2 (1977)		
		17 (1978)	15 (1978)	
		> 2 (1979)	9 (1979)	
		> 2 (1980)		
(Resin F. Black WP)	No CAS Number	38 (1978)		
	Composition unknown			
		US		
Pigment Orange 13	3520-72-7	65 (1971)		Colourant for plastics,
		82 (1972)	4 (1972)	rubber, printing inks,
C.I. 21110	3H-Pyrazol-3-one, 4,4′-	148 (1973)	5 (1973)	paper coatings, floor
	((3,3′-dichloro(1,1′-bi-	197 (1974)	4 (1974)	coverings, interior
	phenyl)-4,4′-diyl)bis(azo))-	95 (1975)	5 (1975)	paints, chalks and
	bis(2,4-dihydro-5-methyl-2-	121 (1976)	1 (1976)	crayons, and for mass
	phenyl-	104 (1977)	4 (1977)	dyeing of viscose
		108 (1978)	1 (1978)	Leathercloth dye
		164 (1979)	3 (1979)	
		86 (1980)	6 (1980)	
		Japan		
		160 (1979)		Mostly colourant for inks
		150 (1980)		Small amount for resins

		US		
Pigment Orange 34	15793-73-4	55 (1971)		Colourant for printing and
		56 (1972)	< 1 (1972)	finishing inks (metal-can
C.I. 21115	3H-Pyrazol-3-one, 4,4′-((3,3′-dichloro (1,1′-biphenyl)-4,4′-diyl)bis(azo))bis(2,4-dihydro-5-methyl-2-(4-methylphenyl)-	41 (1973)	6 (1973)	coatings and decorative
		27 (1974)	14 (1974)	metal finishes on cans and
		45 (1975)		foils), textile printing,
		40 (1976)	44 (1976)	paper coatings, floor
		34 (1977)	6 (1977)	coverings, chalks and
		38 (1978)	4 (1978)	crayons
		43 (1979)	11 (1979)	
		40 (1980)	16 (1980)	
		Japan		
		3 (1979)		Mostly colourant for
		3 (1980)		resins
		US		
Pigment Red 38	6358-87-8	60 (1971)		Colourant for rubber,
		84 (1972)	< 1 (1972)	floor products, lacquers,
C.I. 21120	1H-Pyrazole-3-carboxylic acid, 4,4′-((3,3′-dichloro-(1,1′-biphenyl)-4,4′-diyl)-bis(azo))bis(4,5-dihydro-5-oxo-1-phenyl), diethyl ester	77 (1973) (sales)	1 (1973)	emulsion paints and
		> 1 (1974)	4 (1974)	printing inks
		> 1 (1975)	2 (1975)	
		64 (1976)	29 (1976)	
		93 (1977)		
		> 1 (1978)		
		75 (1979)	6 (1979)	
		37 (1980)		
		Japan		
		13 (1979)		Colourant for inks and PVC
		12 (1980)		resins

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1 000 kg (year)	US Imports[b] 1 000 kg (year)	Use[c]
		US		
Pigment Yellow 12	6358-85-6	2547 (1971)	58 (1971)	Colourant for oil- and
		2939 (1972)	45 (1972)	aliphatic-based printing
C.I. 21090	Butanamide, 2,2-((3,3′-	3813 (1973)	53 (1973)	inks, lacquers, rubber,
	dichloro(1,1′-biphenyl)-	3641 (1974)	2 (1974)	linoleum, paints and
	4,4′-diyl)bis(azo))bis(3-	2737 (1975)	28 (1975)	textile printing
	oxo-*N*-phenyl))-	3555 (1976)	16 (1976)	
		3936 (1977)	13 (1977)	
		5021 (1978)	1 (1978)	
		5945 (1979)	45 (1979)	
		5218 (1980)	28 (1980)	
		Japan		
		1800 (1979)		Colourant for printing
		1750 (1980)		inks
		US		
Pigment Yellow 13	5102-83-0	> 4 (1971)	< 1 (1971)	Colourant for printing
		> 4 (1972)	11 (1972)	inks, interior paints,
C.I. 21100	Butanamide, 2,2′-((3,3′-	> 4 (1973)	22 (1973)	textiles, rubber and
	dichloro(1,1′-biphenyl)-	> 5 (1974)	8 (1974)	plastics (e.g., flexible
	4,4′-diyl)bis(azo))bis-	109 (1975)	1 (1975)	vinyl products)
	(*N*-2-dimethylphenyl)-3-oxo-	173 (1976)	9 (1976)	
		167 (1977)	2 (1977)	
		232 (1978)	4 (1978)	
		216 (1979)	9 (1979)	
		331 (1980)	2 (1980)	
		Japan		
		3 (1979)		Colourant for water-based
		2 (1980)		colours

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1000 kg (year)	US Imports[b] 1000 kg (year)	Use[c]
		US		
Pigment Yellow 14	5468-75-7	1034 (1971)	4 (1971)	Colourant for textiles,
		971 (1972)	12 (1972)	printing inks, rubber,
C.I. 21095	Butanamide, 2,2′-((3,3′-	1346 (1973)	10 (1973)	paints and plastics
	dichloro(1,1′-biphenyl)-	1636 (1974)		
	4,4′-diyl)bis(azo))bis-	835 (1975)	5 (1975)	
	(*N*-(2-methylphenyl)-	1362 (1976)		
	3-oxo-	1475 (1977)	< 1 (1977)	
		1421 (1978)	2 (1978)	
		1853 (1979)	3 (1979)	
		1345 (1980)		
		Japan		
		1500 (1979)		Colourant for resins
		1450 (1980)		
		US		
Pigment Yellow 17	4531-49-1	259 (1971)	1 (1971)	Colourant for plastics
		260 (1972)	1 (1972)	(e.g., rigid vinyl
C.I. 21105	Butanamide, 2,2′-((3,3′-	261 (1973)	< 1 (1973)	products and polyolefins),
	dichloro(1,1′-biphenyl)-	379 (1974)	5 (1974)	printing inks, textiles,
	4,4′-diyl)bis(azo))bis-(*N*-	188 (1975)		interior paints, rubber
	(2-methoxyphenyl)-3-oxo-	348 (1976)		and artists' paints
		455 (1977)		
		622 (1978)		
		489 (1979)		
		274 (1980)		
		Japan		
		150 (1979)		Colourant for printing
		120 (1980)		inks and resins

Colour Index Name and Number	Chemical Abstract Services Number and Name	Production[a] 1000 kg (year)	US Imports[b] 1000 kg (year)	Use[c]
		US		
Pigment yellow 55	635-37-3		< 1 (1972)	Colourant for textiles, printing inks and plastics
C.I. 21096	Butanamide, 2,2′-[(3,3′-dichloro(1,1′-biphenyl)-4,4′-diyl)bis(azo)]bis-(*N*-(4-methylphenyl)-3-oxo-	> 0.5 (1974) > 0.5 (1975) > 1 (1976) > 1 (1977) > 1 (1978) > 0.5 (1979) > 0.5 (1980)		
		Japan		
		130 (1979) 110 (1980)		Colourant for emulsion paints

a Data include US production in 1971-1980 and Japanese production in 1979 and 1980. (Data for other countries were not available to the Working Group.) US data include the minimum production (indicated by >) based on the number of producing companies when separate data are not reported. US data are from US International Trade Commission (1973-1981a) and National Institute for Occupational Safety and Health (1980). Japanese data are estimates.

b Data are for imports through the principal US customs districts and are from US International Trade Commission (1973-1981b).

c From The Society of Dyers and Colourists (1971) and JRB Associates, Inc. (1979), except for information on uses in Japan

References to Appendix

JRB Associates, Inc. (1979) *Survey of the Manufacture, Import, and Uses for Benzidine, Related Substances and Related Dyes and Pigments*. Prepared for the US Environmental Protection Agency, McLean, VA

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US International Trade Commission (1973-1981b) *Imports of Benzenoid Chemicals and Products, 1971-1980*, Washington DC, US Government Printing Office

THE MONOGRAPHS

BENZYL CHLORIDE

This substance was considered by a previous Working Group, in February 1976 (IARC, 1976). Since that time new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

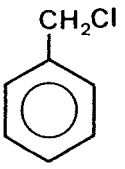
Chem. Abstr. Services Reg. No.: 100-44-7

Chem. Abstr. Name: Benzene, (chloromethyl)-

IUPAC Systematic Name: alpha-Chlorotoluene

Synonyms: Chloromethyl benzene; chlorophenylmethane; Ω-chlorotoluene; α-tolyl chloride; tolyl chloride

1.2 Structural and molecular formulae and molecular weight



C_7H_7Cl Mol. wt: 126.6

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Colourless to slightly yellowish liquid (Manufacturing Chemists Association, 1974)

(*b*) *Boiling-point*: 179.3°C

(*c*) *Melting-point*: -39°C

(*d*) *Density*: d_{20}^{20} 1.1002

(*e*) *Refractive index*: n_D^{20} 1.5391

(*f*) *Spectroscopy data*: λ_{max} 217 nm (in ethanol); nuclear magnetic resonance, infrared and mass spectral data have been reported (Grasselli and Ritchey, 1975).

(*g*) *Solubility*: Insoluble in water; miscible with chloroform, diethyl ether and ethanol

(*h*) *Volatility*: Vapour pressure, 60 mm at 100.5°C (Gelfand, 1979)

(*i*) *Stability*: Decomposes in hot water to benzyl alcohol (US Environmental Protection Agency, 1980a). Decomposes rapidly when heated in the presence of iron (Windholz, 1976); combustible (flash-point, 67°C, closed cup) (Manufacturing Chemists Association, 1974)

(*j*) *Reactivity*: Undergoes reactions both at the sidechain containing the chlorine and at the aromatic ring (Gelfand, 1979). Reacts with steam and oxidizing agents (US Environmental Protection Agency, 1980a). Shows alkylating activity towards 4-(4-nitrobenzyl)pyridine (Druckrey *et al.*, 1970; Neudecker *et al.*, 1980).

(*k*) *Conversion factor*: ppm = 0.193 x mg/m^3

1.4 Technical products and impurities

Benzyl chloride available in the US has the following specifications: a minimum of 98.5% active ingredient; maxima of 1.0% benzal chloride, chlorotoluenes and toluene; distillation range (95%, minimum), 3°C, including 179.4°C; refractive index (n_D^{25}), 1.5360-1.5370; and specific gravity ($d_{15.5}^{15.5}$), 1.105-1.110 (Stauffer Chemical Co., undated). One stabilized grade of benzyl chloride contained a fixed amount of sodium carbonate solution or propylene oxide (Manufacturing Chemists Association, 1974).

Benzyl chloride is available in Japan as a transparent liquid with a minimum purity of 97.0% and a distillation range of 178.0-181.0°C.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Benzyl chloride was first prepared by the reaction of benzyl alcohol with hydrochloric acid (Ringk and Theimer, 1978). Its commercial production is based on the controlled

chlorination of toluene under conditions which do not favour ring chlorination. (Gelfand, 1979).

This compound has been produced commercially in the US since at least 1919 (US Tariff Commission, 1921) and in Japan since 1936. Two US companies, with a combined annual production capacity of about 75 million kg, have been producing approximately 46 million kg of benzyl chloride annually in recent years. Another US company is believed to produce this chemical solely for use as an intermediate in the production of derivatives. Separate US production data were last reported in 1972, when three companies reported total production of 36.5 million kg (US Tariff Commission, 1974).

In 1979, 18 thousand kg of benzyl chloride were imported through the principal US customs districts (US International Trade Commission, 1980a).

This compound is believed to be produced commercially by two companies in Italy and two in Spain, and by one company in Belgium, one in France, one in the Federal Republic of Germany and one in the UK. Total production in western Europe in 1979 is estimated to have been 40 million kg.

About 7.2 million kg of benzyl chloride were produced by the two producing companies in Japan in 1979, and exports were approximately 800 thousand kg.

(*b*) *Use*

The US use pattern for benzyl chloride in 1978 was as follows: benzyl phthalates, 62%; benzyl alcohol, 11%; benzyl quaternary ammonium salts, 10%; and pharmaceuticals, 8%.

Butyl benzyl phthalate (which represents over 90% of the benzyl phthalates produced in the US) is the subject of a separate monograph in this volume. Benzyl alcohol is used as a dyeing aid, a photographic developer and as an intermediate in drugs and perfumes.

Benzyl chloride is used to convert a variety of tertiary amines to quarternary ammonium chlorides, $[R_3NCH_2C_6H_5]^+Cl^-$. The most important of these, made from dimethyl alkyl amines containing long-chain (C_{10} to C_{18}) alkyl groups, are bactericides used widely in industries and institutions. Total production of one such product, benzyldimethyl(mixed alkyl)ammonium chloride, by six US companies in 1979 was 5.0 million kg (US International Trade Commission, 1980b).

Among the pharmaceutical products made from benzyl chloride, the most important are believed to be benzathine penicillin G (*N*,*N*′-dibenzylethylenediamine dipenicillin G) and phenobarbital [see IARC, 1977].

Although the Society of Dyers and Colourists (1971) indicated that two dyes can be prepared from benzyl chloride, only one of these, C.I. 42536 (Basic Violet 13), appears to have been produced commercially in the US; its production by one company was last reported in 1975 (US International Trade Commission, 1977).

Among the reported minor uses of benzyl chloride is the production of certain benzyl esters (e.g., the acetate and benzoate) by reaction with salts of the acids (Ringk and Theimer, 1978). It has been used as an irritant gas in chemical warfare (von Oettingen, 1955), and can be used in the manufacture of synthetic tannins and as a gum inhibitor in petrol (US Environmental Protection Agency, 1980a).

The 6.4 million kg of benzyl chloride used in Japan in 1979 were distributed as follows: bactericides, 47%; benzyl alcohol and benzyl acetate, 31%; and other applications, 22%.

Occupational exposure to benzyl chloride has been limited by regulation or recommended guidelines in at least 12 countries. These standards are listed in Table 1. In the Federal Republic of Germany, benzyl chloride has been included in a list of substances for which recent experimentation has demonstrated a carcinogenic potential but which still need further study. In Sweden, benzyl chloride is included in a list of carcinogenic substances for which limit values are specified (International Labour Office, 1980).

Table 1. National occupational exposure limits for benzyl chloride[a]

Country	Year	Concentration		Interpretation[b]	Status
		mg/m^3	ppm		
Australia	1978	5	1	TWA	Guideline
Belgium	1978	5	1	TWA	Regulation
Finland	1975	5	1	TWA	Regulation
German Democratic Republic	1979	10	—	Maximum (30 min)	Regulation
		5	—	TWA	
Federal Republic of Germany	1979	5	1	TWA	Guideline
The Netherlands	1978	5	1	TWA	Guideline
Romania	1975	5	—	TWA	Regulation
		8	—	Maximum	
Sweden	1978	5	1	TWA	Guideline
Switzerland	1978	5	1	TWA	Regulation
USSR	1977	0.5	—	Maximum	Regulation
USA					
OSHA	1975	5	1	TWA	Regulation
ACGIH	1981	5	1	TWA	Guideline
NIOSH	1980	5	—	Ceiling (15 min)	Guideline
Yugoslavia	1971	5	1	Ceiling	Regulation

a From American Conference of Governmental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980); National Institute for Occupational Safety and Health (NIOSH) (1980); US Occupational Safety and Health Administration (OSHA) (1980).
b TWA, time-weighted average.

The US Environmental Protection Agency (EPA) (1979) requires that notification be made whenever discharges containing 45.4 kg or more of benzyl chloride are made into waterways. The EPA has also identified benzyl chloride as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with regulations of a Federal hazardous waste management programme. Still bottoms from the distillation

of benzyl chloride and distillation or fractionating column bottoms from the production of chlorobenzenes are included in a list of hazardous wastes from specific sources; benzyl chloride is one of the hazardous constituents present in those wastes (US Environmental Protection Agency, 1980b,c).

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzyl chloride are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

2.2 Occurrence

(*a*) *Natural occurrence*

Benzyl chloride has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

It has been estimated that approximately 3000 workers in the US are potentially exposed to benzyl chloride (National Institute for Occupational Safety and Health, 1978). Occupations involving potential exposure to benzyl chloride are listed in Table 2.

Table 2. Occupations involving potential exposure to benzyl chloride[a]

Algicide makers	Petrol additive workers
Benzyl alcohol workers	Germicide makers
Benzyl chloride workers	Motor fuel blenders
Benzyl ester makers	Perfume makers
Butyl benzyl phthalate workers	Pharmaceutical workers
Drug makers	Photographic-developer makers
Dye-intermediate makers	Quaternary-ammonium-compound workers
Dye makers	Resin makers
Dyers	Rubber makers [see IARC, 1982]
Disinfectant makers	Tannin makers [see IARC, 1980]
Extreme-pressure-lubricant makers and users	Wetting-agent makers
Analytical chemists	Synthetic organic chemists

a From National Institute for Occupational Safety and Health (1978)

In 1947, benzyl chloride vapours were detected at concentrations of up to 2590 mg/m^3 [500 ppm] in pharmaceutical factories where certain halides were used as intermediates (National Institute for Occupational Safety and Health, 1978). In surveys made in 1975-1976 of two factories engaged in the production of benzyl chloride, average concentrations in the air ranged from <0.05 to 0.20 mg/m^3 [<0.01-0.04 ppm] with a maximum of

23.5 mg/m³ [4.7 ppm] (Cohen *et al.*, 1978). Concentrations greater than 10 mg/m³ [>2 ppm] have been associated with benzyl chloride production (National Institute for Occupational Safety and Health, 1978).

Trace amounts of benzyl chloride have been found in personal air samples taken at spray painting booths in a US automobile manufacturing factory (McGlothlin, 1979).

(*c*) *Water and sediments*

Benzyl chloride has been identified in surface water, oil refinery effluents and other industrial effluents (Hushon *et al.*, 1980). It has also been identified in water samples taken from the Delaware River in Pennsylvania and in New Jersey (Sheldon and Hites, 1978).

2.3 Analysis

Typical methods for the analysis of benzyl chloride in air and water are summarized in Table 3.

Table 3. Methods for the analysis of benzyl chloride

Sample matrix	Sample preparation	Assay procedure[a]	Limits of detection	Reference
Air	Pass through charcoal column	CG/FID	not given	Cohen *et al.* (1978)
Water	Extract with dichloromethane; add potassium hydroxide solution or pass helium throught the sample; condense in polymer adsorbant	GC/MS	not given	Jungclaus *et al.* (1978); Sheldon and Hites (1978)

a Abbreviations: GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(*a*) *Skin application*

Mouse: A series of skin-painting experiments were conducted in one strain of mice (Fukuda *et al.*, 1981). In the first experiment, two groups, each consisting of 10 or 11 female ICR mice, 3 weeks old [body weights unspecified], received skin applications of 10 μl benzene [8.8 mg] (controls) or of 10 μl [11.1 mg] reagent-grade benzyl chloride [purity and impurities unspecified] [about 280-740 mg/kg bw, assuming a body weight of 15-40 g] thrice weekly for the first four weeks and twice weekly thereafter, until the mice were killed at week 43 [total dose of benzyl chloride, 977 mg/animal]. No death occurred, and no tumour was found in benzene-treated controls or in test animals. [The Working Group noted the short duration of the experiment.]

In the second experiment, two groups of 20 female ICR mice, 7 weeks old [body weights unspecified], received skin applications of 25 μl benzene [22 mg] (controls) or of 25 μl of a 9.2% solution of benzyl chloride in benzene [v/v, 2.6 mg/treatment; about 65-130 mg/kg bw, assuming a body weight of 20-40 g] twice weekly for 50 weeks [total dose of benzyl chloride, 255 mg/animal]; the mice were killed at week 82. By that time, 20% of the control group and 50% of the test group were dead. Three mice in the test group had developed squamous-cell carcinomas of the skin and one had developed a leiomyosarcoma of the uterus; 2 mice in the treated group and 2 in the control group had lung adenomas. The difference in skin cancer incidence between the groups was not statistically significant. [The Working Group noted the limited size of the experimental groups.]

(*b*) *Subcutaneous and/or intramuscular administration*

Rat: Eight 14-week-old BD rats [sex unspecified] were given weekly s.c. injections of 80 mg/kg bw purified benzyl chloride in arachis oil for 51 weeks (total dose, 3.9 g/kg bw). Six developed sarcomas at the injection site, with an average induction time of 500 days; lung metastases were observed in most animals. Of 14 rats given 40 mg/kg bw weekly for 51 weeks (total dose, 2.1 g/kg bw), 3 developed sarcomas at the injection site, with an average induction of 500 days. Arachis oil alone did not 'usually' produce local tumours at the injection site in otherwise untreated animals (Preussmann, 1968; Druckrey *et al.*, 1970).

(*c*) *Intraperitoneal administration*

Mouse: Three groups of 20 A/He mice of both sexes, 6-8 weeks old, received a total of 8-12 i.p. injections of benzyl chloride [more than 98% pure, impurities unspecified] in

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[1] The Working Group was aware of a study in progress involving mice and rats treated by oral administration (IARC, 1981).

tricaprylin as thrice weekly injections (total doses, 0.6, 1.5 and 2 g/kg bw). All survivors were killed 24 weeks after the first injection. Lung tumours were found in 4/15, 7/16 and 2/8 surviving mice in the three groups, respectively, with averages of 0.26, 0.50 and 0.25 lung tumours per mouse. The incidences were reported to be not statistically different from that in controls that received tricaprylin alone (0.22 lung tumours per mouse) or no treatment (0.21 lung tumours per mouse) (Poirier *et al.*, 1975). [An unbiased assessment of the lung tumour incidence with increasing dose was not possible on the basis of the reported figures, due to obvious intercurrent mortality. The Working Group also noted that a negative effect observed in the strain A mouse assay cannot be taken as evidence of lack of carcinogenesis.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

In rats, the s.c. LD_{50} of benzyl chloride in oil solution is 1000 mg/kg bw (Druckrey *et al.*, 1970). The LD_{50}s in mice and rats following oral administraton have been reported as 1620 and 1230 mg/kg bw, respectively (Vernot *et al.*, 1977), and as 780 and 625 mg/kg bw, respectively (Rudnev *et al.*, 1979).

Rats treated orally with 0.06 mg/day benzyl chloride for six months showed no effects; while with 0.6 mg/day, blood levels of inorganic phosphate, alkaline phosphatase, cholinesterase and succinic dehydrogenase were increased. Benzyl chloride was also a sensitizing agent in rats (Rudnev *et al.*, 1979) and in guinea-pigs (von Oettingen, 1955). Vinogradov (1979) reported that the minimum sensitizing dose for benzyl chloride in rats is 0.0006 mg/kg given in 30 daily oral administrations.

In two-hour exposures, the LC_{16}, LC_{50} and LC_{84} values for benzyl chloride were 440, 740 and 1200 mg/m^3 [85, 143 and 232 ppm], respectively, in rats and 230, 390 and 620 mg/m^3 [44, 75 and 120 ppm], respectively, in mice. The decreasing order of toxicity among the chlorinated toluenes was: benzotrichloride > benzal chloride > benzyl chloride. Thus, in a single two-hour exposure of mice and rats to 100 mg/m^3, all three compounds produced central nervous system excitation, irritation of the eyes and respiratory mucosae and slowed respiration in the order of activity described above. Hyperaemia of the extremities was also noted; at 1000 mg/m^3 [125 ppm] mice exhibited motor automatism and rats showed twitching of peripheral muscles. When rats were exposed to 100 mg/m^3 of each of the compounds in an inhalation chamber continuously for one month, degree of weight loss was related to the same order of activity as in the acute studies. While benzyl chloride did not cause decreases in blood cell counts, benzotrichloride cause leucopenia and mild anaemia. Benzotrichloride, but not benzal or benzyl chloride, caused decreases in renal function (Mikhailova, 1964).

In the course of a carcinogenicity bioassay in which the three compounds were dissolved in benzene and applied to the clipped backs of specific-pathogen-free ICR female mice, irritation of the eyes, skin and respiratory tract was observed. Benzotrichloride was the most and benzal chloride the least irritating. The skin initially exhibited erythema and swelling, then alopecia and induration. In some cases, marked keratinization, ulcers and/or necrosis of the skin were observed (Fukuda *et al.*, 1981).

Effects on reproduction and prenatal toxicity

In groups of 20 Wistar rats given oily solutions of benzyl chloride orally from 1-19 days of gestation, 208 mg/kg bw produced a high incidence of embryolethality; 0.006 mg/kg bw also increased embryolethality, but 0.0006 and 0.00006 mg/kg bw had no adverse effect. No malformation was observed. In rats given 208 mg/kg bw [period not stated], postnatal development of offspring was retarded and their resistance to anoxia and fertility impaired; no adverse postnatal effect was seen in rats treated with 0.00006 mg/kg bw (Leonskaya, 1980). [The Working Group noted the wide dose range reported in this study.]

Absorption, distribution, excretion and metabolism

Benzyl chloride is adsorbed through the lungs and gut (von Oettingen, 1955).

Following its s.c. injection in rats and rabbits or its oral administration to dogs, it reacts with tissue proteins and is metabolized into benzyl mercapturic acid (*N*-acetyl-*S*-benzyl cysteine) (von Oettingen, 1955). Stekol (1938) had shown earlier that the mercapturic acid was formed on the side chain rather than on the ring; and Knight and Young (1958) found that the urine of rats given benzyl chloride does not contain an acid-labile precursor of benzyl mercapturic acid indicating that it is converted *in vivo* directly into the mercapturic acid. The percentages of the dose excreted as mercapturic acid in urine after administration of benzyl chloride to rats (Barnes *et al.*, 1959), rabbits (Bray *et al.*, 1958) and guinea-pigs (Goodwin, 1976) were 27%, 49% and 4%, respectively.

In addition to the mercapturic acid, benzoic acid in its free or glycine-conjugated form has been recovered from the urine of benzyl chloride-treated animals. In rabbits, 37% of a dose was converted to benzoic acid, 17% of which was free and 20% conjugated (Bray *et al.*, 1958). In rats, 30% of the dose was recovered as the hippuric acid derivative (Maitrya and Vyas, 1970).

Mutagenicity and other short-term tests

[The purity of benzyl chloride was not specified in any of the following studies.]

Benzyl chloride is a direct alkylating agent and has been found to be biologically active without metabolic activation (Preussmann, 1968; Neudecker *et al.*, 1980). It was mutagenic in *Salmonella typhimurium* strains TA1535 (McCann *et al.*, 1975; Rosenkranz and Poirier, 1979; Simmon, 1979a) and TA100 (McCann *et al.*, 1975; McMahon *et al.*, 1979; Yasuo *et al.*,1978; Neudecker *et al.*, 1980) and in *Escherichia coli* strain WP2 hcr (Yasuo *et al.*, 1978). No mutagenic activity was detected in *S. typhimurium* TA1535 in a host-mediated assay (Simmon *et al.*, 1979). Benzyl chloride was also reported to be positive in the *E. coli* pol A test (Fluck *et al.*, 1976; Rosenkranz and Poirier, 1979).

It increases mitotic recombination in *Saccharomyces cerevisiae* D3 (Simmon, 1979b) and transforms Syrian hamster embryo cells *in vitro* (Pienta *et al.*, 1978; Pienta, 1980); the lowest transforming dose was 0.1 μg/ml.

(*b*) *Humans*

Concentrations of 160 mg/m^3 [32 ppm] in air cause severe irritation of the eyes and respiratory tract (von Oettingen, 1955). No data on the absorption, distribution, excretion,

metabolism, teratogenicity or mutagenicity of this compound to humans were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data clearly relating cancer to exposure to benzyl chloride were available to the Working Group. However, benzoyl chloride manufacturing workers, among whom an excess of respiratory cancer has been reported (six cases in total) were also potentially exposed to benzyl chloride (Sakabe *et al.*, 1976; Sakabe and Fukuda, 1977). These reports are considered in detail in the monograph on benzoyl chloride.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzyl chloride was tested in mice by skin application and in rats by subcutaneous injection. Sarcomas at the injection site were observed in rats. A few skin carcinomas were observed in a limited number of mice, but their incidence was not statistically significant.

Benzyl chloride is a direct-acting mutagen to bacteria. It induces mitotic recombination in yeast and transforms hamster embryo cells.

The available data are inadequate to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzyl chloride may occur during its manufacture and during its use in the production of benzyl phthalates, benzyl alcohol, quaternary ammonium salts, pharmaceuticals and benzyl esters; but no data were available on levels of exposure.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study involving exposure to benzyl chloride alone was available to the Working Group. Six cases of respiratory cancer have been reported

among benzoyl chloride manufacturing workers in two small plants, who were also potentially exposed to benzyl chloride. The cases occurred in relatively young workers, three of whom were nonsmokers.

4.3 Evaluation[1]

There is *limited evidence* that benzyl chloride is carcinogenic in experimental animals.

Although the epidemiological data were inadequate to evaluate the carcinogenicity of benzyl chloride alone, they provide *limited evidence* that employment in the production of benzoyl chloride and its chlorinated toluene precursors, which involves exposure to benzoyl chloride, represents a carcinogenic risk to man.

No evaluation could be made of the carcinogenicity to man of benzyl chloride itself.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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BENZAL CHLORIDE

1. Chemical and Physical Data

1.1 Synonyms and trade names

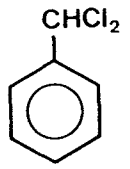
Chem. Abstr. Services Reg. No.: 98-87-3

Chem. Abstr. Name: Benzene, (dichloromethyl)-

IUPAC Systematic Name: alpha, alpha-Dichlorotoluene

Synonyms: Benzyl dichloride; benzylene chloride; benzylidine chloride; chlorobenzal; (dichloromethyl)benzene; dichlorophenylmethane; dichlorotoluene

1.2 Structural and molecular formulae and molecular weight



$C_7H_6Cl_2$ Mol. wt: 161.0

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Colourless, oily liquid (Hawley, 1981)

(*b*) *Boiling-point*: 205.2°C

(*c*) *Melting-point*: -16.4°C

(*d*) *Density*: d^{14} 1.2557

(*e*) *Refractive index*: n_D^{20} 1.5502

(*f*) *Spectroscopy data*: λ_{max} 260, 265, 272 nm (in cyclohexane); nuclear magnetic resonance, infra-red and mass spectral data have been reported (Grasselli and Ritchey, 1975).

(*g*) *Solubility*: Insoluble in water; soluble in diethyl ether and ethanol

(*h*) *Volatility*: Vapour pressure, 60 mm at 123.6°C (Gelfand, 1979)

(*i*) *Stability*: Hydrolysed to benzaldehyde under both acid and alkaline conditions (Gelfand, 1979)

(*j*) *Reactivity*: Undergoes reactions both at the sidechain containing the chlorines and at the aromatic ring (Gelfand, 1979)

(*k*) *Conversion factor*: ppm = 0.152 x mg/m^3

1.4 Technical products and impurities

Benzal chloride is available in the US as a technical grade with a minimal purity of 95% (Stauffer Chemical Co., 1980).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Benzal chloride was first prepared by the reaction of phosphorus pentachloride with benzaldehyde in 1848. Commercial production is based on the controlled chlorination of toluene under conditions which do not favour ring chlorination (Gelfand, 1979).

Benzal chloride has been produced in the US since at least 1919 (US Tariff Commission, 1921). Two US companies reported combined production of 0.91-9.1 million kg in 1977 (US Environmental Protection Agency, 1981). At the present time, only one US company is believed to produce commercial quantities of this compound. Imports of benzal chloride through the principal US customs districts were last reported in 1971, when they totalled 4.6 thousand kg (US Tariff Commission, 1972).

This chemical is believed to be produced by one company each in Belgium, France, the Federal Republic of Germany and the United Kingdom. Total production in those countries in 1979 is estimated to have been 5 million kg.

Benzal chloride is produced in Japan only as an unisolated intermediate.

(*b*) *Use*

Benzal chloride is used almost exclusively for the manufacture of benzaldehyde. Although an estimated 3.5-4.0 million kg benzaldehyde are produced in the US annually, most is made by direct oxidation of toluene rather than by hydrolysis of benzal chloride. Benzaldehyde is used in the manufacture of perfume and flavour chemicals, dyes and pharmaceuticals.

Although it has been reported that benzal chloride can be converted to cinnamic acid by heating with sodium acetate (Ringk, 1979), cinnamic acid is not being produced commercially in the US at this time.

In the Federal Republic of Germany, benzal chloride has been included in a list of substances for which recent experimentation has demonstrated a carcinogenic potential but which still require further study. Levels of benzal chloride in the working environment in the USSR may not exceed a maximum allowable concentration of 0.5 mg/m^3 (International Labour Office, 1980).

The US Environmental Protection Agency (1980) has identified benzal chloride as a toxic waste, and requires that persons who generate, transport, treat, store or dispose of it comply with Federal hazardous waste management programme regulations. As part of the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of benzal chloride are subject to a variety of labelling, packaging, quantity and shipping regulations consistent with its designation as a hazardous material.

2.2 Occurrence

(*a*) *Natural occurrence*

Benzal chloride has not been reported to occur as such in Nature.

(*b*) *Water and sediments*

Benzal chloride has been identified in surface waters; the concentrations were not reported (Hushon *et al.*, 1980).

2.3 Analysis

A method has been described for the determination of benzal chloride in workplace air, using gas chromatography after adsorption on a polymeric adsorbant and desorption with carbon tetrachloride. The minimal detectable concentration was about 70 μg/m^3 (10 ppb) (Matsushita and Kanno, 1979).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Skin application*

Mouse: A series of skin-painting experiments in one strain of mice were conducted by Fukuda *et al.* (1981). In the first experiment, two groups of 10 female ICR mice, 3 weeks old [body weights unspecified], received skin applications of 10 μl [8.8 mg] benzene (controls) or 10 μl [12.6 mg] reagent-grade benzal chloride [purity and impurities unspecified] alone [about 315-840 mg/kg bw, assuming body weights of 15-40 g] thrice weekly for four weeks, and twice weekly thereafter, until the mice were killed, at week 43 [total dose, about 1109 mg/animal]. No death occurred in either group; no tumour was found in controls; 2 benzal chloride-treated mice developed a skin papilloma. [The Working Group noted the short duration of the experiment.]

In the second experiment, two groups, each consisting of 19 or 20 female ICR mice, 7 weeks old [body weight unspecified] were given skin applications of 25 μl [22 mg] benzene (controls) or 25 μl of a 9.2% solution of benzal chloride in benzene [v/v; 2.9 mg/treatment; about 75-150 mg/kg bw, assuming body weights of 24-40 g] twice weekly for 50 weeks [total dose, about 289 mg/animal]. All mice were killed at week 82. Five of the 20 controls had died by that time; no skin tumour was observed in any control. Of the 19 benzal chloride-treated mice, 14 (74%) had died by the end of the experiment; 12 (63%) developed tumours: 9 with squamous-cell carcinomas of the skin ($P < 0.01$), 2 with a skin fibrosarcoma, and 1 with a lymphoma; 5 treated mice and 2 controls had a lung adenoma.

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Oral LD_{50} values in mice and rats were 2460 and 3250 mg/kg bw, respectively (Vernot *et al.*, 1977).

The LC_{16}, LC_{50} and LC_{84} values for two-hour exposures to benzal chloride were 160, 400 and 1000 mg/m^3 [24, 61 and 152 ppm], respectively, for rats, and 80, 210 and 550 mg/m^3 [12, 32 and 84 ppm], respectively, for mice. The order of decreasing toxicity among the chlorinated toluenes was: benzotrichloride > benzal chloride > benzyl chloride. Thus, in a single two-hour exposure of mice and rats to 100 mg/m^3 [15.2 ppm], all three compounds produced central nervous system excitation, irritation of the eyes and respiratory mucosae and slowed respiration in the order of activity described above. Hyperaemia of the extremities was also noted; at 1000 mg/m^3 [125 ppm], mice exhibited

motor automatism, and rats showed twitching of peripheral muscles. When rats were exposed to 100 mg/m^3 of each of the compounds in an inhalation chamber, continuously for one month, the degree of weight loss was related to the same order of activity as in the acute studies. While benzyl chloride did not cause decreases in blood cell counts, benzotrichloride caused leucopenia and mild anaemia. Benzotrichloride, but not benzal or benzyl chloride, caused decreases in renal function (Mikhailova, 1964).

In the course of a carcinogenicity bioassay in which the three compounds were dissolved in benzene and applied to the clipped backs of specific-pathogen-free ICR female mice, irritation of the eyes, skin and respiratory tract were observed. Benzotrichloride was the most and benzal chloride the least irritating. The skin initially exhibited erythema and swelling, then alopecia and induration. In some cases, marked keratinization, ulcers and/or necrosis of the skin were oberved (Fukuda *et al.*, 1981).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

Benzal chloride (redistilled commercial material, purity and impurities not specified) has been reported to be mutagenic in reversion assays using *Salmonella typhimurium* strain TA100 [the only strain tested] and in *Escherichia coli* strain WP2 hcr; mutagenicity required metabolic activation by a microsomal fraction of the livers of rats induced by Arochlor 1254. It was also positive in a *rec*-assay using *Bacillus subtilis* (Yasuo *et al.*, 1978).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data clearly relating cancer to exposure to benzal chloride were available to the Working Group. However, benzoyl chloride manufacturing workers, among whom an excess of respiratory cancer has been reported (six cases in total), were also potentially exposed to benzal chloride (Sakabe *et al.*, 1976; Sakabe and Fukuda, 1977). These reports are considered in detail in the monograph on benzoyl chloride.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

In one experiment in which benzal chloride was tested by skin application in female mice, it produced squamous-cell carcinomas of the skin. In a concurrent experiment in which it was tested for a shorter duration, a low incidence of skin papillomas was observed.

In one study, benzal chloride was mutagenic to bacteria with metabolic activation and was positive in a *rec*-assay with *Bacillus subtilis*.

No data were available to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzal chloride has and probably still does occur during its manufacture and conversion to benzaldehyde.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study involving exposure to benzal chloride alone was available to the Working Group. Six cases of respiratory cancer have been reported among benzoyl chloride manufacturing workers in two small plants, who were also potentially exposed to benzal chloride. The cases occurred in young workers, three of whom were nonsmokers.

4.3 Evaluation[1]

There is *limited evidence* that benzal chloride is carcinogenic in experimental animals.

Although the epidemiological data were inadequate to evaluate the carcinogenicity of benzal chloride alone, they provide *limited evidence* that employment in the production of benzoyl chloride and its chlorinated toluene precursors, which involves exposure to benzal chloride, represents a carcinogenic risk to man.

No evaluation could be made of the carcinogenic risk to man of benzal chloride itself.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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BENZOTRICHLORIDE

1. Chemical and Physical Data

1.1 Synonyms and trade names

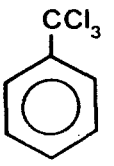
Chem. Abstr. Services Reg. No.: 98-07-7

Chem. Abstr. Name: Benzene, (trichloromethyl)-

IUPAC Systematic Name: *alpha*, *alpha*, *alpha*-Trichlorotoluene

Synonyms: Benzenyl chloride; benzenyl trichloride; benzoic trichloride; benzylidyne chloride; benzyl trichloride; phenyl chloroform; phenylchloroform; phenyltrichloromethane; toluene trichloride; (trichloromethyl)benzene; 1-(trichloromethyl)benzene; α,α,α-trichloromethylbenzene; trichlorophenylmethane; trichlorotoluene; Ω,Ω,Ω-trichlorotoluene

1.2 Structural and molecular formulae and molecular weight



$C_7H_5Cl_3$ Mol. wt: 195.5

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Colourless, oily liquid (Gelfand, 1979)

(*b*) *Boiling-point*: 220.6°C

(*c*) *Melting-point*: -4.75°C

(*d*) *Density*: d^{20} 1.3723

(*e*) *Refractive index*: n_D^{20} 1.5580

(*f*) *Spectroscopy data*: λ_{max} 262, 267, 274 nm (in cyclohexane); nuclear magnetic resonance, infra-red (Grasselli and Ritchey, 1975) and mass spectral data have been reported.

(*g*) *Solubility*: Insoluble in water; soluble in benzene, diethyl ether and ethanol

(*h*) *Volatility*: Vapour pressure, 60 mm at 130.0°C (Gelfand, 1979)

(*i*) *Stability*: Unstable; hydrolyses in the presence of moisture (Windholz, 1976)

j) *Reactivity*: Undergoes reactions both at the sidechain containing the chlorines and at the aromatic ring (Gelfand, 1979)

(*k*) *Conversion factor*: ppm = 0.125 x mg/m^3

1.4 Technical products and impurities

Benzotrichloride is available in the US as a clear liquid containing a minimum of 99% active ingredient with a minimal freezing-point of -5°C (Hooker Chemical Corp., 1981). Additives such as phosphorus trichloride are reportedly used to complex metallic impurities (Gelfand, 1979).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Benzotrichloride was first prepared by the reaction of benzoyl chloride and phosphorus pentachloride. Commercial production is based on the chlorination of toluene at 100-140°C in the presence of ultra-violet light (Gelfand, 1979).

Benzotrichloride has been produced commercially in the US since at least 1919 (US Tariff Commission, 1921). Separate production data were last reported in 1961, when three US companies reported a combined production of 169 thousand kg (US Tariff Commission, 1962). One of the two present US manufacturers of benzotrichloride reported that its production in 1977 was 4.5-22.7 million kg (US Environmental Protection Agency, 1981). On the basis of the estimated quantity of benzotrichloride required to produce only its major derivative, benzoyl chloride, annual US production is believed to be around 18 million kg.

In 1980, US imports of benzotrichloride through the principal customs districts totalled 11.8 thousand kg (US International Trade Commission, 1981).

Benzotrichloride is believed to be produced by two companies in the Federal Republic of Germany and by one company each in Belgium, France and the United Kingdom. Total production in western Europe in 1979 is estimated to have been 22 million kg.

On the basis of the estimated quantity of benzotrichloride required to produce only its major derivative, benzoyl chloride, annual Japanese production is believed to be approximately 3.9 million kg.

(b) Use

Benzotrichloride is used exclusively as a chemical intermediate. By far its most important derivative is benzoyl chloride, which is the subject of a separate monograph in this volume. About 18 million kg benzotrichloride are estimated to be used annually in the US for the production of benzoyl chloride.

Benzotrichloride is also used as a dye intermediate. According to The Society of Dyers and Colourists (197l), eight dyes and pigments can be prepared from it. Five of these have been produced in commercial quantities in the US in recent years. Separate production data were last reported for Vat Yellow 2, 81/2%, in 1976, when total production by three companies amounted to 298 thousand kg (US International Trade Commission, 1977a), and for Basic Green 4 (Malachite Green), in 1975, when four companies produced 145 thousand kg (US International Trade Commission, 1977b).

Benzotrichloride is also used to make benzotrifluoride, an important intermediate in the manufacture of herbicides (e.g., fluometuron); pharmaceuticals (e.g., trifluprom azine and fenfluramide); the lampreycide, 4-nitro-3-(trifluoromethyl)phenol; and antimicrobial agents (e.g., fluorosalan and cloflucarban) (Boudakian, 1980).

Benzotrichloride is also used to produce hydroxybenzophenone ultra-violet stabilizers (e.g., 4-alkoxy-2-hydroxybenzophenones), which are used to prevent discolouration and degradation in a variety of plastics (Gelfand, 1979). Another reported use for benzotrichloride is in the production of ion-exchange resins (Hooker Chemical Corp., 1981), but no evidence was found that it is presently used commercially for this purpose. Benzoic acid was formerly produced by the hydrolysis of benzotrichloride, but this process is not now used in the US on a commercial scale.

In Japan, benzotrichloride is believed to be used almost exclusively for the production of benzoyl chloride.

Four countries have been reported to limit occupational exposure to benzotrichloride by regulation. Their standards are listed in Table 1. In the Federal Republic of Germany, benzotrichloride has been included in a list of substances for which recent experimentation has demonstrated a carcinogenic potential but which still require further study (International Labour Office, 1980).

The US Environmental Protection Agency (1980) has identified benzotrichloride as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with Federal hazardous waste management programme regulations. Still bottoms from the distillation of benzyl chloride are included in a list of hazardous wastes

from specific sources, and benzotrichloride is one of the hazardous constituents present in this waste. As part of the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of benzotrichloride are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

Table 1. National occupational exposure limits for benzotrichloride[a]

Country	Year	Concentration mg/m^3	Interpretation[b]	Status
Hungary	1974	1	TWA[c]	Regulation
Romania	1975	5	TWA	Regulation
		8	Maximum	
USSR	1977	0.2	Maximum	Regulation
Yugoslavia	1971	1	TWA	Regulation

a From International Labour Office (1980)
b TWA, time-weighted average
c Skin irritant notation added

2.2 Occurrence

(*a*) *Natural occurrence*

Benzotrichloride has not been reported to occur as such in Nature.

(*b*) *Water and sediments*

Benzotrichloride has been identified in surface waters; the concentrations were not reported (Hushon *et al.*, 1980).

2.3 Analysis

A method for the determination of benzotrichloride in workplace air, using gas chromatography after adsorption on a polymeric adsorbant and desorption with carbon tetrachloride, has been described. The minimal detectable concentration was about 80 µg/m^3 (10 ppb) (Matsushita and Kanno, 1979).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

In a study reported as an abstract, groups of 40 female ICR mice, 9 weeks old, were given intragastric doses of 0, 0.0315, 0.125, 0.5 or 2 μl [0, 0.043, 0.17, 0.7 or 2.7 mg]/animal benzotrichloride twice weekly for 25 weeks. At 18 months after the start of treatment all surviving mice were killed. The incidences of malignant tumours were 0/35 in controls, and 2/37, 11/38, 28/40 and 27/35 in the treated groups, respectively. The incidences of squamous-cell carcinomas of the forestomach and adenocarcinomas of the lung were: 0/35, 0/37, 2/38, 22/40 and 24/35, and 0/35, 1/37, 9/38, 16/40 and 10/35 in the control and treated groups, respectively (Fukuda *et al.*, 1978).

(*b*) *Skin application*

Mouse: A series of skin-painting experiments in one strain of mice were conducted by Fukuda *et al.* (1981). In the first experiment, three groups of 19-22 female ICR mice, 14 weeks old [body weights unspecified], received skin applications of 25 μl [22 mg] benzene (controls), 25 μl [34.4 mg] reagent-grade benzotrichloride [purity and impurities unspecified] [about 860-1380 mg/kg bw, assuming body weights of 25-40 g] or 25 μl [17.2 mg] of a 50% solution of benzotrichloride in benzene [v/v; about 430-690 mg/kg bw, assuming body weights of 25-40 g] twice weekly for three weeks, and once weekly thereafter, until the mice were killed at week 32 [total doses of benzotrichloride, 1170 and 585 mg/animal]. Mortality at termination was 0, 10 and 46% in the control, low- and high-dose groups, respectively. By that time, 21 of the 22 animals that received the higher dose had developed tumours: 12 mice had a carcinoma (11 a squamous-cell carcinoma, 1 a basal-cell carcinoma) and 4 a papilloma of the skin, 8 had adenomas and 1 a carcinoma of the lungs, and 6 mice had a lymphoma. Of the 19 animals that received the lower dose, 17 had tumours: 6 a carcinoma and 5 a papilloma of the skin, 8 an adenoma and 2 a carcinoma of the lungs and 1 a lymphoma. No tumour was found in benzene-treated controls.

In the second experiment, three groups of 10 female ICR mice, 3 weeks old [body weights unspecified], received skin applications of 10 μl [8.8 mg] benzene (controls), 10 μl [13.7 mg] reagent-grade benzotrichloride [about 340-920 mg/kg bw, assuming body weights of 15-40 g] and 10 μl [6.9 mg] of a 50% solution of benzotrichloride in benzene [v/v; about 170-460 mg/kg bw, assuming body weights of 15-40 g] thrice weekly for four weeks and twice weekly thereafter until the mice were killed, either at week 43 [controls and low-dose animals; total dose, 580 mg/animal] or at week 24 [high-dose animals; total dose, 685 mg/animal]. Mortality at termination was 0, 60 and 80% in the control, low- and high-dose groups, respectively. Except for 1 mouse that received the higher dose, all benzotrichloride-treated animals had developed tumours, including 3 papillomas and 11 carcinomas of the skin (one was a basal-cell carcinoma), 11 pulmonary adenomas (3 multiple) and 2 carcinomas, 8 lymphomas, 2 carcinomas of the lips and a carcinoma of the forestomach. No tumour was found in benzene-treated controls.

In the third experiment, two groups of 20 female mice of the same strain, 7 weeks old [body weights unspecified], received skin applications of 25 µl [22 mg] benzene (controls) or 25 µl [3.2 mg] of a 9.2% solution of benzotrichloride in benzene [v/v; about 80-160 mg/kg bw, assuming body weights of 20-40 g] twice weekly for 50 weeks [total dose, 316 mg/animal]. The mice treated with benzotrichloride were killed at week 57 and the controls at week 82. Mortality at termination was 20% in the control group and 35% in the test group. A total of 48 tumours were found in 18 of the 19 mice treated with benzotrichloride; 13 mice had a carcinoma (12 squamous-cell carcinomas, 1 sebaceous carcinoma) and 5 a papilloma of the skin, and 2 had a carcinoma and 9 multiple adenomas of the lung; there were also 19 carcinomas or papillomas of the lips, tongue, oesophagus or stomach. Only 2 lung adenomas were found in benzene-treated controls.

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

Benzotrichloride was lethal to rats when given at 125 ppm [1000 mg/m^3] over a four-hour period. The oral LD_{50} in rats was 6 g/kg bw (Smyth *et al.*, 1951).

The LC_{16}, LC_{50} and LC_{84} values for two-hour exposures to benzotrichloride were 90, 150 and 240 mg/m^3 [11, 19 and 30 ppm], repectively, for rats, and 30, 60 and 120 mg/m^3 [4, 8 and 15 ppm], respectively, for mice. The order of decreasing toxicity among the chlorinated toluenes was: benzotrichloride > benzal chloride > benzyl chloride. Thus, in a single two-hour exposure of mice and rats to 100 mg/m^3 [12.5 ppm], all three compounds produced central nervous system excitation, irritation of the eyes and respiratory mucosae and slowed respiration in the order of activity described above. Hyperaemia of the extremities was also noted; at 1000 mg/m^3 [125 ppm] mice exhibited motor automatism and rats showed twitching of peripheral muscles. When rats were exposed to 100 mg/m^3 of each of the compounds in an inhalation chamber, continuously for one month, the degree of weight loss was related to the same order of activity as in the acute studies. While benzyl chloride did not cause decreases in blood cell counts, benzotrichloride caused leucopenia and mild anaemia. Benzotrichloride, but not benzal or benzyl chloride, caused decreases in renal function (Mikhailova, 1964)

In the course of a carcinogenicity bioassay in which the three compounds were dissolved in benzene and applied to the clipped backs of specific-pathogen-free ICR female mice, irritation of the eyes, skin and respiratory tract was observed. Benzotrichloride was the most and benzal chloride the least irritating. The skin initially exhibited erythema and swelling, then alopecia and induration. In some cases, marked keratinization, ulcers and/or necrosis of the skin were observed (Fukuda *et al.*, 1981).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

Benzotrichloride [purity and impurities unspecified] has been reported to be mutagenic in reversion assays using *Salmonella typhimurium* strains TA1535, TA98 and TA100 and in *Escherichia coli* strain WP2 hcr; mutagenicity required metabolic activation by a microsomal fraction of the livers of rats induced by Arochlor 1254. The authors stated that slight activity was observed in WP2 hcr in the absence of activation, but no data were given. It was also positive in a *rec*-assay using *Bacillus subtilis* (Yasuo *et al.*, 1978).

(*b*) *Humans*

Benzotrichloride has been reported to be highly irritating to skin and mucous membranes (Windholz, 1976).

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data clearly relating cancer to exposure to benzotrichloride were available to the Working Group. However, benzoyl chloride manufacturing workers, among whom an excess of respiratory cancer has been reported (six cases in total), were also potentially exposed to benzotrichloride (Sakabe *et al.*, 1976; Sakabe and Fukuda, 1977). These reports are considered in detail in the monograph on benzoyl chloride.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzotrichloride was tested in a series of three studies by skin application to female mice. It produced squamous-cell carcinomas of the skin in all three experiments; upper-digestive-tract tumours were also observed in two of the three experiments. An increase in the incidence of tumours at other sites was reported. In a study reported as an abstract, oral administration of benzotrichloride produced malignant tumours of the forestomach and lung in female mice.

Benzotrichloride was mutagenic to bacteria with metabolic activation, and was positive in a *rec*-assay with *Bacillus subtilis*.

No data were available to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzotrichloride has and probably still does occur during its manufacture and use as an intermediate in the manufacture of benzoyl chloride, dyes, ultra-violet stabilizers and other derivatives.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study involving exposure to benzotrichloride alone was available to the Working Group. Six cases of respiratory cancer have been reported among benzoyl chloride production workers in two small plants, who were also potentially exposed to benzotrichloride. The cases occurred in young workers, three of whom were nonsmokers.

4.3 Evaluation[1]

There is *sufficient evidence* that benzotrichloride is carcinogenic in mice.

Although the epidemiological data were inadequate to evaluate the carcinogenicity of benzotrichloride alone, they provide *limited evidence* that employment in the production of benzoyl chloride and its chlorinated toluene precursors, which involves exposure to benzotrichloride, represents a carcinogenic risk to man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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BENZOYL CHLORIDE

1. Chemical and Physical Data

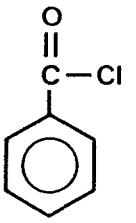
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 98-88-4

Chem. Abstr. and IUPAC Systematic Names: Benzoyl chloride

Synonyms: Benzene carbonyl chloride; benzenecarbonyl chloride; benzoic acid, chloride; α-chlorobenzaldehyde

1.2 Structural and molecular formulae and molecular weight



C_7H_5ClO Mol. wt: 140.6

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Transparent, colourless liquid (Hawley, 1981)

(*b*) *Boiling-point*: 197.2°C

(*c*) *Melting-point*: 0°C

(*d*) *Density*: d_4^{20} 1.2120

(*e*) *Refractive index*: n_D^{20} 1.5537

(*f*) *Spectroscopy data*: Infra-red, nuclear magnetic resonance and mass spectral data have been reported (Grasselli and Ritchey, 1975).

(*g*) *Solubility*: Decomposes in water and ethanol; miscible with diethyl ether; soluble in benzene and carbon disulphide

(*h*) *Volatility*: Vapour pressure, 0.4 mm at 20°C (Verschueren, 1977)

(*i*) *Stability*: Decomposes in water (Hawley, 1981); generates phosgene on heating (Hooker Chemical Corp., 1979); combustible (flash-point, 72.2°C) (Hawley, 1981)

(*j*) *Reactivity*: Reacts vigorously with steam, hot water, alkalis and oxidizers (Hooker Chemical Corp., 1979)

(*k*) *Conversion factor*: ppm = 0.174 x mg/m^3

1.4 Technical products and impurities

Benzoyl chloride is available in the US as a clear, colourless liquid with a minimum of 99.0% active ingredient and a minimal freezing-point of -0.62°C (Velsicol Chemical Corp., 1979).

In western Europe, benzoyl chloride must have a minimum of 99.6% active ingredient and maxima of 0.1% benzotrichloride, benzal chloride, benzyl chloride and chlorotoluenes.

In Japan, it must have a minimum of 99.5% active ingredient and a minimal freezing-point of -0.7°C. The principal impurities are believed to be benzotrichloride and benzoic acid.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Benzoyl chloride was first prepared by the chlorination of benzaldehyde in 1832. It can also be prepared by treatment of benzoic acid with phosphorous pentachloride, phosgene, or thionyl chloride or by the partial hydrolysis of benzotrichloride (Windholz, 1976). The most commonly used method (and probably the method used for commercial production) is reported (Williams, 1978) to be the reaction of benzoic acid with benzotrichloride, which is the subject of a separate monograph in this volume.

Benzoyl chloride has been produced commercially in the US since at least 1919 (US Tariff Commission, 1921) and in Japan since about 1947. Since only two US companies have reported commercial production for many years, separate production data are not available (see preamble, section 8(*b*)(ii)), but total production was estimated to have been 8.4 million kg in 1974 (Stanford Research Institute, 1977). One of the two present US manufacturers of benzoyl chloride reported that its production in 1977 was 4.5-22.7 million kg (US Environmental Protection Agency, 1981).

In 1980, US imports of benzoyl chloride through the principal customs districts totalled 110 thousand kg (US International Trade Commission, 1981).

Production of benzoyl chloride in western Europe is estimated to be 15-40 million kg per year. There is one company each in France, the Federal Republic of Germany and the United Kingdom.

An estimated 4 million kg of benzoyl chloride were produced by the three Japanese producing companies in 1980, and a small amount was exported. Approximately 60% of the production was based on the reaction of benzoic acid with benzotrichloride and the remainder on the partial hydrolysis of benzotrichloride with water.

(*b*) *Use*

Benzoyl chloride is used as a chemical intermediate to produce a variety of derivatives. No information on the quantities used for different applications in recent years was available to the Working Group; however, the following pattern of use was reported in the US for 1974: benzoyl peroxide, 57%; herbicides, 39%; dyes, 3%; and plasticizers and drugs, 1% (Stanford Research Institute, 1977). Total production of benzoyl peroxide in 1979 (all of which is believed to have been made from benzoyl chloride) by five US companies amounted to 4.0 million kg (US International Trade Commission, 1980); benzoyl peroxide is used as an initiator and crosslinking agent in the manufacture of a variety of polymers.

At the present time, the only herbicide produced commercially from benzoyl chloride in the US is chloramben (3-amino-2,5-dichlorobenzoic acid; Amiben^{R}), which is used principally as a pre-emergent herbicide on soya beans. The only US manufacturer is estimated to have had a production capacity of about 14 million kg per year in 1979.

According to the Society of Dyers and Colourists (1971), 18 dyes and pigments can be prepared from benzoyl chloride. However, only one of these, Vat Yellow 3, 12-1/2%, has been produced in commercial quantities in the US in recent years; and the single manufacturer last reported production in 1977 (US Environmental Protection Agency, 1981). Several chemicals listed in the *Colour Index* as starting materials for vat-dye synthesis are benzoyl derivatives of substituted anthraquinones that are believed to be produced from benzoyl chloride, but this could not be verified.

No evidence was found that benzoyl chloride is currently used to make benzoate plasticizers or pharmaceuticals; the local anaesthetic, hexylcaine hydrochloride (1-cyclohexylamino-2-propanol benzoate hydrochloride), which can be made from benzoic acid or benzoyl chloride (Swinyard, 1975; Windholz, 1976), is believed to be produced commercially in small quantities. Benzoyl chloride is used in the manufacture of benzophenone, a chemical used as a fixative in perfumes in soaps and as a chemical intermediate (Papa and Sherman, 1981). It has also been used to produce benzoyl

derivatives of a variety of chemicals and, to a limited extent, to treat cellulosic yarns and to improve the fastness of dyed fibres or fabrics (Williams, 1978).

In Japan, approximately 80% of the benzoyl chloride used is in the synthesis of benzoyl peroxide; the remainder is used for a variety of purposes, including pesticide and dye synthesis.

Two countries have been reported to limit occupational exposure to benzoyl chloride by regulation. In Romania, the mean concentration expressed as a time-weighted average for a working shift should not exceed 5 mg/m^3; and at no time may the concentration exceed a maximum concentration of 10 mg/m^3. The maximum allowable concentration in the USSR is 5 mg/m^3 (International Labour Office, 1980).

The US Environmental Protection Agency (1979) requires that notification be given whenever discharges containing 454 kg or more of benzoyl chloride are made into waterways. As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzoyl chloride are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

2.2 Occurrence

Benzoyl chloride has not been reported to occur as such in Nature. No other data on its occurrence in the environment were available to the Working Group.

2.3 Analysis

No data on methods for the analysis of benzoyl chloride were available to the Working Group.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Skin application*

Mouse: A series of skin-painting experiments in one strain of mice were conducted by Fukuda *et al.* (1981). In the first experiment, three groups of 10 female ICR mice, 3 weeks old [body weights unspecified], received skin applications of 10 μl [8.8 mg] benzene (controls), 10 μl [12.1 mg] reagent-grade benzoyl chloride [purity and impurities unspecified] [about 300-800 mg/kg bw, assuming body weights of 15-40 g] or 10 μl [6.1 mg] of a

50% solution of benzoyl chloride in benzene [v/v; about 150-400 mg/kg bw, assuming body weights of 15-40 g] thrice weekly for four weeks and twice weekly thereafter until the mice were killed at week 43 [total doses of benzoyl chloride, 1065 and 533 mg/animal]. No death occurred and no tumour was found in the control group; 2 animals that received the lower dose had a skin tumour: 1 papilloma and 1 squamous-cell carcinoma; 3 lung adenomas were seen in the high-dose group. [The Working Group noted the short duration of the experiment.]

In the second experiment, two groups of 20 female ICR mice, 7 weeks old [body weights unspecified], were given skin applications of 25 µl [22 mg] benzene (controls) or 25 µl of a 9.2% solution [2.8 mg] of benzoyl chloride in benzene [v/v; about 70-140 mg/kg bw, assuming body weights of 20-40 g], twice weekly for 50 weeks [total dose, 278 mg/animal]. The mice were killed at week 82. Mortality at termination was 5% in the test group and 20% in the control group. Two mice of the test group had a squamous-cell carcinoma of the skin; no skin tumour was found in benzene-treated controls. Lung adenomas were seen in 5 treated and 2 control animals. In neither experiment was the skin tumour incidence in test groups statistically significantly higher than that in controls. [The Working Group noted the limited size of the experimental groups.]

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

In the course of a carcinogenicity bioassay (Fukuda *et al.*, 1981), specific-pathogen-free female ICR mice were treated with 50% benzoyl chloride dissolved in benzene by skin painting intermittently for 9.8 months. The mice displayed marked irritation of the eyes, skin and respiratory tract, demonstrated excitation and attempted to scratch the painted area. In this regard, benzoyl chloride was only slightly less effective than benzotrichloride (see monograph on benzotrichloride).

No data were available to the Working Group on effects on reproduction and prenatal toxicity or on absorption, distribution, excretion and metabolism.

Mutagenicity and other short-term tests

Benzoyl chloride [purity and impurities unspecified] was not mutagenic in *Salmonella typhimurium* strains *his* G46, *his* C3076, *his* D3052, TA1535, TA1536, TA1537, TA98 or TA100, nor in *Escherichia coli* strains WP2 and WP2 *uvr*A$^-$ with or without metabolic activation (Yasuo *et al.*, 1978; McMahon *et al.*, 1979). However, Yasuo *et al.* (1978) drew attention to the rapid hydrolysis of this compound in aqueous media. Chiu *et al.* (1978) reported that benzoyl chloride [purity and impurities not specified] was weakly mutagenic to *Salmonella* strain TA98 in the absence of metabolic activation. [The Working Group noted that the induced reversion rate was less than two-fold greater than background and concluded that these results were equivocal.]

Benzoyl chloride was negative in a *rec*-assay using *Bacillus subtilis* (Yasuo *et al.*, 1978).

(b) Humans

Benzoyl chloride is a strong lachrymator. It is irritating to skin, eyes and mucous membranes (Williams, 1978; Hooker Chemical Corp., 1979).

3.3 Case reports and epidemiological studies of carcinogenicity in humans

(a) Case reports

Sakabe *et al.* (1976) reported four cases of respiratory cancer among the employees of a small chemical plant in Japan in which benzoyl chloride was produced from 1954 to 1972. One of the cases was a squamous-cell carcinoma of the bronchus in a 44-year-old nonsmoker; another two cases of lung cancers occurred in smokers, also 44 years old. The histological types of the latter two cases are unknown. The fourth case was a malignant lymphoma of the maxillary sinus. The length of exposure to the benzoyl chloride manufacturing process ranged from about six to 15 years; and the induction-latent period from start of exposure until diagnosis was 6-18 years (the shortest period relating to one of the lung cancer cases in a smoker).

Sakabe and Fukuda (1977) reported two further cases of lung cancer in another small plant where benzoyl chloride was produced. One of the patients was a 40-year-old smoker with seven years of exposure and an induction-latent period of 13 years from start of exposure to diagnosis; the other was a 35-year-old nonsmoker with a squamous-cell carcinoma, who had had about one year of exposure to the benzoyl chloride production process starting about 11 years prior to diagnosis. Both cases had had earlier exposures, of 17 and four years, respectively, to benzoyl peroxide production stemming from the benzoyl chloride manufacturing process.

All cases in the two reports seem to have been exposed not only to benzoyl chloride but also to precursors in the production process, particularly toluene, chlorine, hydrogen chloride and benzotrichloride, and to a lesser extent also to benzal chloride and benzyl chloride.

(b) Epidemiological studies

An epidemiological evaluation was made of the reports by Sakabe *et al.* (1976) on malignancies among benzoyl chloride manufacturing workers. The plant was reported to employ an average of 20 workers. When female office workers were included in a cohort evaluation of lung cancer deaths, 2188 person-years were obtained from 147 individuals. The expected number of cases was 0.22 (as derived from national rates), whereas 2 were observed ($p = 0.02$). When only benzoyl chloride workers were considered, 670 person-years were obtained from 41 individuals; 2 lung cancer deaths were observed *versus* 0.06 expected ($p = 0.002$). [Since no induction-latent period was allowed for, the expected numbers of cases tend to be overestimates, also diluted by office workers in one of the comparisons; in addition, the third case of lung cancer, still alive at the time of presentation of this report, was not included among the observed cases.]

The second report (Sakabe and Fukuda, 1977) contained no epidemiological evaluation, but the factory population from which the two cases were derived varied from 13 individuals in 1952 to 40 in 1963. [The expected number of cases would therefore be of the same magnitude as that in the first report.]

[Epidemiological evidence suggestive of lung cancer was the finding of cases in relatively young nonsmokers in numbers greater than expectation. However, these studies were of exposure to production processes rather than to single chemicals; ascertainment of the cases was through reports of clusters rather than as a result of systematic study of the industry; and they are based on small numbers of cases and person-years at observation.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzoyl chloride was tested in two sets of experiments by skin application to female mice. A few skin carcinomas were observed in treated mice, but their incidence was not statistically significant.

There is no clear evidence that benzoyl chloride is mutagenic in bacterial systems; it was negative in a *rec*-assay with *Bacillus subtilis*.

No data were available to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzoyl chloride has and probably still does occur during its manufacture and use in the production of benzoyl peroxide, herbicides and other derivatives.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study involving exposure to benzoyl chloride alone was available to the Working Group. Six cases of respiratory cancer have been reported among benzoyl chloride production workers in two small plants. The cases occurred in young workers, three of whom were nonsmokers.

4.3 Evaluation[1]

There was inadequate evidence for the carcinogenicity of benzoyl chloride in experimental animals.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

Although the epidemiological data were inadequate to evaluate the carcinogenicity of benzoyl chloride alone, they provide *limited evidence* that employment in the production of benzoyl chloride and its chlorinated toluene derivatives represents a carcinogenic risk to man.

No evaluation could be made on the carcinogenicity to man of benzoyl chloride itself.

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Yasuo, K., Fujimoto, S., Katoh, M., Kikuchi, Y. and Kada, T. (1978) Mutagenicity of benzotrichloride and related compounds. *Mutat. Res., 58*, 143-150

BENZENE

This substance was considered by a previous Working Group, in June 1974 (IARC, 1974). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 71-43-2

Chem. Abstr. and IUPAC Systematic Names: Benzene

Synonyms: (6)-Annulene; benzin[1]; benzine[1]; benzol; benzole; benzolene; bicarburet of hydrogen; carbon oil; coal naphtha; cyclohexatriene; mineral naphtha; motor benzol; phene; phenyl hydride; pyrobenzol; pyrobenzole

Trade Name: Polystream

1.2 Structural and molecular formulae and molecular weight

C_6H_6  Mol. wt: 78.1

1.3 Chemical and physical properties of the pure substance

From Purcell (1978), unless otherwise specified

(*a*) *Description*: Colourless liquid

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[1] These names are no longer used for benzene; they were used for many years to describe a low-boiling petroleum fraction predominantly containing aliphatic hydrocarbons (Purcell, 1978).

(*b*) *Boiling-point*: 80.1C

(*c*) *Melting-point*: 5.5°C

(*d*) *Density*: d_4^{20} 0.8787 (Weast, 1979)

(*e*) *Refractive index*: n_D^{20} 1.5011 (Weast, 1979)

(*f*) *Spectroscopy data*: λ_{max} 243, 249, 256 and 261 nm (in ethanol) (Weast, 1979); mass spectra and carbon-13 nuclear magnetic resonance spectra have been tabulated (NIH/EPA Chemical Information System, 1980).

(*g*) *Identity and purity test*: Conversion to *meta*-dinitrobenzene, which is recrystallized and found to be identical with a standard sample in melting-point tests

(*h*) *Solubility*: Slightly soluble in water (1.8 g/l at 25°C); miscible with acetic acid, acetone, chloroform, diethyl ether and ethanol (Weast, 1979)

(*i*) *Viscosity*: 0.6468 cP at 20°C

(*j*) *Volatility*: Vapour pressure, 100 mm at 26.1°C

(*k*) *Stability*: Stable; combustible (flash-point, -11.1°C)

(*l*) *Reactivity*: Undergoes substitution, addition and cleavage of the ring

(*m*) *Conversion factor*: ppm = 0.313 x mg/m^3

1.4 Technical products and impurities

Benzene is available in the US in three grades: refined, nitration grade and industrial grade, all of which must be free of hydrogen sulphide and sulphur dioxide. Only the refined grade is required to contain no more than 0.15% non-aromatics and 1 mg/kg thiophene. The refined and nitration-grade products must have a distillation range of not more than 1°C including 80.1°C, a specific gravity of 0.8820-0.8860 (15.56/15.56°C), and contain no trace of acidity. The minimum solidification points are 5.35°C (dry basis) for the refined grade and 4.85°C (anhydrous basis) for the nitration grade. The industrial grade must have a distillation range of not more than 2°C including 80.1°C and a specific gravity of 0.875-0.886 (Purcell, 1978). One manufacturer lists the following typical composition for its nitration-grade benzene: 99.9+% purity, 0.03% nonaromatics, 0.02% water, 0.01% toluene, 0.1-0.2 mg/kg thiophene and no xylene (USS Chemicals, 1980).

Benzene available in Japan has the following specifications: boiling-range, 80.1 $\pm$ 1°C; freezing-point, a minimum of 5.2°C; specific gravity, 0.882-0.886 (15/4°C); a maximum of 0.001 g thiophene per 100 ml; and a maximum of 0.0005 g carbon disulphide per 100 ml.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

Recent reviews on benzene include those by Hancock (1975) and Purcell (1978).

(a) Production

Benzene was first isolated by Faraday in 1825 from a liquid condensed by compressing oil gas; Mitscherlich first synthesized it in 1833 by distilling benzoic acid with lime. Benzene was first recovered commercially from light oil derived from coal-tar in 1849, and from petroleum in 1941 (Purcell, 1978).

It is recovered commercially from both petroleum and coal sources; those of petroleum were the basis for an estimated 92% of US production in 1978. Petroleum sources include refinery streams (primarily catalytic reformate), pyrolysis gasoline (a by-product of the manufacture of ethylene by cracking naphtha or gas oil), and toluene hydrodealkylation. Coal-derived benzene is recovered from light oil produced in coke manufacture.

Catalytic reforming (the source of approximately 44% of the benzene produced in the US in 1978) converts the naphthenes and paraffins in naphtha to a product rich in aromatic hydrocarbons. When a high yield of benzene is desired, a suitable naphtha fraction is reformed under severe conditions, and approximately half of the benzene contained in the reformate is recovered by solvent extraction (e.g., with sulpholane or tetraethylene glycol). The rest of the benzene is left in the reformate, which is used in the production of gasoline.

In western Europe, benzene is produced in almost equal quantities from catalytic reformate, pyrolysis gasoline and toluene hydrodealkylation; coke-oven operations provide less than 10% of total production.

Pyrolysis gasoline is believed to be the single largest source of benzene in Japan, and a significant percentage of the total capacity is based on the use of multiple feeds. Coalderived benzene is estimated to constitute less than 10% of the total.

Preliminary data indicate that US production of all grades of benzene by 33 companies in 1980 totalled 1563 million gallons (5217 thousand tonnes) (US International Trade Commission, 1981). In 1979, 31 US companies reported a total production of 1° and 2° benzene (which includes refined, nitration and industrial grades) of 5351 thousand tonnes (US International Trade Commission, 1980). It is estimated that almost as much additional benzene was produced in both years but was not isolated from the various streams (catalytic reformate, etc) and was used for fuel purposes.

US imports of benzene in 1980 totalled 94.7 million gallons (316 thousand tonnes) (US Department of Commerce, 1981a), and exports were 11.8 million gallons (39.4 thousand tonnes) (US Department of Commerce, 1981b).

An estimated 4800 thousand tonnes of benzene were produced in western Europe in 1979. Annual production capacity in 1980 is estimated to have been at least 6877 thousand tonnes. This compound is produced by 66 companies in 11 western European countries, the major producers being the UK (nine producers), the Federal Republic of Germany (16) and The Netherlands (four). Recent production of benzene in thousands of tonnes by COMECON countries is estimated to have been as follows: USSR, 1538 (1977); Czechoslovakia, 195 (1979); Romania, 162 (1979); Bulgaria, 61 (1979); Hungary, 34 (1979) and Poland, 15 (1979).

About 2170 thousand tonnes of benzene were produced in Japan in 1979, approximately 185 thousand tonnes of which were derived from coal. The combined annual production capacity of the 22 Japanese producers in 1980 is estimated to have been 2882 thousand tonnes. Japanese exports of benzene in 1979 were about 173 thousand tonnes.

World production of benzene in 1977 is estimated to have been over 12 million tonnes, making it the fourth or fifth largest volume organic chemical produced on a worldwide basis. The areas with the largest production, apart from the US, Europe and Japan, are Canada and South America.

(*b*) *Use*

The use pattern for recovered benzene in the US in 1978 was as follows: ethylbenzene/styrene, over 50%; cumene/phenol, close to 20%; cyclohexane, 15-16%; nitrobenzene/aniline, 4-5%; and maleic anhydride, chlorobenzenes, detergent alkylate and other uses, 2.5-3.0% each.

Over 97% of all US production of ethylbenzene is based on the alkylation of benzene with ethylene; all but minor amounts of the ethylbenzene produced are dehydrogenated to styrene. The latter, an important monomer for a variety of polymers (both plastics and elastomers), was the subject of an earlier IARC monograph (IARC, 1979a). Preliminary data indicate that US production of styrene in 1980 totalled 3135 million kg (US International Trade Commission, 1981).

Benzene is alkylated with propylene to produce cumene (isopropylbenzene), all but minor quantities of which are oxidized to cumene hydroperoxide, which is split into phenol and acetone. Phenol, essentially all of which is derived from cumene in the US, is an intermediate in the manufacture of phenol-formaldehyde resins (See monograph on formaldehyde in this volume for further information), bisphenol A (used in the manufacture of epoxy resins), and caprolactam [the subject of an earlier IARC monograph, IARC, 1979b]. Acetone (60% of which was derived from cumene in the US in 1979) is an important solvent and chemical intermediate. Its most important derivative in the US is methyl methacrylate, a monomer for acrylic resins, which was the subject of an earlier IARC monograph (IARC, 1979c).

Approximately 85% of all cyclohexane produced in the US is made by the catalytic hydrogenation of benzene. Cyclohexane is a chemical intermediate for three chemicals used in the manufacture of nylon fibres and resins: caprolactam [see IARC, 1979b], adipic acid and hexamethylenediamine.

Of the other chemicals derived from benzene, the following have been the subjects of IARC monographs: aniline (IARC, 1982a), *ortho*- and *para*-dichlorobenzenes (this volume), hexachlorobenzene (IARC, 1979d), hexachlorocyclohexane (IARC, 1979e), and the two dihydroxybenzenes, hydroquinone and resorcinol (IARC, 1977).

In the past, benzene was used widely as a solvent, but the amounts used now for this purpose are believed to be relatively small and decreasing.

Use of an estimated 4864 thousand tonnes of recovered benzene in western Europe in 1979 was as follows: ethylbenzene/styrene, 48%; cumene, 20%; cyclohexane, 14%; nitrobenzene/aniline, 7%; detergent alkylate, 4%; maleic anhydride, 3%; chlorobenzenes, 2%; and other uses, 2%.

The use pattern in Japan in 1980 for recovered benzene was as follows: ethylbenzene/styrene, 51%, cyclohexane, 24%; cumene/phenol, 13%; detergent alkylate, 4%; maleic anhydride, 2%; and other uses, 6%.

Fifteen countries have been reported to limit occupational exposure to benzene by regulation or recommended guideline. Their standards are listed in Table 1. Benzene is

Table 1. National occupational exposure limits for benzene[a]

Country	Year	Concentration mg/m³	Concentration ppm	Interpretation[b]	Status
Australia	1978	30	10	TWA[c]	Guidelinge
Belgium	1978	30	10	TWA[c]	Regulation
Czechoslovakia	1976	50	—	TWA	Regulation
		80	—	Ceiling (10 min)	
Finland	1975	32	10	TWA[c]	Regulation
Hungary	1974	20	—	TWA[d]	Regulation
Italy	1978	30	10	TWA[c]	Guideline
Japan	1978	80	25	Ceiling	Guideline
The Netherlands	1978	30	10	TWA[c]	Guideline
Poland	1976	30	—	Ceiling[c]	Regulation
Romania	1975	50	—	Maximum[c]	Regulation
Sweden	1978	15	5	TWA[c]	Guideline
		30	10	Maximum (15 min)	
Switzerland	1978	6.5	2	TWA[c]	Regulation
USA[a]					
OSHA	1980	—	10	TWA	Regulation
		—	25	Ceiling	
		—	50	Peak[e]	
ACGIH	1981	30	10	TWA	Guideline
		75	25	STEL	
NIOSH	1980	3.2	1	Ceiling (60 min)	Guideline
USSR	1980	5	—	Ceiling[c]	Regulation
Yugoslavia	1971	50	15	Ceiling[c]	Regulation

a From American Conference of Governmental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980); National Institute for Occupational Safety and Health (NIOSH) (1980); US Occupational Safety and Health Administration (OSHA) (1980)
b TWA, time-weighted average; STEL, short-term exposure limit
c Skin irritant notation added
d May be exceeded 5 times per shift as long as average does not exceed value
e Peak limit above ceiling - 10 minutes

recognized as being carcinogenic by six countries (Finland, the Federal Republic of Germany, Italy, Japan, Sweden and Switzerland) and is designated as being a suspected carcinogen in two others (Australia and the USA) (International Labour Office, 1980).

A ban on all consumer products (except gasoline and solvents or reagents for laboratory use) containing benzene as an intentional ingredient or as a contaminant constituting 0.1% or more by volume was proposed by the US Consumer Products Safety Commission in 1978. In 1981, the Commission withdrew its proposed ban on the basis of information that benzene, as currently used in consumer products, did not present a significant risk to consumers. Data from contacts in industry and information obtained from manufacturers, importers and labellers of such products indicated that benzene is not currently used intentionally in consumer products (US Consumer Product Safety Commission, 1981).

The US Environmental Protection Agency (EPA) (1979) requires that notification be given whenever discharges containing 454 kg or more of benzene are made into waterways. In 1980, the EPA proposed a national standard for benzene emissions from maleic anhydride plants that would prohibit detectable emissions from new sources and limit emissions from existing sources to 0.3 kg per 100 kg of benzene fed to the reactor (US Environmental Protection Agency, 1980a).

The EPA has also identified benzene as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. Bottom sediment sludge from the treatment of waste-waters from wood-preserving processes involving use of creosote and/or pentachlorophenol, water or caustic cleaning wastes from painting equipment, tank cleaning from paint manufacture, emission control dust or sludge from paint manufacture, and distillation or fractionating column bottoms from the production of chlorobenzenes are included in a list of hazardous wastes in which benzene was identified as one of the hazardous constituents (US Environmental Protection Agency, 1980b,c). However, in early 1981, the second and third of these four wastes were removed from the list, and the following wastes were added: the combined waste-water streams generated from nitrobenzene/aniline production and the separated aqueous stream from the reactor product washing step in the production of chlorobenzenes (US Environmental Protection Agency, 1981a).

The EPA proposed a national emission standard for 'fugitive emissions' (from supposedly sealed installations) of benzene in early 1981, which would prohibit detectable benzene emissions from processing equipment (e.g., pumps, valves) that contains materials which have a benzene concentration of 10% or more by weight (US Environmental Protection Agency, 1981b). The EPA has also proposed a regulation to limit effluent discharges into publicly owned treatment works of benzene from beehive cokemaking operations. The proposed limit is 63.8 mg benzene per thousand kg of product [0.0638 ppm] (US Environmental Protection Agency, 1981c).

The Bureau of Alcohol, Tobacco and Firearms of the US Department of the Treasury (1981) lists benzene among the approved denaturants for three of the prescribed formulae for denaturing alcohol.

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzene are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

The Commission of the European Communities (1980) prohibits the use of benzene in products intended for use as toys (e.g., children's ballons).

2.2 Occurrence

(*a*) *Natural occurrence*

Benzene is a natural constituent of crude oil (Brief *et al.*, 1980).

(*b*) *Occupational exposure*

There is or has been occupational exposure to benzene in numerous industries because of its presence as a component of many fuels and as an impurity in organic chemicals made from it. For example, it is present in straight-run petroleum distillates and in coal-tar distillates (Ayers and Muder, 1964). It has been estimated that about two million workers in the US are potentially exposed to benzene (Brief *et al.*, 1980).

The early uses of benzene, particularly as a solvent, resulted regularly in concentrations in workplace air of about 1600 mg/m^3 [500 ppm] and sometimes concentrations in excess of 3200 mg/m^3 [1000 ppm]. Table 2 gives examples of concentrations of benzene reported in workplace air in various industries since 1935.

Concentrations of benzene found in the air around certain operations in various US rubber tyre factories are given in Table 3.

In four different central telephone offices in the US, benzene was present in the workplace air in concentrations ranging from 1.3 to 28.8 μg/m^3 [0.41-9 ppb]. When levels at three of the offices were 58, 16 and 2.6 μg/m^3 [18, 5 and 0.8 ppb], outside air contained 3, 9.6, and 0.6 μg/m^3 [1, 3 and 0.2 ppb] of benzene, respectively. In one US telephone business office, a level of 16 μg/m^3 [5 ppb] was found (Oblas *et al.*, 1980).

(*c*) *Air*

Rural background concentrations of benzene have been reported to range from 0.3-54 μg/m^3 [0.1-17 ppb]. It has been suggested that these levels are related to biological sources; for example, ambient benzene concentrations increase after forest fires and oil seeps (Brief *et al.*, 1980). The general urban atmosphere reportedly contains 0.05 mg benzene/m^3 [0.02 ppm] of air. Assuming a daily inhalation of 24 m^3 of air and a benzene

Table 3. Concentrations of benzene in the US tyre industry, 1973-1977[a]

Operation	Personal breathing sample		Area sample	
	mg/m^3	ppm	mg/m^3	ppm
Cement mixing	0.6-15.4	0.2-4.8	0-52.8	0-16.5
Extrusion	< 0.3-16.3	< 0.1-5.1	1.3-14.7	0.4-4.6
Tyre building	0.3-7.7	0.1-2.4	0.3-6.4	0.1-2.0
Curing preparation	< 0.3-18.9	< 0.1-5.9	1.3-3.8	0.4-1.2
Inspection and repair	< 0.3-6.1	< 0.1-1.9	0.03-7.0	0.01-2.2
Maintenance	0.6-1.5	0.2-0.5	1.5-4.8	0.5-1.5
Warehouse	—		0.03-1.5	0.01-0.5

a From Van Ert *et al.* (1980)

Table 2. Occupational exposures to benzene in various industries

Industry	Concentration (mg/m^3 air) [ppm] Range	Other	Year	Reference[a]
Rubber coating (churn room)	304-832 [95-260]		1935-1937	NIOSH (1974)
(spreader machine)	208-640 [65-200]		1935-1937	NIOSH (1974)
Rubber factory	320[b] [100]	1600 [500][c]	1942	EPA (1980d)
Rubber coating	83-416 [25-125]		1961	EPA (1980d)
	64-80 [20-25][b,d]	448 [140][c]	1960-1963	NIOSH (1974)
Rubber raincoat factory	438-698 [137-218]		1977	EPA (1980d)
Printing	32-3392 [10-1060]		1939	NIOSH (1974)
Artificial leather, rubber goods or shoe manufacture	320-1600 [100-500]		1936-1939	NIOSH (1974)
Leatherette factory	150-1000 [47-310]		1953-1957	NIOSH (1974)
	80-150 [25-47][e]		1953-1957	NIOSH (1974)
Shoe factory	1017-1504 [318-470)		1956	NIOSH (1974)
	100-500 [31-156]		1960-1963	NIOSH (1974)
	130-140 [41-44][f]		1964	NIOSH (1974)
	480-2080 [150-650]		1966-1978	EPA (1980d)
Chemical plant[g]	0.3-1.3 [0.1-0.4]		1979	Evans and Wilcox (1979)
Petroleum refinery (quality control lab)	2-103 [0.6-32][h]		1978	Markel and Elesh (1979)
Waste-water treatment plant at petroleum factory	0.06-1.5 [< 0.02-0.51] (personal samples)		1978	Gorman and Slovin (1980)
	0.06-21.7 [< 0.02-7.22] (area samples)		1978	Gorman and Slovin (1980)
Coal liquefaction plant	0.06 [0.02]		1980	Anon. (1980)
Australian Air Force workshop	33-116 [10-35][b]	4480 [1400][c]	1947	EPA (1980d)
Paint manufacture	trace-362 [trace-78.5]		1963	NIOSH (1974)
Painting of metal products	0.5-10 [0.17-3.1]		1978	McQuilkin (1980)

a NIOSH, National Institute for Occupational Safety and Health; EPA, US Environmental Protection Agency

b Average *c* Peak

d Following removal of benzene from use in the industry; naphtha solvents containing up to 7.5% benzene were being used

e Following improved control measures

f Following replacement of a technical benzene with toluene in shoe adhesives

g Producing hexabromocyclododecane and dialkylaminoethyl chloride hydrochlorides

h Maximum measured at benzene distillation operation

retention of 50%, human beings would inhale 0.6 mg of benzene per day (National Research Council, 1980). Table 4 is a summary of the results of several studies conducted since 1963 on concentrations of benzene in ambient air. Estimated annual emissions of benzene to the air in the US from various sources are summarized in Table 5.

Table 4. Levels of benzene in ambient air

Location (year)	Concentration		Source
	$\mu g/m^3$	ppb	
Los Angeles Basin, USA (1965)	16-70	5-22	Howard and Durkin (1974)
Central Los Angeles, USA (1963)	48-192	15-60	Howard and Durkin (1974)
Los Angeles Basin, USA (1968)	48	182	Howard and Durkin
	182	57[b]	(1974)
Los Angeles, USA (1977)	19	6[a]	Martin *et al.* (1980)
Riverside, California, USA (1973)	22-26	7-8	Howard and Durkin (1974)
Dallas, Texas, USA (1977)	5	1.6[a]	Martin *et al.* (1980)
Chicago, Illinois, USA (1977)	18	6[a]	Martin *et al.* (1980)
Toronto, Ontario, Canada (1973)	42	13[a]	Howard and Durkin
	31	98[b]	(1974)
Vancouver, B.C., Canada (1965)	3-32	1-10	Howard and Durkin (1974)
Delft, The Netherlands	3	0.93[a]	Brief *et al.*
	26	8[b]	(1980)
The Hague, The Netherlands	29	9[a]	Brief *et al.*
	93	29[b]	(1980)
Zurich, Switzerland	112	35[a]	Brief *et al.*
	237	74[b]	(1980)
Zurich, Switzerland (1971)	173	54	Howard and Durkin (1974)
Prague, Czechoslovakia	0.3	0.1[a]	Brief *et al.* (1980)
London, UK	573	179[b]	Brief *et al.* (1980)
London airport, UK	293	92	Thorburn and Colenutt (1979)
Uxbridge, Middlesex, UK	278-358	87-112	Colenutt and Thorburn (1980)
Rural location in UK	194	61	Thorburn and Colenutt (1979)

a Average
b Maximum

Table 5. Annual emissions of benzene to air from various sources[a]

Source	Emission (thousand tonnes)
Component of gasoline[b]	40.0-80.0
Production of other chemicals	44.0-56.0
Indirect production of benzene[c]	23.0-79.0
Production of benzene from petroleum	1.8-7.3
Solvents and miscellaneous sources	1.5
Imports of benzene	0.013

a From JRB Associates, Inc. (1980)
b Production, storage, transort, vending and combustion
c Coke ovens, oil spills, nonferrous metals manufacturing, ore mining, wood processing, coal mining, and textile industry

Concentrations of benzene in air at various locations in Texas, USA, were found to be as follows: in an oil-field, 9.6-512 μg/m³ [3-160 ppb]; near a crude-oil tank farm, 12.8-41.6 μg/m³ [4-13 ppb]; near an oil refinery, 6.4-41.6 μg/m³ [2-13 ppb]; in a remote, non-industrial area, 9.6-12.8 μg/m³ [3-4 ppb] (Oldham *et al.*, 1979). In a survey of 17 US states, five million people were estimated to be exposed to 0.3-3.0 μg/m³ [0.1-1.0 ppb] benzene from petroleum refineries, and 3000 people to 3-13 μg/m³ [1.1-4.0 ppb] (Suta, 1980).

Average benzene concentrations in 24-hour air samples taken near coke-oven operations at a steel plant in Pennsylvania, USA, were in the range of 4-19 μg/m³ [1-6 ppb] (Fentiman *et al.*, 1979). In a survey of 12 US states, 300 000 people were estimated to be exposed to benzene in the air from coke-oven operations (Suta, 1980).

Table 6 is a summary of concentrations of benzene found in air samples taken near US chemical factories where benzene was used.

Table 6. Benzene concentrations in the air near US chemical manufacturing factories

Source	Concentration μg/m³	ppb	Reference
Nitrobenzene manufacture	3-11	1-4	Fentiman *et al.* (1979)
Cumene manufacture	25-51	9-19	Fentiman *et al.* (1979)
Maleic anhydride manufacture	2-32	1-10	Fentiman *et al.* (1979)
From pyrolysis gas	6-35	2-13	Fentiman *et al.* (1979)
Detergent alkylate manufacture	2.7-55.4	1-18	Fentiman *et al.* (1979)
Other factories using benzene	1.9-108.8	0.6-34	Suta (1980)

In a survey of 22 US states, it was estimated that about six million people were exposed to 0.3-3.0 μg/m³ [0.1-1.0 ppb] benzene from chemical factories, about one million to 3.0-13.0 μg/m³ [1.1-4.0 ppb], about 200 000 to 13.0-32.0 μg/m³ [4.1-10.0 ppb] and about 80 000 people to more than 32.0 μg/m³ [10.0 ppb] (Suta, 1980).

The benzene content of ambient air sampled in the vicinity of one solvent reclamation plant was found to be 74 mg/m³ [23 ppm] (Howard and Durkin, 1974).

US gasolines contain an average of 0.8% benzene and European gasolines contain an average of 5% (US Environmental Protection Agency, 1980d). Several studies that have been made of the levels of benzene in the air at gasoline service stations and loading facilities are summarized in Table 7. Suta (1980) has estimated that 37 million people in the US are exposed to benzene in the air from self-service gasoline stations.

Benzene comprises about 2.15% of total hydrocarbon emissions from a gasoline engine, or about 4% of automotive exhaust (US Environmental Protection Agency, 1980d). Data on benzene found in ambient air in areas associated with automobile use are summarized in Table 8. Brief *et al.* (1980) also reported that levels of benzene in the air near major roadways correlate with traffic levels. It has been reported that older automobiles (1975 and earlier models) emit larger quantities of benzene in exhaust than do newer, catalyst-equipped automobiles (Briggs *et al.*, 1977).

(*d*) *Water and sediments*

Benzene has been detected in lake, river and well water, raw and finished drinking-water, and in effluents from oil and coal processing, chemical factories, raw sewage and sewage treatment plants (Shackelford and Keith, 1976; Hushon *et al.*, 1980).

Assuming an intake of 2 l/day and a level of 1 μg benzene per l of US drinking-water, the dose of benzene to humans from water would be 2 μg/day (National Research Council, 1980).

Annual emissions of benzene to water in the US from various sources are summarized in Table 9.

Concentrations of benzene in waste-water from coal preparation plants have been reported to be in the range of 0.3-48 μg/l (Randolph *et al.*, 1979). Concentrations in effluents from plants which manufacture or use benzene have been found to be in the range of <1-179 μg/l; river and stream water near the plants contained <1-13 μg/l, while samples taken further downstream contained 2 μg/l or less (Fentiman *et al.*, 1979).

Benzene concentrations in water in several countries are summarized in Table 10.

(*e*) *Soil and plants*

Benzene has been found at concentrations of <2 to 191 μg/kg in soil samples taken near factories where benzene was used or produced (Fentiman *et al.*, 1979).

(*f*) *Food, beverages, feed*

Benzene has been reported in several foods: eggs, 500-1900 μg/kg; Jamaican rum, 120 μg/kg; irradiated beef, 19 μg/kg; heat-treated or canned beef, 2 μg/kg (US Environmental

Table 7. Benzene in the air near gasoline facilities and operations in the US and Europe

Sampling site	Average concentration mg/m³	ppm	Reference[a]
Service stations	0.4	0.123	NRC (1980)
	0.6-10.2	0.2-3.2	NIOSH (1980)
	0.08-10	0.02-3.2	Brief *et al.* (1980)
	0.001-0.007	0.0003-0.0023	Fentiman *et al.* (1979)
Residential neighbourhood upwind of service station	0.004-0.006	0.0013-0.002	Fentiman *et al.* (1979)
Downwind of service station during refuelling of underground tanks	0.032-0.069	0.01-0.024	Fentiman *et al.* (1979)
Bulk-loading facilities	0.3-24.6	0.1-7.7	NIOSH (1974)
	4.5-31.7	1.4-9.9	Brief *et al.* (1980)
Loading and discharging of road tankers	4.5-30.1	1.4-9.4	NIOSH (1974)
Loading of rail tankers	5-8	1.6-2.5	NIOSH (1974)
Distribution facility	0.003-0.032	0.001-0.01	Oldham *et al.* (1979)

a NCR, National Research Council; NIOSH, National Institute for Occupational Safety and Health

Table 8. Benzene in air associated with automobile use

Sampling site	Concentration µg/m³	ppb	Reference
Residential neighbourhood	5	1.5	Fentiman *et al.* (1979)
Central business district	12	3.8	Fentiman *et al.* (1979)
Busy highway leading into business district	9-28	3-8.6	Fentiman *et al.* (1979)
Roadway intersection	16-150	5-47	Suyama *et al.* (1980)
Highway	490	153	Thorburn and Colenutt (1979)
Urban area with much traffic	393	123	Thorburn and Colenutt (1979)
Urban streets, parking garages, car repair shops, inside automobiles, and in a home above a parking garage	25-600	7.8-190	Jonsson and Berg (1980)

Table 9. Annual benzene emissions to water in US[a]

Source	Emissions (tonnes)
Indirect production of benzene[b]	200-11 000
Solvent and miscellaneous uses	1450
Production of chemicals other than benzene	1000
Production of benzene from petroleum	630
Imports of benzene	13

a From JRB Associates, Inc. (1980)
b Coke ovens, oil spills, nonferrous metal manufacture, ore mining, wood processing, coal mining and textile manufacture

Table 10. Benzene concentrations in water samples

Location	Concentration (μg/l)	Reference[a]
Lake (UK)	6.5-8.9	Colenutt and Thorburn (1980)
Stream (UK)	18.6	Colenutt and Thorburn (1980)
River (UK)	6.8	Colenutt and Thorburn (1980)
Rainwater (UK)	87.2	Colenutt and Thorburn (1980)
Drinking-water (Czechoslovakia)	0.1	EPA (1980d)
Drinking-water (US)	0.1-0.3	EPA (1980d); NCR (1977, 1980); Coleman *et al.* (1976)
Groundwater (US)	> 100	EPA (1980d)
Subsurface brine	10 000	Ochsner *et al.* (1979)
Subsurface water[b]	24 000	Ochsner *et al.* (1979)

a EPA, US Environmental Protection Agency; NCR, National Research Council
b Taken near extensive gas and oil deposits

Protection Agency, 1980d). In another study, levels in irradiated beef were <0.1 mg/kg (Federation of American Societies for Experimental Biology, 1979). Benzene has also been detected (no levels were reported) in the following foods: haddock, cod, red beans, roasted filberts and peanuts, potato tubers, blue and Cheddar cheese, cayenne pineapple, strawberries, black currants, hothouse tomatoes, soya bean milk, cooked chicken, boiled beef and canned beef stew (Chang and Peterson, 1977; US Environmental Protection Agency, 1980d).

Conventional cooking may produce an increase in the benzene content of food (Chang and Peterson, 1977; Federation of American Societies for Experimental Biology, 1979).

The National Research Council (1980) estimated that the average US urban dweller may receive about 850 μg of benzene daily from food and air and that the dietary intake of benzene may be as high as 250 μg/day. Levels of 24 to 60 μg/m^3 benzene [8-20 ppb] have been found in the breath of individuals without specific benzene exposure, suggesting that the source may be the diet.

(*g*) *Tobacco and tobacco smoke*

Benzene has been identified in cigarette smoke at levels of 47-64 ppm [150-204 mg/m^3] (Lauwerys, 1979).

(*h*) *Pyrolysis products*

Benzene is one of the volatile components resulting from the burning of various shipboard materials; the following concentrations have been reported: wall insulation material, 60 mg/m^3 [18.8 ppm]; polyvinyl chloride cable jacket, 9 mg/m^3 [2.8 ppm]; and hydraulic fluid, 800 mg/m^3 [250 ppm] (Zinn *et al.*, 1980).

Benzene has been reported to be a thermal degradation product of polyvinyl chloride food-wrapping film when it is cut with a hot wire; concentrations measured during this operation ranged from 5 to 20 ng per cut (Boettner and Ball, 1980).

2.3 Analysis

Several methods for the analysis of benzene were described in the earlier monograph (IARC, 1974). Typical methods for the analysis of benzene in various matrices are summarized in Table 11.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(*a*) *Oral administration*

Rat: Three groups of 30 or 35 male and 30 or 35 female Sprague-Dawley rats, 13 weeks old, received 50 or 250 mg/kg bw benzene [purity unspecified] dissolved in pure olive oil by stomach tube once daily on 4 or 5 days each week during 52 weeks. Groups of 30 male and 30 female controls received olive oil only. The rats were allowed to live until spontaneous death or were killed at 144 weeks, the end of the experiment; average survival times were unspecified. Of females of the control, low- and high-dose groups,

[1] The Working Group was aware of a study in progress of oral administration of benzene to rats and mice (IARC, 1981).

Table 11. Methods for the analysis of benzene

Sample matrix	Sample preparation	Assay procedure[a]	Limits of detection	Reference
Aircraft fuels	Add to pentane; mix and centrifuge	GC/MS	not given	Warner and Kenan (1979)
Air	Trap onto adsorbent; desorb thermally	GC/MS	not given	Jonsson and Berg (1980)
	Trap onto adsorbent; desorb thermally	GC/FID	0.3 μg/m³ [0.09 ppb]	Baxter *et al.* (1980)
	Trap in charcoal tube; desorb with carbon disulphide	GC/FID	0.3 μg/m³ 0.09 ppb	Baxter *et al.* (1980)
	Inject air directly into chromatograph	GC/MS	not given	Fujii *et al.* (1979)
	Pass through silica gel treated with 5 % cerium sulphate in fuming sulphuric acid	Indicator tube (visual analysis)	5 μg/l (± 15 %)	Koljkowsky (1969)
	Collect on silica gel; compare with absorbence in iso-octane	S	< 100 μg/l	Maffett et al. (1956)
Water	Sparge sample with helium; trap on porous polymeric adsorbent; desorb thermally	GC/MS	< 5 μg/l	Pereira and Hughes (1980)
	Pass nitrogen through sample and through a carbon adsorbent; desorb with carbon disulphide	GC/FID	not given	Colenutt and Thorburn (1980)
	Sparge with helium; trap in Tenax GC tube	GC/MS	0.1 μg/l	Fentiman *et al.* (1979)
Landfill vapours	Adsorb on carbon in glass tubes; desorb with carbon disulphide	GC/FID	not given	Colenutt and Davies (1980)
Soil	Sparge with nitrogen; trap in Tenax GC tube	GC/FID	0.1 μg/kg	Fentiman *et al.* (1979)
Blood	Dissolve in pentane	GC/FID	0.8-4.8 μg/l	Ghimenti *et al.* (1978)
	Mix with sodium citrate solution	GC/FID	20 μg/l	Pinigina and Mal'tseva (1978)
Adipose tissue, brain, kidney, liver, lung, muscle, pancreas, and spleen	Treat with chloro-benzene, ethanol and water at 60°C; inject vapour phase into gas chromatograph	GC/MS	not given	Nagata *et al.* (1978)

a Abbreviations: GC/MS, gas chromatography/mass spectrometry; GC/FID, gas chromatography/flame ionization detection; S, spectrophotometry

0/30, 2/30 and 8/32, respectively, developed Zymbal gland carcinomas [Cochran-Armitage test for positive trend: p = 0.001; Fisher exact test for control *versus* high-dose group: p = 0.003]; 3/30, 4/30 and 7/32 developed mammary gland carcinomas; and 1/30, 2/30 and 1/32 developed leukaemias [type unspecified]. No such tumours were found in males, except that leukaemias occurred in 4/33 high-dose males [Cochran-Armitage test for positive trend: p = 0.008; Fisher exact test: p < 0.069]. The background incidence of Zymbal gland carcinomas in several thousand male and female rats of the same strain was said by the authors to be about 0.7%. The average latent period of the mammary gland carcinomas was 88 weeks in each of the test groups *versus* 110 weeks in the control group. In the high-dose group, two females had a skin carcinoma, one male had a hepatoma, and one male had a subcutaneous angiosarcoma; no such tumour was seen in the control or low-dose group (Maltoni and Scarnato, 1979).

(*b*) *Skin application*

In many experiments in which a variety of chemicals were applied to the skin of mice as solutions in benzene, a large number of control animals were treated with benzene alone. In none has there been any indication that benzene has induced skin tumours; however, not all possible tumour sites were examined in all of the experiments. Some of the most pertinent studies were carried out by Burdette and Strong (1941), Kirschbaum and Strong (1942), Neukomm (1962), Coombs and Croft (1966), Laerum (1973) and Fukuda *et al.* (1981).

(*c*) *Inhalation*

Mouse: Anaemia, lymphocytopenia and bone-marrow hypoplasia were found in 50 male AKR/J mice, 6 weeks of age at the start of the study, which were exposed to 300 mg/m^3 [100 ppm] benzene for 6 hours/day, 5 days/week, for life. The exposure ended at 505 days with the death of the last test animal. The incidence or induction time of the viral-induced lymphomas commonly seen in this strain of mice was not influenced by exposure to benzene, the incidences being 29/49 and 24/50 in the test and control group, respectively (Snyder *et al.*, 1980).

Two groups of 40 male C57BL/6J mice, 6 weeks old, were exposed to atmospheres containing 0 or 900 mg/m^3 [300 ppm] benzene for 6 hours/day, 5 days/week, for life. The exposure ended after 488 days with the death of the last test mouse. In addition to anaemia, lymphocytopenia, neutrophilia and bone-marrow hyperplasia, 6 of 40 mice exposed to benzene developed lymphocytic lymphoma with thymic involvement ($p < 0.01$ for lymphomas, according to Peto's log-rank method), 1 plasmacytoma and 1 haematocytoblastic leukaemia. The average survival time of the 8 tumour-bearing mice was 262 days. Two of the 40 control animals died from lymphocytic lymphoma with no thymic involvement after 282 and 608 days, respectively. The differences in incidence and induction time of tumours between the groups were statistically significant (Snyder *et al.*, 1980). [The Working Group noted that the thymus was not examined routinely.]

Male Charles River CD-1 mice [number unspecified] were exposed for 6 hours/day, 5 days/week, for life to atmospheres containing benzene at levels of 0 (control), 100 ppm [320 mg/m^3] or 300 ppm [958 mg/m^3]. Two mice in the high-exposure group developed myelogenous (myeloid) leukaemia (Snyder *et al.*, 1978a).

Rat: There was no evidence of a leukaemic response in 45 male 6-week-old Sprague-Dawley rats exposed to an atmosphere containing 900 mg/m^3 [300 ppm] benzene for 6 hours/day, 5 days/week, for life. Exposure was terminated at week 99 when the last test animal died. The controls were 27 males of the same strain and age (Snyder *et al.*, 1978b).

(*d*) *Subcutaneous and/or intramuscular administration*

Mouse: Lignac (1932) reported the occurrence of different types of leukaemia in 8/33 male and female albino mice injected subcutaneously with 0.001 ml benzene [0.88 mg/kg bw] (chemically pure, thiophene-free) in 0.1 ml olive oil weekly for 17-21 weeks (total dose, about 1 mg/kg bw). The time between first injection and death of the 8 tumour-bearing mice ranged from 4 to 11 months. [The Working Group noted that no controls were used and that the study could therefore not be evaluated.]

Of 20 mice [sex unspecified] of the high leukaemia F strain given weekly s.c. injections of 0.001 ml benzene [purity unspecified] in sesame oil [0.88 mg/kg bw], 6 (30%) developed leukaemia at 200-300 days of age. Of 212 untreated mice, 29 (14%) developed leukaemia before 300 days of age (Kirschbaum and Strong, 1942) [Fisher exact test: $p = 0.06$.]

Groups of 30 male AKR, DBA/$_2$, C3H or C57BL6 mice were given weekly s.c. injections of 0.001 ml benzene (purity unspecified) in 0.1 ml olive oil [0.88 mg/kg bw] for life. No tumours other than those that occur normally in such animals were found in mice of the DBA/$_2$, C3H or C57BL6 strains, the maximum lifespan beng 730 days. Leukaemia was seen in both treated and control AKR mice (Amiel, 1960).

Weanling male C57BL/6N mice were given s.c. injections of corn oil (20 mice) or of a 30% solution of benzene (99% pure) in corn oil (80 mice), or were not treated (20 mice). The injections were given twice weekly for 44 weeks, and then once weekly until 54 weeks. Mice given the 30% benzene solution received 0.05, 0.1, 0.1 and 0.2 ml for the first 4 weeks, respectively, followed by 0.2 ml until the last injection (total dose of benzene, about 4.9 g/animal). Vehicle controls received 0.025, 0.05, 0.05 and 0.1 ml for the first 4 weeks, respectively, followed by 0.1 ml until the last injection. At 104 weeks after the first injection all surviving mice were killed. The numbers of tumour-bearing mice were 26/45, 10/16 and 12/20 in the benzene, vehicle control and untreated groups, respectively. The incidences of granulocytic leukaemia were 8/45, 1/16 and 2/20, respectively; and of lymphomas, 6/45, 4/16 and 3/20. Tumours also occurred in the liver, stomach and lungs, but there was no significant difference from controls in tumour types or multiplicity of tumours (Ward *et al.*, 1975).

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The oral LD_{50} of reagent-grade benzene in male Sprague-Dawley rats was reported to be 0.93 (0.71-1.23) g/kg bw (Cornish and Ryan, 1965); however, Kimura *et al.* (1971) reported oral LD_{50}s in male Sprague-Dawley rats of 3.4 g/kg bw in young adults (80-160 g) and 4.9 g/kg bw in older animals (300-470 g). An oral LD_{50} of 5.6 g/kg bw was reported

in male Wistar rats (Wolf *et al.*, 1956). Withey and Hall (1975) reported an oral LD_{50} in male Sprague-Dawley rats of 5.96 g/kg bw (95% confidence limits, 5.08-7.00). The i.p. LD_{50} of benzene in mice was 0.34 ml/kg bw (0.299 g/kg bw) (95% confidence limits, 0.28-0.42 ml/kg bw [0.25-0.37 g/kg bw]) (Kocsis *et al.*, 1968); the i.p. LD_{50} in female Sprague-Dawley rats was 2.94 g/kg bw (95% confidence limits, 2.45-3.53) (Drew and Fouts, 1974).

In acute inhalation experiments, 3/8 male Long Evans rats died within 24 hours after exposure to 130 000 mg/m^3 [40 000 ppm] benzene for five 20-35-min periods. Death also occurred in 2/10 rats exposed to 33 000 mg/m^3 [10 000 ppm] benzene for 12.5-30 min daily for 1 or 12 days (Furnas and Hine, 1958). The LC_{50} in female Sprague-Dawley rats was 13 700 ppm (95% confidence limits, 13 050-14 380) [43 770; 41 690-45 940 mg/m^3] following a single four-hour exposure (Drew and Fouts, 1974).

Decreases in circulating blood cells of animals treated with benzene have been reported frequently (Snyder and Kocsis, 1975): Decreased leucocyte levels have been seen in rabbits (Santesson, 1897; Weiskotten *et al.*, 1915; Selling, 1916; Kissling and Speck, 1972), rats (Latta and Davies, 1941; Nomiyama, 1962; Gerarde and Ahlstrom, 1966) and mice (Nomiyama and Minai, 1969) given benzene subcutaneously. Uptake of radioactive iron into red cells, as a measure of erythrocyte production, was also decreased following s.c. administration of benzene (Lee *et al.*, 1974).

The severity of myelotoxicity is related to the dose, duration of treatment and test species. Doses of 0.4-2.2 g/kg bw per day are effective from within a few days (higher doses) to weeks (repeated lower doses). Rats are the most resistant species; rabbits and mice are relatively more sensitive (Lee *et al.*, 1974; Andrews *et al.*, 1977; Snyder *et al.*, 1978b; Andrews *et al.*, 1979; Sammett *et al.*, 1979; Longacre *et al.*, 1980, 1981a). Longacre *et al.* (1980, 1981a,b) have shown that DBA/2 and CD-1 are more sensitive than C57BL/6 mice.

Selling (1916) produced marrow aplasia in rabbits by giving benzene subcutaneously, in studies that were instrumental in initiating the concept of chemically induced aplastic anaemia. Later, Kissling and Speck (1972) succeeded in reproducing these results.

Weiskotten *et al.* (1920) first demonstrated that inhalation of 240 ppm [767 mg/m^3] benzene for 10 hours/day for 2 weeks could induce leucopenia in rabbits. Slight leucopenia was reported in rats, guinea-pigs and rabbits exposed to 280 mg/m^3 [88 ppm] for 7 hours/day for up to 269 days. Leucopenia was also seen in rats given 132 daily oral doses of 10 mg/kg bw during 187 days (Wolf *et al.*, 1956). No effect on the blood picture was seen in rats, guinea-pigs and dogs exposed continuously to 56 mg/m^3 [17.6 ppm] for up to 127 days (Jenkins *et al.*, 1970). Slight leucopenia has been reported in rats exposed to 140 mg/m^3 [44 ppm] benzene for 5 hours/day on 4 days/week for 8 weeks (Deichmann *et al.*, 1963). Leucopenia has also been produced in rats exposed to 400 ppm [1278 mg/m^3] for 7 hours/day for 14 weeks (Boje *et al.*, 1970) or to 1000 ppm [3195 mg/m^3] for 2 weeks (Ikeda and Ohtsuji, 1971).

Sprague-Dawley rats and AKR mice exposed to benzene (300 ppm [958 mg/m^3]) for 6 hours/day, 5 days/week for life had lymphocytopenia, with little evidence of anaemia. AKR mice were more sensitive to benzene-induced leucopenia than were rats (Snyder *et al.*, 1978b). Lifetime exposure of C57BL/6J mice to 100 or 300 ppm [320 or 958 mg/m^3] benzene produces anaemia, lymphocytopenia and neutrophilia associated with a relative increase in the number of immature leucocytes and a decrease in mature leucocytes in circulation (Snyder *et al.*, 1980). Subcutaneously administered benzene led to a selective

depression in B-lymphocytes in rabbits, whereas T lymphocytes were more resistant (Irons and Moore, 1980).

Several groups are studying the effects of benzene on bone-marrow cell cultures. In marrow cells taken from BDF_1 mice exposed to 4680 ppm [14 950 mg/m^3] for 8 hours there was a significant depletion of colony-forming cells of the CFU-C (colony-forming units, leucocyte percursors) type one day after exposure, but recovery was noted by seven days. The effect was enhanced by multiple exposures. There was also evidence of depression of CFU-S (erythroid precursors) in the spleen colony-forming assay (Uyeki *et al.*, 1977). In CD-1 mice exposed to 1.1-4862 ppm [3.3-15 534 mg/m^3] benzene for 6 hours/day on 5 days a week, concentrations of 103 ppm [329 mg/m^3] and higher produced a significant decrease in the cellularity of the marrow and the spleen; splenic but not marrow GM-CFU-C (granulocyte macrophage-colony-forming unit-committed macrophage precursors) were also depressed. When exposure was to 9.6 ppm [31 mg/m^3] for 50 days, no change in marrow activity was seen; but splenic cellularity and CFU-S were elevated. When the dose was raised to 302 ppm [965 mg/m^3] for 26 weeks, marrow and spleen cellularity and CFU-S and marrow GM-CFU-C were decreased (Green *et al.*, 1981a,b).

In rabbits (Moeschlin and Speck, 1967; Kissling and Speck, 1972) and rats (Boje *et al.*, 1970) treated with benzene, there was decreased uptake of tritiated thymidine into bone-marrow DNA.

After repeated s.c. administration of benzene to rats, there was an increase in the number of bone-marrow cells in the G_2 and M phases of the cell cycle. No inhibition of DNA synthesis was reported, but benzene treatment resulted in an increase in cell proliferative activity, as measured by cytofluorometry and ^{3}H-thymidine uptake (Irons *et al.*, 1979).

Effects on reproduction and prenatal toxicity

Rats, guinea-pigs and rabbits exposed to 80-88 ppm [256-281 mg/m^3] for 7 hours per day for 30-40 weeks had increased testicular weight and degeneration of the seminiferous tubules, as well as other signs of toxicity (Wolf *et al.*, 1956). Alteration of oestrous cycles has been reported in rats exposed to 1.6 or 9.4 ppm [5 or 30 mg/m^3] for 4 months (Avilova and Ulanova, 1975), but there was no effect on their subsequent fertility or litter size. Gofmekler (1968) showed that continuous exposure of female rats to 210 ppm [670 mg/m^3] for 10-15 days completely prevented pregnancy; it was not stated if this was due to failure to mate or to other causes. Exposure to lower concentrations, 0.3-20 ppm (1-63.3 mg/m^3) was without effect. In C3H(JAX) mice whose ovaries were painted directly with benzene and which were later mated, a high incidence of subcutaneous haemorrhages and tail defects was observed in the offspring, which persisted through four generations (Sridharan *et al.*, 1963).

A single s.c. injection of 3 ml/kg bw benzene on one of days 11-15 of gestation to CFI mice caused cleft palate, agnathia and micrognathia in the offspring (Watanabe and Yoshida, 1970). [No controls were used, and it is very likely that these effects were produced by the stress of the injection.] Several other studies in pregnant mice exposed to benzene - 2 and 4 ml/kg bw subcutaneously (Matsumoto *et al.*, 1975), 0.3-1.0 ml/kg bw orally (Nawrot and Staples, 1979) or 500 ppm [1597 mg/m^3] by inhalation for 7 hours/day (Murray *et al.*, 1979) - all failed to show any teratogenic effect, although reduced fetal weight and occasional embryolethality were observed. Similarly, several

inhalation studies in rats have shown embryolethality and reduced fetal weight but only occasional teratogenic effects: Sprague-Dawley rats exposed to 10, 50 or 500 ppm [32, 160 or 1600 mg/m^3] for 7 hours/day had a low incidence of brain and skeletal defects but no embryolethality at 50 or 500 ppm, and no abnormality or embryolethality at the lower levels (Kuna and Kapp, 1981). No teratogenic effect was seen in pregnant rats exposed to 10 or 40 ppm [32 or 128 mg/m^3] for 6 hours/day (Murray *et al.*, 1979), to 313 ppm [1000 mg/m^3] for 24 hours/day (Hudak and Ungvary, 1978) or for 6 hours/day (Green *et al.*, 1978) or to 400 mg/m^3 [125 ppm] for 24 hours/day (Tatrai *et al.*, 1980).

No teratogenic effect has been reported in rabbits injected subcutaneously with 0.25 ml/kg of a 40% benzene solution daily during pregnancy (Desoille *et al.*, 1963) or in rabbits exposed by inhalation to 500 ppm [1600 mg/m^3] for 7 hours/day on days 6-18 of pregnancy (Murray *et al.*, 1979).

Absorption, distribution, excretion and metabolism

Lazarew *et al.* (1931) claimed that rabbits absorbed benzene through the skin. Exhalation is a major route of excretion of unchanged benzene in dogs (Schrenk *et al.*, 1941), rabbits (Parke and Williams, 1953a), mice (Andrews *et al.*, 1977) and rats (Rickert *et al.*, 1979). In rats, excretion *via* the lung follows a biphasic pattern, suggesting a two-compartment model for distribution, with an initial t1/2 of 0.7 hour (Rickert *et al.*, 1979). Simultaneous administration of benzene with toluene (Andrews *et al.*, 1977; Sato and Nakajima, 1979) or with piperonyl butoxide (Timbrell and Mitchell, 1977) increases the excretion of unchanged benzene in the breath, presumably because of interference with benzene metabolism.

Benzene metabolism was recently reviewed by Snyder *et al.* (1981)

Metabolism occurs most rapidly in the liver, where benzene is converted to benzene oxide (Jerina and Daly, 1974) by mixed-function oxidases (Gonasun *et al.*, 1973). Benzene oxide then rearranges spontaneously to form phenol, reacts enzymatically with glutathione to yield a premercapturic acid (Jerina *et al.*, 1968), or is hydrated *via* epoxide hydratase (Oesch *et al.*, 1977) to the dihydrodiol (Sato *et al.*, 1963), which is then oxidized to catechol (Ayengar *et al.*, 1959; Jerina *et al.*, 1968; Vogel *et al.*, 1980). Another major metabolite, hydroquinone [see IARC, 1977], may be formed by further reaction of phenol with the mixed-function oxidase; but that pathway has yet to be clarified. Other reported metabolites, such as trihydroxylated benzene (Parke and Williams, 1953a; Greenlee *et al.*, 1981) and muconic acid (Parke and Williams, 1953a), appear to be formed by as yet unresolved pathways.

Conjugated phenolic metabolites of benzene appear in the urine mainly as ethereal sulphates and glucuronides (Parke and Williams, 1953b; Williams, 1959). In fasted rats, formation of ethereal sulphate conjugates is decreased (Cornish and Ryan, 1965). Less than 5% of metabolites are recovered in the urine as phenylmercapturic acid (Parke and Williams, 1953a; Longacre *et al.*, 1981b).

The metabolism of benzene in liver homogenates can be stimulated by treating animals with enzyme-inducing agents. Benzene, phenobarbital, 3-methylcholanthrene, dimethyl sulphoxide, chlordiazepam, diazepam and oxazepam all induce benzene hydroxylase activity (Snyder and Remmer, 1979). Carbon monoxide, aniline, metyrapone, SKF-525A, aminopyrine, cytochrome *c* (Gonasun *et al.*, 1973), aminotriazole (Hirokawa and Nomiyana, 1962) and toluene (Andrews *et al.*, 1977) inhibit benzene metabolism *in vitro*.

Myelotoxic effects of benzene were alleviated by pretreating rats with phenobarbital (Ikeda and Ohtsuji, 1971; Gill *et al.*, 1979) or with either of two polychlorinated biphenyls: 2,4,5,2′,4′,5′-hexachlorobiphenyl or 3,4,3′,4′-tetrachlorobiphenyl (Greenlee and Irons, 1981). The available evidence supports the concept that benzene toxicity is caused by one or more metabolites of benzene (Snyder *et al.*, 1981,1982). Parmentier and Dustin (1948) demonstrated that benzene metabolites containing two or three hydroxyl groups inhibited mitosis. Toluene, which inhibits benzene metabolism (Ikeda *et al.*, 1972; Sato and Nakajima, 1979), protected animals against benzene-induced myelotoxicity (Andrews *et al.*, 1977). Benzene toxicity could be correlated with the appearance of benzene metabolites in bone marrow (Andrews *et al.*, 1977; Rickert *et al.*, 1979; Greenlee *et al.*, 1981; Longacre *et al.*, 1981a,b). Although it is clear that benzene can be metabolized in bone marrow (Andrews *et al.*, 1979; Irons *et al.*, 1980), the observation (Sammett *et al.*, 1979) that partial hepatectomy protects against benzene toxicity suggests that a metabolite formed in liver is essential for benzene toxicity.

Irons and coworkers in a series of papers (see below) have stressed the importance of polyhydroxylated derivatives of benzene and their semiquinones (Irons *et al.*, 1982). They have shown that hydroquinone inhibits rat brain microtubule polymerization (Irons and Neptun, 1980); that hydroquinone and *para*-benzoquinone are the most potent inhibitors of T- and B-lymphocyte function, as measured in mouse spleen cells in culture (Wierda *et al.*, 1981); that hydroquinone inhibits lectin-stimulated lymphocyte agglutination in rat spleen preparations *in vitro* (Pfeifer and Irons, 1981); and that *para*-benzoquinone is the metabolite most likely to be responsible for suppression of lymphocyte transformation and microtubule assembly in rat spleen cells in culture (Irons *et al.*, 1982). However, administration of these compounds to animals does not produce the typical picture of benzene toxicity, i.e., leucopenia, anaemia, thrombocytopenia and, eventually, aplastic anaemia. Engelsberg and Snyder (1982) have administered the major metabolites of benzene to mice and failed to observe decreases in red cell production, using the ^{59}Fe uptake technique of Lee *et al.* (1974,1981). Goldstein *et al.* (1982) have suggested that ring-opening products may play a role in benzene toxicity. Tunek *et al.* (1981) reported that in mice benzene treatment suppressed subsequent CFU-C formation from bone-marrow cells *in vitro*. Treating the animals with phenol, hydroquinone or benzene dihydrodiol failed to suppress CFU-C. Thus, the toxic metabolites of benzene have yet to be identified.

Lutz and Schlatter (1977) demonstrated radioactivity in a nucleic acid fraction from rat liver following administration of either ^{3}H- or ^{14}C-labelled benzene. It has been shown that benzene binds covalently to protein in liver, bone marrow, kidney, lung, spleen, blood and muscle (Snyder *et al.*, 1978c; Longacre *et al.*, 1981a,b). Less covalent binding was observed to the protein of bone marrow, blood, and spleen of C57BL/6 mice, which are more resistant to the benzene-induced effects on red cell production, than to that of sensitive DBA/2 mice (Longacre *et al.*, 1981b). Irons *et al.* (1980) demonstrated covalent binding of benzene to protein in perfused bone-marrow preparations. Tunek *et al.* (1978) have argued that a metabolite of phenol binds to liver protein more efficiently than does benzene oxide, and they have electrophoretically separated hepatic proteins to which benzene preferentially binds (Tunek *et al.*, 1979). Gill and Ahmed (1981) argue that covalent binding to mitochondria is a prominent feature of benzene metabolism. They reported further that there is relatively more radioactivity in a nucleic acid-rich fraction of a benzene metabolite isolated from mouse bone-marrow cells than in a similar fraction from liver.

Mutagenicity and other short-term tests

Benzene is not mutagenic in bacterial systems. In a detailed study with *Salmonella typhimurium* strains TA98 and TA100, benzene was tested at concentrations of 0.1 to 1.0 µl per plate, with and without microsomal fractions obtained from liver homogenates of 3-methylcholanthrene or phenobarbital-treated rats. In some experiments, 1,1,1-trichloro-2,3-epoxypropane was added to the liver homogenate mixture in the activated bacterial plate assay in an attempt to block the possible biodegradation of an epoxide metabolite. Bone-marrow homogenates from 3-methylcholanthrene-treated rats were also tested in place of liver homogenates. Finally, a host-mediated assay was performed with *Salmonella* strain TA1950, in which mice were given two s.c. injections of 0.1 ml/kg benzene. In none of these studies was there an increase in the reversion rates (Lyon, 1976).

The lack of a mutagenic effect of benzene has since been confirmed with a large range of tester strains. Benzene showed no mutagenic activity in assays with *Salmonella typhimurium* (Dean, 1978; Shahin and Fournier, 1978; Lebowitz *et al.*, 1979) or *Bacillus subtilis* (Tanooka, 1977), no resistance to 8-azaguanine as a marker in *S. typhimurium* (Kaden *et al.*, 1979), and no mutagenicity to *Saccharomyces cerevisiae* (Cotruvo *et al.*, 1977). It was also negative in the *Escherichia coli* pol A test (Rosenkranz and Leifer, 1980). A preliminary study suggests, however, that benzene oxide, a postulated intermediary metabolite of benzene, may be mutagenic in *S. typhimurium* (Kinoshita *et al.*, 1981). [The Working Group considered that the data of the last study are equivocal and must be confirmed.]

No mutagenic effect of benzene was seen in *Drosophila melanogaster* in a sex-linked genetically unstable test system involving a transposable genetic element, in which mutagenicity was measured by the frequency of somatic mutations for eye pigmentation (red sectors). Newly hatched larvae were placed in a medium containing 1.0% or 2.0% benzene (Nylander *et al.*, 1978).

Benzene was not mutagenic in the mouse lymphoma forward mutation assay in L5178Y ($TK^{+/-}$) cells (Lebowitz *et al.*, 1979). Clastogenic effects of benzene on mammalian chromosomes have been observed *in vitro*. A statistically significant increase in chromosomal aberrations, mainly chromatid-type deletions and gaps, has been reported in human lymphocytes (Koizumi *et al.*, 1974; Morimoto, 1976) and in HeLa cells (Koizumi *et al.*, 1974) exposed *in vitro* to 0.2-3.0 mM benzene. Gerner-Smidt and Friedrich (1978), who analysed only 60 cells, could detect no increase in chromosomal aberrations in human lymphocytes treated *in vitro* with similar concentrations of benzene.

In experiments parallel to those described above, Gerner-Smidt and Friedrich (1978) found no enhancement in the frequency of sister chromatid exchange in benzene-treated cultures. Diaz *et al.* (1979) reported that when benzene was present during the first 24 hours of culture, but not later, the frequency of sister chromatid exchange was enhanced; when benzene treatment was carried out in the presence of metabolic activation by rat liver microsomes, a further increase in sister chromatid exchange/cell was observed. Morimoto and Wolff (1980) found that a 72-hour incubation with up to 5 mM benzene did not increase the frequency of sister chromatid exchange or affect cell cycle kinetics, whereas its principal metabolites, catechol and hydroquinone, induced high levels of sister chromatid exchange at much lower concentrations. Phenol produced only a weak effect. The sum of these results suggests that the apparent clastogenic effects of benzene *in vitro* may result from its biologically active metabolites rather than from benzene itself.

Treatment with 0.2-1.0 mM benzene significantly enhanced the number of rings and dicentrics induced in chromosomes of human lymphocytes by administration of 100 rads (1 Gy) of γ radiation (Morimoto, 1976).

In male rats, no dominant lethality was induced by a single i.p. injection of 0.5 ml/kg bw benzene (Lyon, 1976); however, a positive effect was observed in mice given 3000 mg/kg bw (Pavlenko *et al.*, 1979). [Due to the experimental design, the Working Group found it difficult to interpret the latter result.]

An increase in polychromatic erythrocytes with micronuclei (micronucleus test) has been observed in benzene-treated animals in four separate investigations (Lyon, 1976; Diaz *et al.*, 1980; Hite *et al.*, 1980; Meyne and Legator, 1980). Hite *et al.* (1980) gave 0.0625 to 2.0 ml/kg bw per day of benzene in two daily oral doses to male and female Charles River (CD-1) mice. The mice were sacrificed at from 6 hours to 16 days after the second dose, and bone marrow was obtained. Animals sacrificed 6 hours to 5 days after treatment showed significant increases in micronuclei with doses of 0.25 ml/kg per day, and in some cases with 0.125 ml/kg per day. A similar result was reported by Lyon (1976) in rats sacrificed 6 hours after a second dose of 0.05-0.5 ml/kg bw per day of benzene administered intraperitoneally; and by Diaz *et al.* (1980) in male mice (F_1 hybrids from the cross CSW × CS No. 1) sacrificed 6 or 30 hours after s.c. administration of 0.2-2 ml/kg per day. A significant dose-effect correlation was found in both of these studies (Lyon, 1976; Diaz *et al.*, 1980). In another study (Meyne and Legator, 1980), oral administration of benzene induced a higher frequency of micronuclei in female and male Swiss (CD-1) mice than did intraperitoneal injection of the same doses.

Male mice are more sensitive than females to the induction of micronuclei by benzene administered either orally or intraperitoneally (Meyne and Legator, 1980; Siou *et al.*, 1980). Castration of males reduces their sensitivity to that of females (Siou *et al.*, 1980).

Numerous studies have demonstrated the induction of chromosomal aberrations in bone-marrow cells from mice (Meyne and Legator, 1978, 1980), rats (Dean, 1969; Philip and Krogh Jensen, 1970; Lyapkalo, 1973; Lyon, 1976; Dobrokhotov and Enikeev, 1977; Anderson and Richardson, 1979) and rabbits (Kissling and Speck, 1971) treated with single or multiple daily doses of benzene ranging from about 0.2 to 2.0 ml/kg per day and given either subcutaneously or intraperitoneally. Most of the induced aberrations were chromatid breaks or deletions; but chromosome-type aberrations also occurred (Kissling and Speck, 1971; Lyon, 1976), particularly after prolonged exposure, when toxicity, manifested by a drop in the peripheral blood leucocyte count, appeared (Kissling and Speck, 1971). The persistence of these changes has varied: A significantly elevated level of aberrations was seen up to 8 days after a single i.p. injection of 0.5 ml/kg bw in rats (Lyon, 1976), whereas aberrations were significantly increased in mice 24 hours but not 7 days after receiving a similar dose (0.5 ml/kg bw) (Meyne and Legator, 1978). In the former study (Lyon, 1976), double minutes (small supernumerary chromosomal fragments) were seen up to 70 days after benzene treatment.

The route of administration may influence some of the mutagenic effects of benzene. Experiments done in parallel to the micronucleus test described above revealed similar frequencies of chromosomal aberrations after oral and i.p. treatment with benzene (Meyne and Legator, 1980). Exposure of adult male and female DBA/2 mice to 3100 ppm [10 000 mg/m^3] benzene by inhalation for 4 hours significantly increased the frequency of sister chromatid exchange but not of chromosomal aberrations in bone-marrow cells. This treatment also inhibited marrow cellular proliferation, but only in male mice.

Treatment with sodium phenobarbital prior to benzene exposure enhanced the frequency of sister chromatid exchange in female mice, and led to a significant yield of chromatid-type aberrations in animals of both sexes. The authors suggested that different metabolites of benzene might be involved in different biologic endpoints (Tice *et al.*, 1980).

(*b*) *Humans*

Toxic effects

Single exposures to concentrations of 66 000 mg/m^3 [20 000 ppm] commercial benzene have been reported to be fatal in man within 5-10 minutes (Flury, 1928). At lower levels, loss of consciousness, irregular heart-beat, dizziness, headache and nausea are observed (Deutsche Forschungsgemeinschaft, 1974). In cases of acute poisoning, inflammation of the respiratory tract, haemorrhages of the lungs, congestion of the kidneys and cerebral oedema have been observed at autopsy; but even with levels in blood of up to 2 mg/100 ml, no changes were observed in the blood picture (Winek and Collom, 1971).

It has been known since the earliest reports, of Santesson (1897) and Selling (1916), that benzene can cause aplastic anaemia; and the recent reports of Aksoy *et al.* (1972) further support these observations. Early stages in the progression to pancytopenia have been stated to be anaemia (Hunter, 1939; Goldwater, 1941; Helmer, 1944), leucopenia in which lymphocytopenia predominated (Greenburg *et al.*, 1939; Goldwater, 1941), leucopenia in which neutropenia predominated (Hamilton-Paterson and Browning, 1944) and thrombocytopenia (Savilahti, 1956).

Of 60 individuals (58 women and 2 men) who had displayed signs of benzene toxicity and were reevaluated 16 months after use of benzene in the factory where they worked had been terminated, 46 had recovered, 12 still exhibited signs of benzene toxicity and 2 had died (Helmer, 1944). In workers exposed to about 25 ppm [80 mg/m^3] benzene over several years, increases in mean corpuscular volume and minimal decreases in mean haemoglobin and haematocrit levels were seen. These values returned to normal after cessation of exposure (Fishbeck *et al.*, 1978). However, it has not been possible to establish with certainty the degree of exposure below which no adverse haematological effects of benzene in humans would occur.

Smolik and coworkers (Lange *et al.*, 1973a; Smolik *et al.*, 1973) studied a large number of workers exposed to but not seriously intoxicated by benzene and found that serum complement levels, IgG and IgA, were decreased but that IgM levels did not drop and were in fact slightly higher. Immunotoxicological studies of benzene had not previously concerned workers, since early reports on rabbits had described increased susceptibility to tuberculosis (White and Gammon, 1914) and pneumonia (Hirschfelder and Winternitz, 1913; Winternitz and Hirschfelder, 1913), decreased production of red cell lysins, agglutinins for killed typhoid bacilli and opsonins (Simonds and Jones, 1915) and reduction or absence of antibacterial antibodies (Camp and Baumgartner, 1915; Hektoen, 1916). Recently, Wierda *et al.* (1982) have demonstrated that administration of benzene to C57BL/6 mice *in vivo* inhibits the function of B- and T-lymphocytes studied *in vitro*. These observations, taken together with the well-known ability of benzene to depress leucocytes, which themselves play a significant role in protection against infectious agents, may explain why benzene-intoxicated individuals readily succumb to infection and why the terminal event in severe benzene toxicity is often an acute, overwhelming infection.

Lange *et al.* (1973b) also found that levels of leucocyte agglutinins were elevated in selected individuals exposed to benzene. They suggested that in some people benzene toxicity may be accounted for in part by an allergic blood dyscrasia.

Increases in red-cell δ-aminolaevulinic acid were found in 16 of 27 workers exposed to benzene (Kahn and Muzyka, 1973). Similar results were observed in animals (Kahn and Muzyka, 1973). Inhibition of reticulocyte haem was observed *in vitro* (Wildman *et al.*, 1976; Greenblatt *et al.*, 1977).

Effects on reproduction and prenatal toxicity

Benzene crosses the human placenta, and levels in cord blood are similar to those in maternal blood (Dowty *et al.*, 1976). Menstrual disturbances have been reported more frequently in women exposed industrially to benzene and other solvents than in unexposed controls (Michon, 1965; Mikhailova *et al.*, 1971).

Severe anaemia caused by solvents such as benzene or by other factors, such as chloramphenicol, may lead to death of mothers at parturition (Messerschmitt, 1972).

Absorption, distribution, excretion and metabolism

The most frequent route by which humans are exposed to benzene is *via* inhalation. Toxic effects in humans have often been attributed to combined exposure by both respiration and through the skin: e.g., rotogravure workers wash ink from their hands in open vats of benzene (Hunter, 1978).

When benzene was placed on the skin under a closed cup it was absorbed at a rate of 0.4 mg/cm^2 per hour (Hanke *et al.*, 1961), a rate equal to 2% of that of ethylbenzene (Dutkiewicz and Tyras, 1967) and 2-3% of that of toluene (Dutkiewicz and Tyras, 1968).

Following exposure to benzene, it is eliminated unchanged in expired air (Srbova *et al.*, 1950; Hunter, 1968; Sherwood and Carter, 1970; Nomiyama and Nomiyama, 1974a,b; Sato and Nakajima, 1979). In men and women exposed to 52-62 ppm [166-198 mg/m^3] benzene for 4 hours, a mean of 46.9% was taken up, 30.2% was retained and the remaining 16.8% excreted as unchanged benzene in expired air. Pharmacokinetic plots of respiratory elimination were interpreted as indicating that there are three phases to the excretion, described by three rate constants, with no significant differences between men and women (Nomiyama and Nomiyama, 1974,a,b). When humans were exposed to 100 ppm [300 mg/m^3] benzene, it was detected in expired air 24 hours later, suggesting that it is possible to back-extrapolate to the benzene concentration in the inspired air (Hunter, 1968). This interpretation has since been supported (Berlin *et al.*, 1980).

Subjects who inhaled concentrations of 340 mg/m^3 [106 ppm] benzene in air for 5 hours excreted 29% as phenol, 3% as catechol and 1% as hydroquinone in the urine, mostly as ethereal sulphates. Most of the phenol and catechol was excreted within 24 hours, and the hydroquinone within 48 hours (Teisinger *et al.*, 1952). A correlation has been shown in a small number of workers between the concentration of benzene in air during 8-hour exposures and the excretion of phenol in urine (Rainsford and Lloyd Davies, 1965).

The percentage of inorganic sulphate in urine can also be used as an index of benzene exposure (Hammond and Hermann, 1960).

Mutagenicity and chromosomal effects

Numerous studies have been carried out on the chromosomes of bone-marrow cells and peripheral lymphocytes from people known to have been exposed to benzene (Dean, 1978). The populations included in these studies fall into two general categories: (1) patients with either a current or a past history of benzene-induced blood dyscrasias ('benzene haemopathies'), often associated with extensive exposure to benzene; and (2) workers with known current or past exposure to benzene but with no obvious clinical effects. In many of these studies, significant increases in chromosomal aberrations have been seen, which in some cases have persisted for years after cessation of exposure.

Pollini *et al.* (Pollini and Colombi, 1964a,b; Pollini *et al.*, 1964, 1969; Pollini and Biscaldi, 1976, 1977) examined bone-marrow cells and peripheral lymphocytes from workers with current severe blood dyscrasias, and followed several workers by repeated cytogenetic studies up to 12 years after recovery from benzene-induced pancytopenia. Gross chromosomal abnormalities were characteristic of these cells; 70% of the bone-marrow cells and lymphocytes in patients with acute poisoning showed karyotypic abnormalities (Pollini and Colombi, 1964a,b). The authors could not relate the frequency or type of chromosomal alterations to the severity of the blood dyscrasia (Pollini *et al.*, 1964). Five years after poisoning, all of five patients studied still showed stable (C_s) and unstable (C_u) chromosomal aberrations in their lymphocytes, although only 40% of the cells were now abnormal (Pollini *et al.*, 1969). By 12 years (Pollini and Biscaldi, 1977), no cytogenetic abnormalities remained in the four patients studied.

Forni and collaborators (Forni *et al.*, 1971a,b) examined two groups of workers with chronic benzene poisoning; one group included 25 subjects who had recovered from benzene haemopathy one to 18 years previously (plus four others showing acute toxicity at the time of first chromosome examination); and the other group comprised 34 workers in a rotogravure plant who had been exposed in 1952-1953 to concentrations of 125-532 ppm [400-1700 mg/m^3] benzene in air, which had led to toxic effects. Lymphocytes from both groups showed significantly higher levels of C_u and C_s than those from age-matched controls. Although in the first group C_u and C_s were present several years after cessation of benzene exposure, follow-up cytogenetic studies indicated a tendency toward a decrease in C_u and a persistence or increase in C_s (Forni *et al.*, 1971a).

The finding of significant increases in chromosomal aberrations in blood and bone marrow (Forni and Moreo, 1967, 1969) and in lymphocytes from clinically symptomatic subjects exposed to benzene has been confirmed in several other investigations (Hartwich *et al.*, 1969; Sellyei and Kelemen, 1971; Erdogan and Aksoy, 1973; Hudak and Gombosi, 1977; Van den Berghe *et al.*, 1979). Forni and Moreo (1967, 1969) hypothesized that such aberrations are involved in the eventual development of leukaemia in benzene-exposed individuals.

Tough and others (Tough and Court Brown, 1965; Tough *et al.*, 1970) studied workers in three different factories who had been exposed to benzene in the atmosphere for approximately one to 25 years. The workers were apparently asymptomatic as far as evidence of acute benzene toxicity was concerned. The first two groups consisted of a total of 38 workers who had been exposed to 25-150 ppm [80-480 mg/m^3] benzene until two to four years prior to sampling; the incidence of cells with unstable chromosomal aberrations (C_u) in these groups was higher than was expected from the general population. Workers in the third factory had been exposed intermittently to approximately 12 ppm [38 mg/m^3] benzene for 2-26 years; their lymphocytes showed no increase in

chromosomal abnormalities. The authors hesitated, however, to relate these effects to benzene exposure alone, since there was evidence that age and other environmental factors may also have been contributory.

Funes-Cravioto *et al.* (1977) studied lymphocytes from 73 workers in several chemical laboratories and in the printing industry, and found a significantly increased frequency of chromosomal breaks, as compared with cells from 49 control subjects. In 12 subjects studied, the frequency of sister chromatid exchange was also enhanced. Exposure to organic solvents, including benzene, was common to all work environments studied; and, in 29 workers specifically, exposure to benzene had been heavy during the 1960s. However, no particular solvent could be singled out as the direct clastogenic agent. Picciano (1979) examined lymphocytes from 52 workers who had been exposed to less than 10 ppm [<32 mg/m^3] benzene for periods of one month to 26 years. A statistically significant increase in the rate of chromosomal aberrations was found, as compared with 44 people in a control group. Although these workers were also exposed to other aromatic hydrocarbon solvents, the degree of contact with those solvents was less than with benzene.

Three other studies also report increased levels of chromosomal aberrations in asymptomatic workers who had been exposed to benzene (Hartwich and Schwanitz, 1972; Khan and Khan, 1973; Fredga *et al.*, 1979). In one of these studies (Hartwich and Schwanitz, 1972), the mean aberration rate of cells from nine refinery workers exposed to 'relatively low' levels of benzene was significantly elevated when compared with that in controls, but the rate in individual workers was at the upper limit of normal. In a study of lymphocytes from 65 people (Fredga *et al.*, 1979), a statistically significant increase in chromosomal aberrations was found in both petrol- and milk-tanker drivers and in 12 industrial gasworks workers exposed to benzene, but not in petrol tanker crews or petrol station staff. As no other etiological factor could be identified, the increase in the 12 industrial workers studied was regarded as being due to benzene exposure (5-10 ppm; 16-32 mg/m^3). However, Watanabe *et al.* (1980) found no evidence of an increased frequency of chromosomal aberrations or of sister chromatid exchange among nine female workers engaged in painting ceramics who had been exposed to <1-9 ppm [<3-29 mg/m^3] for 1-20 years, or in seven female workers who had been exposed to 3-50 ppm [10-160 mg/m^3] benzene for 2-12 years.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

(a) Case reports

An association between long-term exposure to benzene and the occurrence of leukaemia was suggested as early as 1928 by Delore and Borgomano (1928), who described acute lymphoblastic leukaemia in a worker who had been exposed to benzene for five years.

The industrial exposure to commercial benzene (benzol) of 89 workers involved in the manufacture either of artificial leather or of shoes (which involves the use of rubber cements containing benzene) and their short- and long-term health records were investigated in a series of studies (Bowditch and Elkins, 1939; Hunter, 1939; Mallory *et al.*, 1939). One of the workers, a 28-year old male who had been exposed to commercial

benzene for 10 years, died from acute myeloblastic leukaemia (Hunter, 1939). Mallory *et al.* (1939) reported on 19 cases of prolonged exposure to commercial benzene. Two cases of leukaemia were described: that reported by Hunter (1939) and a lymphoblastic leukaemia in a 12-year old boy who had 'frequently' used a paint remover known to contain commercial benzene.

DeGowin (1963) reported that an indoor house painter who had thinned his paints with benzene for 13 years developed aplastic anaemia; although he was not subsequently exposed to benzene, he developed acute myeloid leukaemia 15 years later.

Tareeff *et al.* (1963) described 16 cases of leukaemia (6 acute and 10 chronic) in workers in the USSR occupationally exposed to benzene for 4-27 years (average, 15 years). In 3/6 acute cases, a latent period of 2-5 years between the cessation of exposure and the development of leukaemia was noted.

Vigliani and Saita (1964) reported 6 cases of leukaemia seen at the Clinica de Lavoro, Milan, between 1942 and 1963. The patients included a spreader and calender operator at a leathercloth factory (9 years' exposure), an assistant operator in a rotogravure firm (5 years' exposure), a spray varnisher (8 years' exposure), a rotogravure operator (11 years' exposure) and two workers using glues containing benzene (19 and 3 years' exposure). At the Institute of Occupational Health in Pavia, 5 cases of leukaemia were seen between 1961 and 1963 in shoemakers exposed to glues containing benzene.

During the period 1950-1965, 50 cases of leukaemia were observed in workers with confirmed exposure to benzene in industries in the Paris region (Goguel *et al.*, 1967). Clinical data were given for 44 previously unpublished cases (37 men and 7 women), which comprised 13 cases of chronic myeloid leukaemia, 8 cases of chronic lymphoid leukaemia and 23 cases of acute leukaemia, of which 2 were erythroleukaemias. Measurements were made of benzene in the blood for 19 of the cases; in 7 of these, the level was high.

Four cases of acute leukaemia were reported in shoemakers in Istanbul exposed to benzene for 6-14 years. Three of the cases were of the myeloblastic type and the fourth a monocytic type (Aksoy *et al.*, 1972). The concentration of benzene in the working environment was reported to range between 15-30 ppm [48-96 mg/m^3] outside working hours and rose to a maximum of 210 ppm [670 mg/m^3] when adhesives containing benzene were being used (Aksoy *et al.*, 1971).

Ludwig and Werthemann (1962) described one case of myeloid leukaemia and one of a tumour-like reticulosis among 44 workers in two chemical factories exposed to benzene and toluene between 1940 and 1961.

Cases of erythromyelosis have also been described in workers with short- and long-term exposure to benzene (Galavotti and Troisi, 1950; Nissen and Soeborg Ohlsen, 1953; Di Guglielmo and Iannaccone, 1958; Rozman *et al.*, 1968; Bryon *et al.*, 1969; Forni and Moreo, 1969).

In a study carried out during 1966-1969 in two Lyon hospitals, 17/140 (12%) patients with acute leukaemia, 9/61 (15%) with chronic lymphoid leukaemia and 4/56 (7%) with myeloid leukaemia were found to have been exposed to benzene or toluene. Five cases of exposure to benzene were found among 124 (4%) control patients without blood disorders (Girard and Revol, 1970).

A number of additional case reports have been the subject of a review (Goldstein, 1977). Most cases of malignancy in which an association with exposure to benzene was reported have been leukaemias, particularly of the acute myelogenous type. There have, however, been a number of discrepant reports: in some, all of the cases were nonmyelogenous leukaemia; in others, cases of acute and chronic lymphatic leukaemia and of monocytic leukaemia were seen. There have been scattered reports of an excess of lymphomas both of the Hodgkin's and non-Hodgkin's variety. Aksoy (1980) summarized 63 cases - 42 leukaemias, 9 malignant lymphomas, 5 lung cancers, 2 myeloid metaplasias, 2 paroxysmal nocturnal haemoglobinurias and 3 multiple myelomas - all of which involved chronic exposure to benzene in Turkey. The author suggested that '... the frequent finding of this association suggests that benzene may not only cause leukaemia but also be involved in other types of malignancy'. [It is impossible to ascribe risk estimates to reports of this type, since most do not describe appropriate control groups, i.e., they are based on reports of case series. Most of the reports do not include information on environmental measurements; i.e., putative exposure to benzene is based on historical information.]

(*b*) *Epidemiological studies*

A case-control study of leukaemia was reported from Japan (Ishimaru *et al.*, 1971). All cases diagnosed as definite or probable leukaemia, resident in Hiroshima or Nagasaki City at the time of the onset of the disease between 1945 and 1967 were included. One control per case was chosen from the Atomic Bomb Casualty Commission Leukemia Registry sampling frame and matched on five characteristics: city, sex, date of birth $\pm$ 30 months, distance from the atomic bomb explosion, and alive and resident in either Hiroshima or Nagasaki at the time of disease onset in the patient. There were 492 leukaemia cases identified; information could be obtained for only 413 matched case-control pairs. In analysing 303 adult pairs with respect to occupations in which there was potential exposure to benzene, the authors arrived at a risk ratio of 2.5 ($p < 0.01$), on the basis of 42 exposure-discordant pairs. Of these, 41 pairs could be used to investigate the degree to which the benzene-associated relative risk was modified by exposure to the atomic bomb radiation. The relative risk was highest in those exposed to 1-99 rads [0.01-0.99 Gy] (5 exposure-discordant pairs). [The Working Group noted a discrepancy between the figures in table 2 and those in table 4 of that paper; they also noted the small numbers involved and the considerable uncertainty involved in using occupation as an index of exposure to benzene. The increased risk may also have been influenced by exposures to substances other than benzene, since in none of the occupations considered would benzene have been the only chemical encountered.]

Aksoy *et al.* (1974; Aksoy, 1977) estimated the incidence of acute leukaemia or 'preleukaemia' among 28 500 shoe-workers in Turkey on the basis of case ascertainment by contact with medical care. Thirty-four cases were identified. They estimated that the incidence of acute leukaemia was significantly greater among workers chronically exposed to benzene, which was used as a solvent by these workers, than in the general population. Occupational exposures were determined by work histories and by environmental measurements. There was said to be exposure only to benzene in small, poorly ventilated work areas; peak exposures to benzene were reported to be 210-650 ppm [670-2075 mg/m^3]. Duration of exposure was estimated to have been 1-15 years (mean, 9.7 years). The annual incidence was estimated to be 13/100 000, giving an approximate relative risk of 2 when compared with the annual estimate for the general population, 6/100 000. [These estimates are limited by the study design characteristics and by uncertainty about the way in which cases were ascertained and how many of the study population were exposed and how many unexposed.]

The distribution of cell types among the leukaemia cases with a history of exposure to benzene was compared with that of patients with no such history (Table 12) (Aksoy, 1977).

Table 12. Comparison of types of leukaemia found in 40 individuals with chronic benzene poisoning and in 50 nonexposed patients

Type of leukaemia	Exposed		Nonexposed	
	Number	%	Number	%
Acute myeloblastic leukaemia	15	37.5	8	16.0
Acute lymphoblastic leukaemia	4	10.0	13	20.0
Preleukaemia[a]	7	17.5	1	2.0
Acute erythroleukaemia	7	17.5	2	4.0
Acute monocytic or myelonomonocytic leukaemia	3	7.5	3	6.0
Acute unidentified leukaemia	1	2.5	0	0.0
Acute promyelocytic leukaemia	1	2.5	0	0.0
Chronic myeloid leukaemia	2	5.0	10	20.0
Chronic lymphoid leukaemia	0	0.0	13	25.0

The ratio of acute non-lymphocytic leukaemia to chronic leukaemia was 27:2 in the exposed group and 13:23 in the non-exposed, suggesting a relative risk of about 24 for acute nonlymphocytic leukaemia - an order of magnitude higher than the value of 2, which was based on the hypothetical incidence rates. Indirect support for the higher figure can be derived from a follow-up of patients with ankylosing spondylitis (Court Brown and Doll, 1957, 1965), in whom the distribution of leukaemia cell types is similar to that reported by Aksoy, which gave a relative risk of about 12 of dying 3 to 9 years after first coming under observation.

[Aksoy (1978) also followed 44 benzene-exposed patients with pancytopenia. Six cases of leukaemia developed within 6 years of follow-up, giving a proportion of 14%.]

Vigliani (1976) summarized the experience at the Institute of Occupational Health of Milan from 1942 to 1975 with 66 cases of benzene haemopathy, 11 of which were leukaemia, and the experience at the Institute of Occupational Health of Pavia from 1959 to 1974, in which 13 cases of leukaemia were ascertained among 135 instances of benzene haemopathy. [The cumulative incidence of leukaemia among individuals with clinically ascertained benzene haemopathy was about 17% in Milan and about 10% in Pavia.] Occupational exposures were identified in rotogravure plants and shoe factories. Benzene concentrations near rotogravure machines were 200-400 ppm [640-1280 mg/m^3], with peaks up to 1500 ppm [4800 mg/m^3]; benzene concentrations in air near workers handling glue in shoe factories were 25-600 ppm [80-1920 mg/m^3], but were 'mostly around 200-500 ppm' [640-1600 mg/m^3]. Estimated latency (years from start of exposure to clinical diagnosis of leukaemia) ranged from 3-24 years (median, 9 years). Vigliani estimated that the relative risk of acute leukaemia was at least 20:1 for workers heavily exposed to benzene in the rotogravure and shoe industries in the provinces studied, when compared with the general population. [The relative risk is based on a non-validated estimate.]

Fishbeck *et al.* (1978) reported on a small series of 10 chemical workers exposed to benzene (at the same company reported on by Ott *et al.*, see below). Data on exposure to benzene and on periodic health surveillance were available for the period 1953-1963. Benzene levels exceeded a time-weighted average of 25 ppm [80 mg/m^3]; in the job with the highest exposure ('operator'), the eight-hour time-weighted average exposure was 30-35 ppm [96-112 mg/m^3], with peaks of 937 ppm [2994 mg/m^3]. The total period of exposure ranged from 3 years 7 months to 29 years 9 months, and averaged 14 years 6 months. All 10 workers had first been exposed more than 16 years before the study. In 1963, all of these workers showed laboratory evidence of changes in peripheral blood, with slight reduction in haemoglobin and increased mean corpuscular volume. The investigators reported that '... monitoring of their health status has shown no persisting, significant adverse health effects'. [These data provide limited information. Given the presence of benzene-induced pancytopenia, the subsequent risk of leukaemia may be in the range of 10-15% in 10 years. The limited power of this study is such that subsequent cumulative risks of up to 25% could not be excluded.]

Thorpe (1974) reported on mortality from leukaemia among workers in eight European affiliates of a large US oil company. Eighteen cases (14 deaths and 4 incident cases assumed to be dead) were reported for the period 1962 to 1971. Estimates of the number of deaths expected in a combined population of 38 000 workers were based on rates for the general populations in the countries represented by the affiliates. The overall SMR (O/E) of 77 was calculated on the basis of a total of 383 276 person-years. No association was found between an index of potential exposure to benzene (based on estimates by the affiliates) and leukaemia mortality rate. Although no statistically significant excess was observed within any geographic affiliate (the highest SMR was 108 in one affiliate), SMRs of 121 and 60 were observed in all exposed and in all nonexposed workers, respectively. The 95% confidence interval was 37-205 for the exposed. [The study suffers from problems of ascertainment, specificity and validity of diagnoses and the 'healthy worker effect' in the calculation of SMRs. Further, it was impossible to identify the leukaemia type in the majority of instances (12 of the 18 cases). The population at risk was constructed from personnel files, the completeness of which, particularly for work processes and their potential benzene exposure, was not validated. Cases were ascertained from periodic health examinations, absenteeism records and insurance claims by methods idiosyncratic to each affiliate, not designed for research purposes, and of indeterminate completeness and accuracy. Limited data were reported on potential exposure to benzene, and no information was given on the induction-latent period. The power of this study is limited, since only eight of the cases were observed among exposed workers.]

(c) Epidemiological studies of mixed exposures

A number of epidemiological studies of workers potentially exposed to benzene among other agents have shown statistically significant excesses of leukaemia. As the actual exposure to benzene in these studies is often not documented, the relevance of the studies to the issue of benzene carcinogenicity remains undecided. The Working Group chose to include them not only because of their possible relevance in respect to benzene but also because they draw attention to the more general problem of the relationship between exposure to 'solvents' and cancer.

McMichael *et al.* (1975), Monson and Nakano (1976) and Tyroler *et al.* (1976) have summarized studies on the US rubber industry which disclosed excesses of mortality from chronic lymphatic leukaemia, myelogenous leukaemia and lymphosarcoma [see IARC,

1982b]. [There was historical evidence that exposure was to a mixture of agents, including benzene.]

Brandt *et al.* (1978) performed a case-control study of 50 male Swedish patients aged 20-65 seen at the University Hospital, Lund, in 1969-1977 with acute nonlymphocytic leukaemia and reported an excess history of exposure to petroleum products among cases. [No further details were provided regarding potential exposure to benzene or other environmental factors.]

Another Swedish case-control study (Flodin *et al.*, 1981), based on 42 cases of acute myeloid leukaemia from the University Hospital of Linkoping and 244 other deaths from the same area as controls, indicated an approximately six-fold, statistically significantly increased risk from exposure to solvents of various types, including petroleum products. [This study seems to confirm the results obtained in the study by Brandt *et al.*] Estimated background radiation from building materials (stone, concrete, wood, etc) in homes and workplace buildings significantly increased the risk of acute myeloid leukaemia; in particular, there seemed to be a strong effect of the combination of solvent exposure and high background radiation.

In a case-control study at the University Hospital of Umea, comprising 169 cases of malignant lymphoma (60 of which were of the Hodgkin's type) and 338 controls, frequencies of exposure to a number of agents were investigated, including phenoxy acids and organic solvents (Hardell *et al.*, 1981). A risk ratio of 4.6 (95% confidence limits, 1.9-11.4) was reported with regard to combined, 'high-grade' exposure to styrene, trichloroethylene, perchlorethylene and benzene; only one of the cases was explicitly reported to have been exposed to benzene. High-grade exposure to unspecified solvents resulted in a risk ratio of 2.8 (95% confidence limits, 1.6-4.8). [The Working Group noted that the latter exposure might also have involved exposure to benzene in combination with other agents; and it is unclear whether such exposure was associated with development of lymphoma.]

Greene *et al.* (1979) reported that a proportionate cancer mortality study among employees of the US Government Printing Office showed a 'significantly higher proportion of deaths from multiple myeloma, leukemia, Hodgkin's disease....' The excess deaths 'from leukemia occurred primarily in bindery workers who may have had exposure to benzene'. The authors noted the limitations in the methods used, including small numbers, use of the proportionate mortality ratio technique and limitations of ascertainment and diagnosis, but indicated the consistency of their study with the findings of others.

Rushton and Alderson (1981) reported the results of a case-control study nested in a cohort study. All death certificates with mention of leukaemia in men employed over a period of 25 years at eight petroleum refineries in the UK were studied. Potential occupational exposure to benzene among these cases was contrasted with that of two sets of controls selected from the total refinery population - one control was matched for refinery and year of birth, the other for refinery, year of birth and length of service. Job history was used to classify potential benzene exposure into low, medium and high categories. Although there was no overall excess of deaths from leukaemia in the refinery workers, compared with expectations based on national rates (see Rushton and Alderson, 1980), and there was no excess of 'cytological types of leukaemia which have been shown to be particularly associated with benzene exposure', the risk for men with medium or high exposure relative to the risk for those with low benzene exposure did approach statistical significance when length of service was taken into account.

Ott *et al.* (1978) reported the mortality experience of 594 individuals occupationally exposed to benzene in chemical manufacture, using a retrospective cohort analysis of the period 1940-1973. Three deaths due to leukaemia (one myelogenous and one myeloblastic) and one due to aplastic anaemia were noted; 0.8 death from leukaemia, excluding lymphocytic or monocytic cell types, was expected on the basis of incidence data from the Third National Cancer Survey [SMR = 375] ($p < 0.05$). Data derived from work histories and industrial hygiene records were used to estimate cumulative exposure doses. [The authors could find no association between the cases and work areas and estimated exposure levels; however, the number of workers in any particular work area was limited, and the power of the study to detect any association between exposure levels and cases was correspondingly low.]

Infante *et al.* (1977a) made a retrospective cohort analysis of 748 workers occupationally exposed to benzene between 1940 and 1949 in two factories engaged in the manufacture of rubber hydrochloride. They achieved 75% vital status ascertainment of the cohort up to 1975. Rinsky *et al.* (1981) continued to follow up the same cohort, also to 1975, and ascertained vital status for 98%. Cases were ascertained from diagnoses as given on death certificates, coded according to the ICD in effect at time of death and converted to those of the 7th revision. Person-years of observation and deaths from 1 January 1950 to 30 June 1975 were used. Expected rates were derived from US white male mortality statistics. The SMR for all causes of death in the more recent analysis (Rinsky *et al.*, 1981) was 111 (180 observed; 161.3 expected). A statistically significant excess mortality from all leukaemias was seen (O/E - 7.0/1.25; SMR = 560). All of the leukaemias were myelogenous or monocytic, constituting a 10-fold excess over expected of deaths from myeloid and monocytic leukaemias combined (O/E = 7/0.7). (Estimates of cell-type distribution were derived from the Connecticut Tumor Registry.) (Infante *et al.*, 1977a). Four additional cases of leukaemia, three of them myelogenous, occurred in workers employed in the two plants, but were not included in the statistical analysis because death occurred after 1975, because the workers were salaried rather than hourly and thus fell outside the cohort definition, or because of inaccuracies in death certificate coding. The median duration of employment for the entire cohort in the two plants was less than one year.

Exposures to airborne benzene vapour at the two plants were evaluated by Infante *et al.* (1977a). They concluded on the basis of monitoring data that worker exposures were generally within the recommended limits in effect at the time of their employment. [The methods employed in the 1940s for measuring benzene concentrations in air, while reasonably accurate, were relatively less sensitive than those available today.] Recommended levels were as follows:

1941 100 ppm [320 mg/m^3] maximum allowable concentration
1947 50 ppm [160 mg/m^3] 8-hour time-weighted average
1948 35 ppm [112 mg/m^3] 8-hour time-weighted average

Tabershaw and Lamm (1977) argued that the exposures were not as low as Infante *et al.* (1977a) had stated. At plant B, workers manufactured rubber hydrochloride only; at plant A they also manufactured tyres, hose, foams, rubber chemicals and metal products. Tabershaw and Lamm argued that environmental exposure was therefore probably mixed and potentially higher at plant A than at plant B.

Infante *et al.* (1977b) and Rinsky *et al.* (1981), in reply to the critique by Tabershaw and Lamm, evaluated past exposures in both plants in further detail, and reported that,

although other solvents were used in various areas of both plants, benzene was found to be the only solvent used in the manufacture of rubber hydrochloride, except for chloroform, which was used between 1936 and 1949 in one plant. Rinsky *et al.* agreed with Tabershaw and Lamm that occasional high excursions occurred in airborne benzene levels (up to several hundred ppm). They found, however, that most such excursions occurred in areas entered only infrequently by workers, and they estimated that workers' actual eight-hour time-weighted average breathing-zone exposure fell generally within accepted limits. To evaluate possible differences between the two plants, Rinsky *et al.* specifically analysed leukaemia mortality in each. They found excess mortality in both plants: in one, 2 cases were observed *versus* 0.58 expected (SMR = 345); and in the other, 5 cases were observed *versus* 0.67 expected (SMR = 746). [The Working Group accepted the central conclusion of Infante *et al.* and of Rinsky *et al.* that excessive mortality from myelogenous and monocytic leukaemia had occurred among workers with occupational exposure to benzene that was generally within accepted limits. However, the possible contribution of the occasional excursions in exposure and of the employment of some workers in other areas of the plant must be noted; and in the opinion of the Working Group those factors may have made some contribution to the observed excess in mortality from leukaemia.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzene has been tested in rats by intragastric administration and inhalation exposure, and in mice by skin application, inhalation exposure and subcutaneous injection. Oral administration to rats resulted in an increase in the incidence of Zymbal-gland carcinomas. Anaemia, lymphocytopenia and bone-marrow hyperplasia and an increased incidence of lymphoid tumours occurred in male mice exposed by inhalation to benzene; in similar inhalation studies with another strain of mice and with rats there was no evidence of a leukaemic response. Experiments involving skin application or subcutaneous injection of benzene did not produce evidence of carcinogenicity, but most of these experiments were inadequate.

Benzene does not induce specific gene mutations in bacterial systems or in *Drosophila melanogaster*. A single report showed no evidence for the induction of point mutation in mammalian cells; however, benzene induced cytogenetic abnormalities (chromosomal aberrations and sister chromatid exchanges) in mammalian cells *in vitro*.

The micronucleus test in mice and rats has been consistently positive. Numerous studies have shown that benzene exposure of experimental animals *in vivo* leads to the induction of chromosomal aberrations in the bone-marrow cells.

Exposure to benzene may damage the testis. Evidence from most studies in mice, rats, guinea-pigs and rabbits suggests that benzene is not teratogenic at doses that are fetotoxic and embryolethal.

4.2 Human data

Workers and the general public are exposed to benzene as a result of a variety of activities in which it is processed, generated or used. Major contributors to benzene

emissions into air include: (1) gasoline production, storage, transport, vending and combustion; (2) production of other chemicals from benzene; and (3) indirect production of benzene (e.g., in coke ovens). The last is the major source of benzene emissions into water.

Chronic human exposure to benzene results in leucopenia, thrombocytopenia, anaemia or combinations of these. At early stages of such blood dyscrasias, these effects appear to be reversible. Exposure to high doses for longer periods of time may lead to pancytopenia, which results from aplasia of the bone marrow and is considered to be an irreversible stage of the disease.

Benzene crosses the human placenta. There is a clear correlation between exposure to benzene and the appearance of chromosomal aberrations in the bone marrow and peripheral lymphocytes of individuals exposed to high levels of benzene (>100 ppm). Such levels of exposure usually lead to clinical symptoms of benzene-induced blood dyscrasias. These aberrations may persist for many years after exposure and after manifestations of haematotoxicity. The results are not so clear with lower levels (<100 ppm). Although aberrations have been reported following chronic exposures to as little as 10 ppm, this has not been a consistent finding. Environmental factors and exposure to other agents may have interacted with benzene in these studies of low exposure.

Many case reports and case series have described the association of leukaemia with exposure to benzene, either alone or in combination with other chemicals. Most cases were acute myelogenous leukaemia, although some were monocytic, erythroblastic or lymphocytic; and some lymphomas have been noted.

Two follow-up studies showed high incidences of leukaemia among individuals ascertained as cases of benzene haemopathy.

A series of epidemiological studies, both cohort and case-control, showed statistically significant associations between leukaemia (predominantly myelogenous) and occupational exposure to benzene and benzene-containing solvents. These results were replicated in a number of countries and different industries. In the epidemiological studies of people exposed primarily to benzene, statistically significant excesses of leukaemia were observed. [See also Annex. Some Aspects of Quantitative Cancer Risk Estimation.]

4.3 Evaluation[1]

There is *limited evidence* that benzene is carcinogenic in experimental animals.

It is established that human exposure to commercial benzene or benzene-containing mixtures can cause damage to the haematopoietic system, including pancytopenia. The relationship between benzene exposure and the development of acute myelogenous leukaemia has been established in epidemiological studies.

Reports linking exposure to benzene with other malignancies were considered to be inadequate for evaluation.

There is *sufficient evidence* that benzene is carcinogenic to man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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BENZIDINE AND ITS SULPHATE, HYDROCHLORIDE AND DIHYDROCHLORIDE

These substances were considered by a previous working group, in December 1971 (IARC, 1972). Since that time new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

Benzidine

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 92-87-5

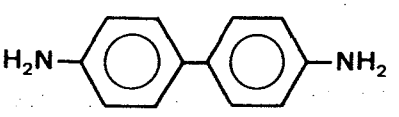
Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine

IUPAC Systematic Name: Benzidine

Synonyms: Benzidine; benzidine base; 4,4′-bianiline; *p,p′*-bianiline; 4,4′-biphenyldiamine; 4,4′-biphenylenediamine; C.I. 37225; C.I. Azoic Diazo Component 112; 4,4′-diaminobiphenyl; 4,4′-diamino-1,1′-biphenyl; *p,p′*-diaminobiphenyl; 4,4′-diaminodiphenyl; *p*-diaminodiphenyl; 4,4′-diphenylenediamine

Trade Name: Fast Corinth Base B

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2$ Mol. wt: 184.2

1.3 Chemical and physical properties of the pure substance

From Verschueren (1977), unless otherwise specified

(*a*) *Description*: Greyish-yellow, white or reddish-grey crystalline powder (Hawley, 1981)

(*b*) *Boiling-point*: 402°C

(*c*) *Melting-point*: 116/129°C (isotropic forms)

(*d*) *Density*: d_4^{20} 1.250

(*e*) *Spectroscopy data*: λ_{max} 287 mm (in ethanol) (Weast, 1979); nuclear magnetic resonance and mass spectral data have been reported (NIH/EPA Chemical Information System, 1980)

(*f*) *Solubility*: Practically insoluble in cold water (400 mg/l at 12°C); slightly soluble in hot water (9400 mg/l at 100°C); soluble in diethyl ether; slightly soluble in ethanol (Weast, 1979)

(*g*) *Stability*: Darkens on exposure to air and light (Windholz, 1976)

(*h*) *Reactivity*: Undergoes chemical reactions characteristic of primary arylamines (e.g., formation of diazonium salts and acyl and alkyl derivatives) (Ferber, 1978)

(*i*) *Conversion factor*: ppm = 0.133 x mg/m^3

1.4 Technical products and impurities

Benzidine is no longer manufactured for sale in the US or Japan.

Benzidine sulphate

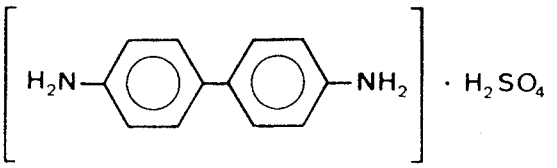
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 531-86-2

Chem. Abstr. Name: (1,1'-Biphenyl)-4,4'-diamine, sulfate (1:1)

IUPAC Systematic Name: Benzidine, sulfate (1:1)

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2.H_2SO_4$ Mol. wt: 282.3

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: White crystalline powder (Hawley, 1981)

(*b*) *Solubility*: Soluble in diethyl ether; slightly soluble in water (98 mg/l at 25°C) (Ferber, 1978), ethanol and dilute acids (Hawley, 1981)

1.4 Technical products and impurities

Benzidine sulphate is no longer manufactured for sale in the US or Japan.

Benzidine hydrochloride

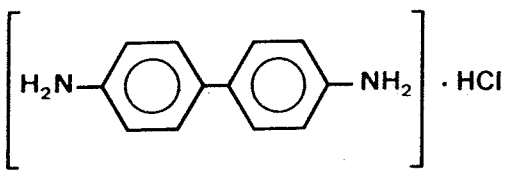
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 14414-63-7

Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine, hydrochloride

IUPAC Systematic Name: Benzidine, hydrochloride

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}H_2.HCl$ Mol. wt: 220.7

1.3 Chemical and physical properties of the pure substance

Solubility: 5.345 g/l in water at 25°C (Ferber, 1978)

1.4 Technical products and impurities

Benzidine hydrochloride is no longer manufactured for sale in the US or Japan.

Benzidine dihydrochloride

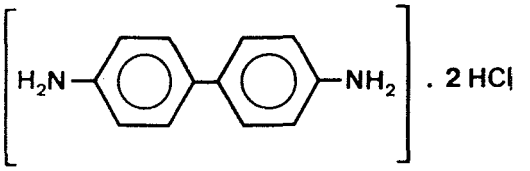
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 531-85-1

Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine, dihydrochloride

IUPAC Systematic Name: Benzidine, dihydrochloride

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2.2HCl$ Mol. wt: 257.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Crystals (Windholz, 1976)

(*b*) *Solubility*: Soluble in water and ethanol (Windholz, 1976)

1.4 Technical products and impurities

Benzidine dihydrochloride is no longer manufactured for sale in the US or Japan.

2. Production, Use, Occurrence and Analysis

Several reviews have been written about benzidine (Shriner *et al.*, 1978; JRB Associates, Inc., 1979; Jones, 1980).

2.1 Production and use

(*a*) *Production*

Benzidine was first synthesized in 1845 by reduction of azobenzene with ammonium sulphide, followed by treatment of the hydrazobenzene with sulphuric acid and treatment with a base to release the free benzidine. Although several methods for the reduction of nitrobenzene can be used, the key reaction in all routes of benzidine synthesis is the rearrangement of the intermediate hydrazobenzene to benzidine (Lurie, 1964). In commercial production, this rearrangement is generally carried out by treating hydrazobenzene with hydrochloric acid (sulphuric acid can also be used); and the resulting benzidine dihydrochloride is used for subsequent reaction rather than converted to the free base (Ferber, 1978).

Benzidine was first produced commercially in Europe in about 1880 (Schwenecke, 1980). This compound and its sulphate were produced commercially in the US for at least 60 years (US Tariff Commission, 1922); commercial production of benzidine hydrochloride was first reported in the US in 1928 (US Tariff Commission, 1930). At present, benzidine dihydrochloride is the only form produced commercially in the US, and this only as an unisolated intermediate which is further converted by the single manufacturer to a variety of dyes. In 1977, one US company reported that its production of benzidine dihydrochloride for captive consumption was in the range of 45.4-454 thousand kg (US Environmental Protection Agency, 1981). Commercial production of benzidine in the US was last reported (by a single company) in 1976 (US International Trade Commission, 1977), and that appears to be the last year of large-scale production of benzidine in the US. An estimated 500 thousand kg were produced in 1974 and 700 thousand kg in 1972 (Ferber, 1978). Even these amounts are small compared with the 1.8 million kg of benzidine produced in the US in 1948 (Boeniger, 1980). A total of 4100 kg were imported through the principal US customs districts in 1980 (US International Trade Commission, 1981).

One company in France probably produces benzidine dihydrochloride as an unisolated intermediate for dyes.

Commercial production of benzidine in Japan was stopped in about 1966. One or more companies in South Korea is believed to produce benzidine dihydrochloride as an unisolated dye intermediate.

The following countries, which are believed to manufacture benzidine-based dyes, may have plants in which benzidine and/or its salts are produced as unisolated dye intermediates: Argentina, Brazil, India, Mexico, the People's Republic of China, Poland, Romania and the USSR.

(b) Use

Although benzidine has had a variety of applications since the first benzidine-based dye, Congo Red, was prepared in 1884, its principal commercial use has remained the production of direct azo dyes. The amount consumed in all its other uses has been insignificant by comparison.

The Society of Dyers and Colourists (1971, 1975) indicates that 254 dyes or pigments can be prepared from benzidine. JRB Associates, Inc. (1979) stated that only one benzidine-based pigment, Pigment Red 39, had been, but was no longer, produced commercially in the US. They also indicated that only 16 out of a list of 232 benzidine-based dyes were being produced in the US or being imported; three of these 16 dyes, Direct Black 38, Direct Blue 6 and Direct Brown 95, are the subjects of monographs in this volume. Data on US production (or sales), imports and principal uses of the remaining 13 dyes during the period 1971-1979 are given in an appendix to the 'General Remarks on the Substances Considered', as are US sales in 1978 of another benzidine-based dye, Resin F Black WP, the composition of which has not been disclosed. The quantities of benzidine-based dyes produced and their relative importance in the dye industry have been decreasing steadily: their position is insignificant compared with the situation in 1948 when 14 million kg of benzidine-based dyes were produced in the US, representing 21% of all dyes manufactured and almost all of the direct dyes on the market that year (Boeniger, 1980).

Benzidine or its salts has also been reported to be used in the following minor applications: for the detection of blood, both in clinics and in criminal investigations - the latter use has existed for over 50 years (Steinberg, 1977); as a hardener (e.g., for polyurethanes) in the rubber industry (see IARC, 1982) and in the adhesives and plastics industries; for the detection of hydrogen peroxide in milk; in security printing (because it reacts with ink erasers to give coloured products); for the detection of a large number of inorganic ions and compounds; for the quantitative determination of nicotine; as a spray reagent for sugars; in the analysis of metals; as a chromogenic spray reagent in thin-layer chromatography of chlorinated organic pesticides; for the detection of bacterial cytochromes; for the determination of naphthalenesulphonic acids and detergents; in the synthesis of nitrosulphone and sulphonic acid derivatives, which can be used as dye intermediates; in the detection of chlorine or pyridine in drinking-water; and in the detection of *meta*- and *para*-cresols (Lurie, 1964; IARC, 1972; Shriner *et al.*, 1978; Auerbach Associates, Inc., 1978; Jones, 1980). It is not known to what extent, if any, benzidine is still used in any of these applications.

Regulations in the US concerning benzidine designate strict procedures to avoid worker contact: mixtures containing 0.1% or more benzidine must be maintained in isolated or closed systems, employees must observe special personal hygiene rules, and certain procedures must be followed for movement of the material and in the case of accidental spills and emergencies (US Occupational Safety and Health Administration, 1980).

Benzidine and its salts have been recognized as carcinogenic by the following 13 countries by regulation or guidelines: Australia, Belgium, Finland, the Federal Republic of Germany, Italy, The Netherlands, Poland, Romania, Sweden, Switzerland, the UK, the USA and Yugoslavia. Those countries which also designate it as a skin irritant are: Australia, Belgium, Italy, The Netherlands, Poland, Romania, Switzerland and the USA. Manufacture of benzidine and its salts is prohibited in Japan; and production of benzidine has been discontinued in the USSR by a decree of the Ministry of Health (International Labour Office, 1980).

The US Environmental Protection Agency (EPA) (1980a) has established the following standards to prevent pollution of navigable waters by industrial discharges: the maximum allowed is 0.1 μg/l; discharges from all benzidine manufacturers and benzidine-based dye applicators shall not contain benzidine concentrations exceeding an average per working day of 10 μg/l, calculated over any calendar month; and various limits apply to the concentrations permissible in any working day. Since the EPA has identified benzidine as a toxic waste, it requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme (US Environmental Protection Agency, 1980b). The EPA has also proposed a regulation requiring notification whenever discharges of hazardous substances are made into waterways. If this regulation is adopted, notification will have to be given to the EPA of any such discharges containing 4.54 kg or more of benzidine (US Environmental Protection Agency, 1980c).

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzidine are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its declaration as a hazardous material.

2.2 Occurrence

(*a*) *Natural occurrence*

Benzidine has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Bell (1973) reported that 17 US companies were using benzidine and that 62 employees were potentially exposed. Zavon *et al.* (1973) studied a benzidine manufacturing plant and found airborne concentrations of $\leqslant$0.007 mg/m^3 at four locations, 0.152 mg/m^3 at a salting-out tub, 0.072-0.415 mg/m^3 at a filter press, and 17.600 mg/m^3 where benzidine was shovelled into drums. A sample from a rafter above the reactors in which nitrobenzene was reduced was found to contain 0.27% benzidine.

Steinberg (1977) reported the results of a 1974 survey of US forensic laboratories, which showed that 54 of 276 laboratories were familiar with the benzidine test for blood. The same report cited several steps in blood testing and the enhancement of fingerprints on a bloody substrate during which laboratory or field workers could be exposed to benzidine-containing solutions.

The National Occupational Hazard Survey (Boeniger, 1980) estimated that about 700 people were exposed occupationally to benzidine. However, many more people may be

Table 1. Methods for the analysis of benzidine

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Bulk direct dyes	Extract (chloroform); elute (choloform-ethanol)	TLC	not given	Genin *et al.* (1977)
	Dissolve (water); extract (benzene); dry extract (sodium sulphate); evaporate; synthesize derivative with heptafluoro-butyric anhydride	GC/ECD	not given	Nony and Bowman (1980a)
Air	Collect sample on glass filter and silica gel; desorb (triethylamine in methanol); elute (60:40 methanol:water)	HPLC/UV	3 μg/m^3	Morales *et al.* (1979)
	Collect on filter paper; extract (sodium carbonate); dilute (chloroform/hexane)	LC/UV	not given	Krajewski *et al.* (1979)
Water				
Wastewaters	Acidify (hydrochloric acid); extract (benzene); add sodium hydroxide to aqueous layer; extract (benzene); perco-late extract through sodium sulphate; evaporate; synthesize derivatives with pentafluoropropionic anhydride or hepta-fluorobutyric anhydride	GC/ECD	0.1 μg/l	Nony and Bowman (1978)
	Add sodium hydroxide; filter; extract (diethyl ether); extract (hydrochloric acid); neutralize (sodium hydroxide); extract (diethyl ether); add methanol; concentrate	GC/FID	2-3 μg/l	Jenkins and Baird (1975)
Aqueous media	Three alternative procedures: (1) direct injection; (2) extract (chloroform), wash and concentrate, dilute (acetate buffer); or (3) adjust pH (phosphate buffer), clean by column chromatography, wash and concentrate, dilute (acetate buffer), concentrate	HPLC/E	(1) 1 μg/l (2) 0.05 μg/l (3) 0.1 μg/l	Riggin and Howard (1979)

Municipal sludge	Dilute (phosphate buffer); extract (chloroform); extract (sulphuric acid); neutralize (sodium phosphate); extract (chloroform); add methanol and concentrate; dilute (acetate buffer)	HPLC/E	10 μg/kg	Warner *et al.* (1980)
Biological samples				
Urine	Add ammonia/ammonium chloride buffer and diethyl ether; centrifuge; repeat ether extraction; add perchloric acid; centrifuge; analyse aqueous layer	HPLC/E	0.01 μg/l	Rice and Kissinger (1979)
Hamster urine	Dissolve (methanol); evaporate; elute (1:1 methanol:potassium phosphate)	HPLC/UV	1 μg/l	Nony and Bowman (1980b)
Monkey urine	Adjust pH; extract (chloroform); extract (hydrochloric acid); add 2,4,6-trinitrobenzenesulphonic acid; extract (chloroform); elute (9:1 chloroform: ethanol)	TLC	not given	Rinde and Troll (1975)
Human urine	Add sodium hydroxide; extract (benzene); dry (sodium sulphate); evaporate; synthesize derivatives with pentafluoropropionic anhydride and heptafluorobutyric anhydride	GC/ECD	1 μg/l	Nony and Bowman (1978)
	Adjust pH (sodium carbonate); extract (diethyl ether or 3:2 diethy ether: benzene); wash (sodium carbonate); extract (hydrochloric acid); add chloramine T; extract (chloroform)	S	10 μg/l	Dangwal *et al.* (1978)

Abbreviations : TLC, thin-layer chromatography; GC/ECD, gas chromatography/electron capture detection; HPLC/UV, high-performance liquid chromatrography/ultra-violet detection; LC/UV, liquid chromatography/ultra-violet spectrometry; GC/FID, gas chromatography/flame-ionization detection; HPLC/E, high-performance liquid chromatography/electrochemical detection; S, spectrometry (colorimetric analysis)

exposed as a result of manufacturing or of using dyes based on benzidine. Twenty-six US-produced dyes based on benzidine were found to contain <1-20 mg/kg benzidine and one had 270 mg/kg. Eight of 33 benzidine-based dye samples obtained from Belgium, Egypt, India, The Netherlands, Poland, Romania, and South Korea were found to contain 38-1254 mg/kg of benzidine, and the others had 24 mg/kg or less. Benzidine was detected in swipe samples taken during a benzidine-dye manufacturing operation; and some of the workers in the factory who were exposed to the dyes were found to have benzidine in their urine (proposed to have arisen from metabolism of the dyes). Similarly, some workers exposed to benzidine-based dyes in a textile dyeing operation were found to excrete benzidine; however, it was detected in the urine of only a very few workers in a paper dyeing operation and in no workers at a leather dyeing plant where benzidine-based dyes were used (Boeniger, 1980).

The National Occupational Hazard Survey estimated that approximately 79 000 workers in 63 occupations were potentially exposed to benzidine-based dyes (National Institute for Occupational Safety and Health, 1980).

(*c*) *Water and sediments*

Effluents from factories where textiles are dyed with benzidine-based dyes were found to contain an average concentration of 3.5 μg/l; those from a leather factory and another manufacturing plant with 'heavy benzidine-dye use' contained 0.25 and 3.5 μg/l, respectively (Jones, 1980).

Benzidine concentrations of up to 233 μg/l were found downstream from a dye plant on the Sumida River in Japan. These were attributed to reduction of the dye molecules to benzidine when either hydrogen sulphide or sulphur dioxide was present in the water (Takemura *et al.*, 1965).

Benzidine has been detected in oil refinery, municipal and industrial effluents and in surface water (Hushon *et al.*, 1980). It has also been found in river water and in raw sewage effluents (Shackelford and Keith, 1976).

2.3 Analysis

Procedures for the detection of benzidine in air, clothing and miscellaneous deposits by colorimetry and paper chromatography have been described (Butt and Strafford, 1956). More recently, analytical methods for the determination of benzidine in air, wastewaters and biological media based on spectrometry, spectrofluorometry, thin-layer chromatography and gas chromatography have been reviewed (Shriner *et al.*, 1978; US Department of Commerce, 1978). An IARC Manual (Egan *et al.*, 1981) gives selected methods for the analysis of aromatic amines and azo dyes, including benzidine.

Typical methods for the analysis of benzidine in various matrices are summarized in Table 1.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(a) Oral administration

Mouse: Benzidine dihydrochloride (certified ACS grade) was fed at a level of 150 mg/kg [ppm] of diet to B6C3F$_1$ mice, from the 6th to 45 weeks of age. Groups of 50 animals were killed at 45, 60, 75 and 90 weeks of age to evaluate the occurrence of liver-cell tumours (LCT). When the treatment was terminated at 45 weeks, 8/50 (16%) mice had LCT, 4% of which were hepatocellular carcinomas; at the successive serial killings at 60, 75 and 90 weeks, the proportions of LCT were 20/50 (40%), 31/50 (62%) and 35/50 (70%), 10%, 28% and 48% of which were hepatocellular carcinomas, respectively. In historical controls, the incidence of LCT was 1/98 (1%) in males and 0/100 (0%) in females (Vesselinovitch *et al.*, 1975).

Benzidine dihydrochloride (certified ACS grade) was fed at a level of 150 mg/kg [ppm] of diet for periods of 39, 54 or 84 weeks to 6-week-old B6C3F$_1$ male mice. All animals were killed at 90 weeks of age. The incidences of mice bearing LCT, mostly hepatocellular carcinomas, are given in Table 2. Thus, a negative relationship was observed between the incidence of LCT and duration of treatment; this may have been related to toxicity (Vesselinovitch *et al.*, 1975).

Table 2. Incidences of liver-cell tumours (LCT) in mice fed benzidine

Duration of treatment (weeks)	Estimated consumption of benzidine (mg/mouse)	Effective no. animals	LCT No.	%
39	117	50	35	70
54	162	50	25	50
84	188	50	22	44

A study was carried out to evaluate the role of age and sex in benzidine hepatocarcinogenesis. Doses of 50 or 100 mg benzidine dihydrochloride (certified ACS grade) per kg of diet [ppm] were given by stomach tube or in food to groups of 50 male and 50 female infant or adult B6C3F$_1$ mice; a further group was treated pre- and postnatally. No effects on survival were noted; all animals were killed at 90 weeks of age. The incidences of LCT are shown in Table 3. Continuous feeding of benzidine in the diet was found to produce LCT in a positive-dose response relationship in animals of both sexes: in 3/50 and 11/50 males receiving 50 and 100 mg/kg, respectively; and 13/50 and 32/50 in females,

[1] The Working Group was aware of a study, completed but not yet published, of s.c. administration in rats (IARC, 1981).

revealing a greater susceptibility of animals of this sex. The incidence of LCT in untreated controls was negligible (1/98 in males and 0/100 in females). Twice-weekly administration of benzidine by stomach tube showed a lesser hepatocarcinogenic effect that continuous feeding: 3/75 and 12/75 of males and 4/75 and 17/75 of females given the low and high dose of benzidine, respectively, developed LCT. The incidences of tumours at other sites (Harderian tumours and lung adenomas) were also affected by benzidine treatment; the compound also had a marginal effect upon the development of lymphoreticular tumours (Vesselinovitch *et al.*, 1975).

Table 3. Incidences of liver-cell tumours (LCT) in mice of different ages fed benzidine

Period of treatment (days of age)	Estimated total intake of benzidine dihydrochloride (mg/mouse)		Incidence of LCT			
			Males		Females	
	Males	Females	No.	%	No.	%
7-27 (by stomach tube)	0.63	0.63	59/89	66	0/82	0
1-27 (150 mg/kg diet to) mother and offspring from delivery to weaning)	4.20	3.36	62/65	95	2/43	5
42-630 (150 mg/kg diet)	187.87	150.29	22/50	44	47/50	94

Groups of B6C3F_1 mice were fed a diet containing 150 mg/kg [ppm] benzidine dihydrochloride (purity unspecified) (1) from the 12th day of gestation (prenatal) to delivery; (2) to mothers with litters from delivery to weaning; (3) to offspring from weaning to 90 weeks of age; (4) during prenatal and preweaning period; or (5) prenatally, during preweaning and in adulthood. Groups of untreated controls were also available. Administration prenatally or during preweaning induced a marked increase in the incidence of LCT in male mice (31 and 95%, respectively) but not in females (3 and 5%, respectively). In mice treated from weaning to 90 weeks of age, the incidences of LCT were 59% in males and 96% in females. In the group treated both prenatally and during preweaning, the incidences of LCT were 100% in males and 25% in females. When mice were treated prenatally, during preweaning and up to 90 weeks of age, the incidences of LCT were very high in animals of both sexes (100% in males and 94% in females) (Vesselinovitch *et al.*, 1979).

In studies designed to assess the biological significance of liver tumours in mice, groups of F_1 (C57BL/6Jf C3Hf/Nctr females x BALB/cStCr1fC3Hf/Nctr males) and F_2 (F_1 females and F_1 males) weanling mice were fed diets containing 0, 30, 60, 120, 200 or 400 mg/kg [ppm] of benzidine hydrochloride (100% pure). Groups of mice were killed after 40, 60 or 80 weeks of treatment. The incidences of LCT (benign and malignant) in the different groups are summarized in Table 4 (Frith *et al.*, 1979, 1980). [The Working Group noted that tumour incidences at the various dose levels could not be estimated.]

Table 4. Incidences of liver-cell tumours (LCT) in mice fed benzidine

Sacrifice period (weeks)	Sex	Controls *Benign* No.	%	*Malignant* No.	%	Treated *Benign* No.	%	*Malignant* No.	%
40	M	0/99	0	0/99	0	4/599	0.7	3/599	0.5
	F	0/96	0	0/96	0	25/594	4.2	15/494	3.0
60	M	0/96	0	1/96	1	27/474	5.7	36/474	7.6
	F	1/96	1	1/96	1	50/566	8.8	201/566	35.5
80	M	2/91	2.2	0/91	0	28/314	8.2	61/341	17.9
	F	0/95	0	0/95	0	26/264	9.8	125/264	47.3

Rat: Two groups, each of 10 female Wistar rats [age not specified], were fed a diet containing 170 mg/kg [ppm] benzidine with casein or a diet containing benzidine with casein hydrolysate-tryptophan. All rats given benzidine plus casein were dead 93-224 days after the start of treatment; and 2/10 had LCT (1 hepatoma seen at 125 days and 1 bile-duct carcinoma at 178 days). Animals fed benzidine-tryptophan survived longer; 3/7 animals examined had LCT (1 carcinoma at 202 days, 1 cholangioma at 236 days and a bile-duct carcinoma at 424 days). None of the animals in the two experimental groups developed bladder tumours (Boyland *et al.*, 1954).

Following preliminary determinations of the maximum tolerated dose, four groups of 10-20 female Sprague-Dawley rats, 40-45 days old, were given total doses of 12, 25, 35 or 50 mg/rat of benzidine by stomach tube. Two control groups, one fed the sesame oil vehicle and the other given 18 mg of dimethylbenz[*a*]anthracene (positive control) were also available. At the end of the nine-month period of observation, when the experiment was terminated, 10/10, 8/10, 0/20 and 4/20 animals were still alive in the four treated groups, respectively. In the positive control group, 19/40 survived, and in the vehicle control group 127/140 were still alive at nine months. Thus, mortality was high in animals fed the two highest doses of benzidine, and only 5 rats (at the highest dose) were autopsied; four of these showed multiple mammary carcinomas. In the groups receiving 12 and 25 mg/rat, 5/10 and 7/9 animals autopsied also showed multiple mammary carcinomas (1 in the group fed 12 mg had a fibroadenoma). All of the positive controls had multiple mammary masses diagnosed as carcinomas, fibroadenomas and hyperplasias; and 5/132 sesame-oil vehicle controls examined also had mammary tumours. In the benzidine-treated groups, the first palpable mammary lesions appeared about 60 days after the first treatment. At this point the mean number of mammary masses per rat showed a dose-response relationship. No effect was reported in organs other than the mammary gland (Griswold *et al.*, 1968). [The Working Group noted that the design of this study was based on the breast tumour induction system of Huggins *et al.* (1959). In this system, benzidine was definitely active in causing mammary cancer.]

It was reported in an abstract that groups of 20 female Fischer 344 rats were given drinking-water containing 100-400 mg/l benzidine dihydrochloride for up to 90 days (estimated daily doses of 10, 21, 28 and 31 mg/kg bw). Rats given the two highest doses

died within nine weeks of exposure. Hepatocellular carcinomas were observed in some rats given 28 mg/kg bw per day (Mennear and Gupta, 1982).

Hamster: Groups of 30 male and 30 female, random-bred Syrian golden hamsters, nine weeks old, were fed diets containing 1000 mg/kg [ppm] benzidine or benzidine dihydrochloride (certified grade) for life. A control group of the same size was also available. No bladder pathology was seen in either the treated or the control group. In the benzidine-treated group, an increased incidence of liver tumours was observed: 19/22 males and 6/26 females developed multiple cholangiomatous tumours, most of which had signs of malignancy; 12 males and 3 females also developed benign and malignant LCT. In the group fed benzidine dihydrochloride, the liver was also the only target organ: 10/20 male and 12/27 female hamsters developed cholangiomas, mostly benign; 7 males and 4 females also developed hepatomas. No liver tumours were seen in females or males of the untreated control group (Saffiotti *et al.*, 1967).

Rabbit: An invasive bladder carcinoma was induced after 2 $\frac{1}{4}$ years in one out of seven animals given oral doses of benzidine (Bonser *et al.*, 1956a).

Dog: Seven dogs were given a total dose of 325 g benzidine over 5 years (200 and then 300 mg per day, on six days a week). Three of the animals developed bladder carcinomas 7, 8 and 10 years after the start of treatment (Spitz *et al.*, 1950; Bonser *et al.*, 1956a).

Frog: A group of five frogs (*Rana temporaria*) received a total oral dose of 60 mg benzidine and were observed for 20 weeks; 1 liver tumour was seen (Khudoley, 1977).

(*b*) *Subcutaneous and/or intramuscular administration*

Mouse: Three groups of 12-24 male, albino Delph mice, 10 weeks of age, were given s.c. injections of 300 mg benzidine base (redistilled) in olive oil three times a week for 45 weeks or received olive oil alone. One group served as untreated controls. The survival rates were good in all groups up to 45 weeks when the experiment was terminated. No changes in the bladder were observed in the benzidine-treated animals. In 2/19 control mice receiving olive oil alone hyperplasia of the bladder was noted. Five of nine mice given benzidine had hepatomas, compared with 3/19 in the olive oil group and 5/17 in the untreated controls (Baker, 1950). [The Working Group noted the short duration of the experiment.] Bonser *et al.* (1956a) obtained similar results.

A group of 54 male and 13 female C3HA mice, 18-20 g, were injected subcutaneously weekly with 6 mg/mouse of benzidine [source and purity not specified] dissolved in 0.2 ml of oil [not specified] over eight months (total dose, 210 mg/mouse). At the appearance of the first tumour (16 months), 22 mice [sex not specified] were still alive. Liver tumours (hepatocellular carcinomas, adenomas and cholangiomas) developed in 13 mice [sex not specified]; and lung adenocarcinomas were found in 2. A further group of 114 females were exposed by the same treatment schedule for 13 months (total dose, 336 mg/mouse). At 16 months, 24 mice were still alive, and 18 developed liver tumours. Hepatomas developed in 1% of historical controls (Prokofjeva, 1971). [The Working Group noted the low survival rates.]

Rat: Groups of Sherman rats, 10 weeks old, average weight 150 g, were administered benzidine (technical and purified grades) or benzidine sulphate (technical grade) subcutaneously once weekly for life. A suitable control group was given the olive oil

vehicle or butyl succinate. The experimental design and data on survival and tumour incidence are summarized in Table 5 (Spitz *et al.*, 1950). [The Working Group noted that the low occurrence of colonic adenocarcinomas was confined to males treated with both grades of benzidine; however, it was impossible to distinguish in which of the two gropus the colonic tumours predominated.]

Table 5. Design and results of experiments in rats given s.c. injections of benzidine and salts

Compound	Average weekly dose (mg)	Total dose (g)	No. of rats at start	No. of rats surviving more than 300 days	Rats with tumours: liver No.	liver %	external auditory canal No.	external auditory canal %	colon No.	colon %
Olive oil	910	92.82	50	28	—	—	—	—	—	—
Technical benzidine	15	1.28	233	36	8	3.4	54	23		
Pure benzidine	15	0.96	152	24	6	3.9	32	21	7	3.8
Benzidine sulphate	15	0.94	153	5	1	0.65	16	10.5		

A group of 25 male and 25 female rats [strain not specified], weighing 100-120 g, were injected subcutaneously with an initial dose of 15 mg benzidine in 0.5 ml of sunflower-seed oil once a week for 14 weeks. Due to severe toxicity, a smaller dose of 10 mg weekly was given for six weeks to each rat, and finally once every 15 days for six weeks. By six months of treatment, each animal had received a total dose of 300 mg benzidine. A group of 50 rats [sex unspecified] served as controls: 25 received s.c. injections of the solvent for six months while the remaining 25 were kept untreated. Of the males surviving at the time of the appearance of the first tumour, 12/15 developed tumours: 2 hepatomas, 4 malignant tumours of the Zymbal gland, 6 sarcomas at the injection site and 2 other sarcomas; 2/5 surviving females developed tumours: 1 malignant tumour of the Zymbal gland and 1 myeloid leukaemia. None of the 25 controls injected with the solvent [sex not specified] developed tumours at the injection site (Pliss, 1964).

A group of 28 rats [from the Rappolovo breeding farm; sex not specified], aged $1\frac{1}{2}$-2 months, were exposed to benzidine [purity, source and vehicle not specified] by weekly s.c. injections of 5 mg/rat for 32-60 weeks (total dose, 170 mg/rat). At 210 days, the time at which the first tumour appeared, 25 rats were still alive. Intestinal tumours developed in four rats between 252 and 318 days (Pliss *et al.*, 1973). [The Working Group noted that no controls were reported.]

Groups of 16 female and 14 male rats [strain and age not specified] were injected subcutaneously with 5 mg/rat benzidine [source and purity not specified] dissolved in 0.5 ml of sunflower oil weekly for about 52 weeks (total dose, 160-260 mg/rat). At 219 days, when the first tumour (a skin epithelioma) was detected, 24 rats [sex not specified] were still alive; all animals were killed at 357 days. Tumours were found in 23 rats (95.8%), with an average latent period of 275 days. Nine rats (39.1%) had multiple primary tumours;

tumours of the Zymbal gland developed in 18 rats (78.3%); 5 had local fibrosarcomas and 1 a local rhabdomyosarcoma (Pliss and Iogannsen, 1974). [The Working Group noted that no untreated or solvent controls were available.]

Groups of 18 male and 16 female ablino non-inbred rats (120-140 g) were injected subcutaneously once a week for about 33 weeks with 5 mg/rat benzidine [source and purity not specified] suspended in 0.5 ml of oil [not specified] (total dose, 170 mg/rat). At 210 days, when the first tumour appeared, 16 males and 12 females were still alive. A total of 26 tumours developed in 14 males; these were 6 local sarcomas, 9 tumours of the Zymbal gland, 9 liver tumours (cystocholangiomas and hepatocellular carcinomas) and 2 intestinal tumours (polyposis and adenocarcinoma). A total of 20 tumours developed in 11 females: 5 local sarcomas, 6 tumours of the Zymbal gland, 4 mammary adenocarcinomas, 1 mammary adenoma, 2 liver tumours (cystocholangioma and hepatocellular carcinoma) and 2 intestinal tumours (Pliss and Volfson, 1974). [The Working Group noted that no untreated or solvent controls were available.]

Groups of 20 female, 100-day-old Sprague-Dawley rats were given weekly s.c. injections of benzidine for 1-24 weeks, at total doses of 150, 250, 450, 625 or 1225 mg/kg bw in sterile saline solution. The length of survival was dose-dependent and ranged from 300 $\pm$ 67 days at the lowest level to 170 days at the highest dose. The total numbers of mammary adenocarcinomas in each of the treated groups were 47, 87, 116, 101 and 66, respectively (Steinhoff, 1974). [The Working Group noted that information on the survival rates in the different groups was not given and that no controls were available.]

Frog: A group of 37 grass frogs (*Rana temporaria*) of both sexes, aged 1-1.5 years, received weekly s.c. injections of 0.2-0.5 ml of a 0.5% solution of benzidine in mineral oil for up to 38 weeks (total dose, 45-114 mg/animal). A group of 120 untreated frogs were observed for 56 weeks (3 of them developed skin cystadenopapillomas); and a further group of 67 frogs were given s.c. injections of 0.2-0.5 ml mineral oil weekly for 42 weeks. Of the treated animals, 6/14 still alive at 16 weeks, when the first tumour appeared, had tumours of the liver and haematopoietic system [not further specified], with an average latent period of 24.8 weeks (Khudoley, 1977).

(c) *Intraperitoneal administration*

Rat: Three groups of 30 female CD rats, 4 weeks of age, were given i.p. injections, twice weekly for 4 weeks, of 0, 10 or 30 μmol/kg bw benzidine in trioctanoin suspensions. All survivors were sacrificed 46 weeks after the first injection. No tumours were seen in the kidney or bladder in treated or control groups. In the benzidine-treated groups, a dose-related increase in the incidence of mammary tumours, benign and malignant, was noted: 3/30 in controls, 7/30 in the low-dose group and 12/29 ($p<0.01$, compared with controls) in the high-dose group. Zymbal gland tumours (adenomas or carcinomas) were observed in 1/30 controls, 1/30 low-dose animals and 7/29 ($p<0.05$, compared with controls) high-dose animals. No tumours of the liver were found at sacrifice; however, cellular-altered foci in the liver were observed in 9/30 controls, 14/30 low-dose animals and 20/29 ($p<0.01$) high-dose rats. An increased incidence of neoplastic nodules in the liver noted in the treated animals was not statistically significant compared with that in the controls (Morton *et al.*, 1981).

(*d*) *Inhalation exposure*

Rat: A group of 48 white outbred rats (Rappolovo stock) of both sexes, weighing 100-120 g, were exposed to 10-20 mg/m^3 [1.3-2.7 ppm] benzidine [source and purity not specified] in inhalation chambers on four hours/day, for five days a week over 20 months (total dose, 27 mg/rat). Control rats [number not specified] were kept in inhalation chambers and exposed to air during the same period. Animals were kept until moribund. The first myelogenous leukaemia was found in a treated rat 13 months after the start of the experiment, at which time 28 rats were still alive. By the end of the study (28 months), 5 myeloid leukaemias, 2 breast fibroadenomas, 1 squamous-cell cancer of the Zymbal gland, 1 hepatoma and 1 breast adenocarcinoma were found in 8 animals. Mammary adenomas were found in 2/21 control rats (Zabezhinsky, 1970). [The Working Group noted the lack of information on the size of particles and on survival of controls.]

(*e*) *Other experimental systems*

Oral administration following implantation of glass beads into the bladder: Following the surgical implantation of glass beads into the urinary bladder of 150 ICR strain female *mice* at 5 weeks of age, the animals were divided into three groups: one group (30 mice) served as controls and was fed a commercial basal diet; the second group (60 mice) received a diet containing 2000 mg/kg [ppm] of benzidine; the third group (60 mice) was fed a diet containing a mixture of 2000 mg/kg [ppm] benzidine and 20 000 mg/kg [ppm] DL-tryptophan. The experimental groups received their diets starting at 6 weeks of age for 20 weeks and were then fed the control diet for 40-43 weeks. The experiment was terminated 63 weeks after the start of treatment. Of the group that received benzidine alone, only 19% of the animals were still alive at the end of the experiment; 65.5% of controls and 49.2% of the group treated with benzidine plus tryptophan were still alive at that time. Hepatomas, diagnosed microscopically, were observed in 34/41 (82.9%) mice treated with benzidine and in 24/51 (47.1%) of mice treated with the benzidine-tryptophan mixture; no hepatomas were seen in the controls. No bladder tumour was found in any of the animals; however, the authors reported hyperplasia in all bladders examined (Miyakawa and Yoshida, 1980).

Mixed in diet added to tank water: Benzidine was mixed into a diet and given to a group of 100 *fish* (guppies) of both sexes, 10-12 months old, at a dose of 300 mg/kg dry diet for 56 weeks, at which point the experiment was terminated. The six fish that survived the treatment period had no detectable tumour; however, signs of hepatotoxicity (focal necrosis, fatty dystrophy and diffuse hyperplasia of hepatocytes) were noted. None of the 120 control guppies fed the standard diet developed tumours or preneoplastic changes (Pliss and Khudoley, 1975). [The Working Group noted the high mortality in the treated group.]

(*f*) *Carcinogenicity of metabolites*

Essentially negative results were obtained in early studies in mice which were given bladder implantations of 3-hydroxybenzidine hydrochloride, 4-amino-3-biphenyl sodium sulphate or 4′-nitro-4-amino-3-hydroxybiphenyl hydrochloride (Bonser *et al.*, 1956b, 1963).

In the study by Morton *et al.* (1981) [see section 3.1 (*c*)], two benzidine metabolites, *N*,*N*′-diacetylbenzidine and *N*-hydroxy-*N*,*N*′-diacetylbenzidine, were also given by i.p. injection twice weekly for four weeks, at dose levels of 10 and 30 μmol/kg bw in

trioctanoin suspensions to groups of 30 4-week-old CD rats. A group of 30 solvent-treated controls was also available. The two metabolites produced similar incidences of tumours of the mammary and Zymbal glands and of preneoplastic changes in the liver as the parent compound, benzidine.

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The acute oral LD_{50} of benzidine administered as a suspension in water to male Wistar rats was 1.57 g/kg (Marhold *et al.*, 1968).

Few data are available on the toxic effects of benzidine; however, the one consistent finding has been that under experimental conditions it causes liver cirrhosis in rats, rabbits and dogs (reviewed in Haley, 1975).

It was reported in an abstract (Rao *et al.*, 1971) that dietary concentrations of 0.01-0.08% [100-800 ppm] benzidine given to C57BL x C3H F_1 mice [sex unspecified] produced concentration-dependent losses of body weight, cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements in the bone marrow and lymphoid cells in the thymus and spleen. All of the lesions were observed with the lowest dose.

Effects on reproduction and prenatal toxicity

Benzidine has been reported to be teratogenic to chicks when injected into hens' eggs (Noto, 1967).

Absorption, distribution, metabolism and excretion

Little is known about the dermal, pulmonary or gastrointestinal absorption of benzidine in experimental animals. The occurrence of systemic toxic manifestations following dietary administration, however, indicated some absorption from the gut; and varying amounts of benzidine or its metabolites were identified in the urine of dogs treated orally or dermally or by inhalation of a benzidine aerosol (Ghetti, 1960).

Uniformly labelled ^{14}C-benzidine (0.2 mg/kg bw) administered intravenously to male Wistar rats and male beagles exhibited multiphasic blood half-lives. The fourth and final phase, predominant within 24 hours after treatment, had an estimated half-life of 65 hours in the rat and 88 hours in the dog. The ^{14}C label of 0.2 mg/kg bw benzidine administered intravenously to rats, dogs and monkeys was rapidly transferred to the excretory organs: liver, gastroinestinal tract, kidney and bladder; significant amounts were also distributed to the lung. By 7 or 14 days after treatment, residues of radioactivity were detected primarily in liver, bile, intestines and kidney (Kellner *et al.*, 1973).

The major routes of excretion for intraperitoneally or intravenously administered benzidine in experimental animals are urine and faeces (Baker and Deighton, 1953; Kellner *et al.*, 1973). Rats excreted approximately 80% of an i.v. dose of 0.2 mg/kg bw benzidine in faeces within 7 days of treatment, while dogs and monkeys excreted only

30%. Urinary excretion predominated in dogs and monkeys (approximately 67% and 50% in urine, respectively, in 7 days), while rats excreted only 17% by this route. Thus, the rats and dogs cleared virtually all of the i.v. dose of benzidine from the body within 7 days. The recovery of only 80% of the administered dose in the urine and faeces of the monkeys may be a consequence of technical losses of the excreta, primarily faeces (Kellner *et al.*, 1973).

The identities of the faecally-excreted materials have not been ascertained. The materials excreted in the urine are largely metabolites of benzidine involving *N*-acetylation, 3-hydroxylation and sulphate and glucuronide conjugations (Bradshaw and Clayson, 1955; Clayson *et al.*, 1959; Fabre *et al.*, 1960; Haley, 1975; Shriner *et al.*, 1978). An interesting species difference is the apparent inability of dogs to acetylate (or otherwise conjugate) the amino groups of benzidine *in vivo* (Bradshaw and Clayson, 1955; Troll and Nelson, 1958).

In-vitro metabolism and macromolecular binding

Benzidine is metabolized *in vitro* by liver cytosol from rats, mice, hamsters or guinea-pigs to *N*-acetylbenzidine and *N,N'*-diacetylbenzidine. Hepatic microsomal preparations *in vitro* convert synthetic *N,N'*-diacetylbenzidine to *N*-hydroxy-*N,N'*-diacetylbenzidine and 3-hydroxy-*N,N'*-diacetylbenzidine by a NADPH-dependent reaction. Liver cytosol catalysed the binding of synthetic *N*-hydroxy-*N*-acetyl-*N'*-[1-^{14}C]-acetylbenzidine to *t*RNA *in vitro*. In all three reactions, liver preparations from hamsters were more active than those from mice or rats; rodent liver therefore contains the enzymes necessary for metabolizing benzidine to a product capable of binding to nucleic acids (Morton *et al.*, 1979). *N*-Hydroxy-*N,N'*-diacetylbenzidine can also be esterified to an electrophilic reactant by hepatic sulphotransferases in the rat and mouse (Morton *et al.*, 1980).

A microsomal preparation from the inner renal medulla of male New Zealand rabbits metabolized ^{14}C-benzidine to products that covalently bound to exogenous *t*RNA and DNA *in vitro*. The reaction was arachidonic acid-dependent (NADPH-independent) and appeared to be mediated by prostaglandin endoperoxide synthetase. The ratio of trichloroacetic acid-precipitable:non-trichloroacetic acid-precipitable:*t*RNA-bound benzidine products was 10:3:1. The addition of glutathione to the incubation medium decreased the formation of trichloroacetic acid-precipitable and *t*RNA-bound materials, with a concomitant increase in the non-trichloroacetic acid-precipitable materials (Zenser *et al.*, 1979, 1980).

Male Wistar rats treated with 32 mg/kg bw ^{3}H-benzidine (specific radioactivity, 30 mCi/mmol) intraperitoneally incorporated radioactivity into liver RNA, DNA and protein. Incorporation was maximal 24 hours after injection, and greatest in RNA. The majority of the radioactivity associated with DNA was stable for at least four weeks. Enzymatic hydrolysis of the DNA and chromatography on Sephadex LH20 yielded five radioactive peaks, the largest of which (in contrast to total DNA binding) had disappeared from the subsequently isolated liver DNA, probably as a consequence of the transfer of radioactivity to other DNA components by an undefined mechanism. The same pattern of peaks was produced by reaction of ^{3}H-*N*-benzoyloxybenzidine with DNA *in vitro*, suggesting that a nitrenium ion is an electrophilic intermediate (Martin and Ekers, 1980).

Mutagenicity and other short-term tests

Benzidine has been found consistently to be mutagenic to *Salmonella typhimurium* strain TA1538 when tested in the presence of an exogenous metabolic activation system

from rats (see, e.g., Ames *et al.*, 1973; Anderson and Styles, 1978) or humans (see, e.g., Haworth and Lawlor, 1978).

The urine of rats fed benzidine was mutagenic to *S. typhimurium* TA1538, TA98 or TA100 when tested in the presence of a rat liver metabolic activation system or to *S. typhimurium* TA1538 in the presence of a rat liver cytosolic fraction; addition of glucuronidase increased the mutagenic activity in TA1538 (Bos *et al.*, 1980; Tanaka *et al.*, 1980).

N-Acetylbenzidine, a urinary metabolite of benzidine, was mutagenic to *S. typhimurium* strains TA98 and TA100 in the presence of a rat liver metabolic system (Tanaka *et al.*, 1980). *N*-Hydroxy-*N,N'*-diacetylbenzidine was mutagenic to *S. typhimurium* TA1538 in the presence of a partially purified *N,O*-acyltransferase preparation (Morton *et al.*, 1979).

Benzidine was negative in the *Escherichia coli* pol A test (Fluck *et al.*, 1976) and in the prophage induction test (Speck *et al.*, 1978), when tested either in the presence or absence of a rat liver metabolic activation system.

Mutagenic activity on the X-chromosome recessives (visibles and lethals) and RNA genes of *Drosophila melanogaster* has been reported (Fahmy and Fahmy, 1977).

Benzidine ($6x10^{-4}$ M for 30 min) inhibited DNA synthesis in HeLa cells *in vitro* without activation (Painter, 1978) and *in vivo* in renal and hepatic cells when given intraperitoneally or intragastrically to suckling 14-18-day-old mice in doses of 15-30% of the LD_{50} (Amlacher and Ziebarth, 1979). Unscheduled DNA synthesis was induced by benzidine (active dose range, 10^{-7}-10^{-3} M) in HeLa cells in the presence of a phenobarbital-induced rat liver activation system (Martin *et al.*, 1978) and in rat hepatocytes (Williams, 1978; Brouns *et al.*, 1979). Benzidine, when tested in the presence of a rat liver metabolic activation system, induced DNA strand breaks in Chinese hamster V79 cells (Swenberg *et al.*, 1976). When measured by the alkaline elution assay, there was a dose-related increase in DNA strand breaks in the livers of rats exposed to benzidine *in vivo* (Petzold and Swenberg, 1978). Benzidine (2.5 μg/ml) transformed BHK21 Cl-13 cells in the presence of an Aroclor 1254-induced rat liver metabolic system (Ashby *et al.*, 1978), and was shown to transform Syrian hamster embryo cells (Pienta, 1980).

Conflicting reports exist on the ability of benzidine to induce micronucleated polychromatophilic rat erythrocytes. It was inactive at doses of up to 250 mg/kg (Trzos *et al.*, 1978), but was positive (with no dose response) when tested at comparable doses (100, 200, 300 mg/kg) (Cihak, 1979). It was also reported to be active when given at the high dose of 409.6 mg/kg dermally or subcutaneously (Urwin *et al.*, 1976).

(*b*) *Humans*

Toxic effects

Exposure to benzidine has been shown to produce a spectrum of lesions of the epithelium of the urinary bladder, which may precede appearance of malignancy. These lesions include hyperaemia, inflammation and papillomata (both sessile and pedunculated) (Muller, 1933; DiMaio, 1937; Douillet *et al.*, 1959; Vigliani and Barsotti, 1961). The presence, grossly visible or occult, of blood in the urine or the development of pain or difficulty in urinating may signal the appearance of such lesions.

The occurrence of such bladder lesions in people exposed to benzidine suggests that relatively early detection of premalignant changes may be possible through medical screening procedures. Some authors have relied on periodic cystoscopic examination of exposed workers for such screening. Another, less invasive approach relies on periodic cytological evaluation of bladder epithelial cells shed in urine (Billiard-Duchesne, 1960; Vigliani and Barsotti, 1961). The value of cytological screening has, however, not been established. Screening of the urine for occult blood may provide an effective, noninvasive means for the early detection of bladder lesions.

Absorption, distribution, excretion and metabolism

Benzidine may enter the body by percutaneous absorption, ingestion or inhalation; percutaneous absorption appears to be the primary route of absorption following occupational exposure (Meigs *et al.*, 1951, 1954; von Ehrlicher, 1958).

Following application of 100 mg benzidine to the skin, <0.02 mg benzidine and metabolites were detected in the urine. Concentrations of 0.27 to 1.60 mg/l were measured in the urine of workers in a chemical plant manufacturing benzidine and substituted benzidines (Meigs *et al.*, 1951).

3-Hydroxybenzidine conjugates constituted 80-90% of the urinary constituents in benzidine-exposed workers; another 5-10% was excreted as diacetylbenzidine, 1-5% as monoacetylbenzidine and 4-6% as parent compound (Sciarini and Meigs, 1961). It was reported in an abstract (Troll *et al.*, 1963) that an *N*-acetyl-*N*-hydroxy derivative of benzidine was found in the urine of six people given 200 mg benzidine [route unspecified].

Mutagenicity and chromosomal effects

An increase in the number of sister chromatid exchanges was reported in peripheral blood lymphocytes of 15 subjects occupationally exposed to benzidine (Bassendowska-Karska, 1980). [The effect reported was small, and the Working Group questioned the significance of this observation.]

3.3 Case reports and epidemiological studies of carcinogenicity in humans

An association between industrial exposure to benzidine and cancer of the bladder has been recognized since the early decades of the twentieth century. Several international bodies, including the International Labour Office (1921) and the IARC (1972, 1979), concluded previously that sufficient evidence exists to consider benzidine a carcinogen in man.

Oppenheimer (1927) described 40 cases of tumours of the urinary bladder among workers in the German dyestuffs industry. Six cases occurred in workers whose principal exposure was to benzidine. The mean duration of employment before development of tumour for the entire series was 17 years (range, 2-41 years). Mean induction period was 18.5 years.

Muller (1933) described 19 cases of papilloma of the bladder and 36 of carcinoma among workers in the Swiss dye industry. Eleven of the tumours occurred in workers with principal exposure to benzidine.

DiMaio (1937) performed cystoscopic examination on 86 workers in the benzidine department of two Italian dye factories. Four of the workers were found to have carcinoma of the bladder and another 7 had papillomas.

Goldblatt (1949) studied the incidence of urinary-tract tumours in two British chemical plants. From 1934-1947, 99 tumours of the upper and lower urinary tract occurred, of which 59 were fatal. Six cases occurred in workers exposed to benzidine alone. Comparison of the observed number of deaths in this plant with the number expected for the entire adult male population of England and Wales showed an excess mortality of more than twenty-fold. The mean age at diagnosis of tumour was 50.5 years; mean induction period was 18.9 years.

Barsotti and Vigliani (1949) reevaluated the two Italian plants previously studied by DiMaio (1937). In workers engaged in production and use of benzidine, they found 14 carcinomas of the bladder and 7 papillomas. Duration of employment before appearance of the tumours ranged from 5 to 26 years.

Scott (1952) described 30 cases of bladder tumours (8 fatal) among 284 workers exposed to benzidine in a British dyestuffs manufacturing plant. Mean duration of exposure was 15.9 years (range, 8-32 years); the distribution of induction periods was the same. For workers who began employment before age 30, mean age at death was 44 years; for those beginning employment at 30 to 40 years, mean age at death was 54 years; and for those entering employment after age 40, mean age at death was 66 years. For all adult males in England and Wales, the mean age at death for cancer of the urinary bladder at the time of this study was 67.5 years.

Aboulker and Smagghe (1953) described 21 cases of bladder tumour among workers in a French dyestuffs plant. Two of these cases occurred in workers exposed to benzidine alone, for a mean duration of exposure of 17.5 years.

Uebelin and Pletscher (1954) described 100 cases of urinary-tract tumours among workers in a Swiss dyestuffs factory. Twenty had exposure to benzidine alone, with an average duration of exposure of 11.6 years (range, 1-29 years); mean induction period for workers exposed to benzidine was 14.8 years (range, 5-29 years).

Douillet *et al.* (1959) described 13 cases of urinary-tract tumours among workers in a French plant where benzidine was manufactured. Duration of exposure to benzidine ranged from 2-21 years; the induction periods ranged from 10-26 years (mean, 16 years).

Billiard-Duchesne (1960) reported 12 cases of bladder tumours among workers in a French factory where benzidine was the only aromatic amine manufactured.

Vigliani and Barsotti (1961) reevaluated the workforce in the two Italian factories evaluated by DiMaio (1937) and by Barsotti and Vigliani (1949) and those of four further factories. They found 17 new cases of carcinoma and 11 of papillomas of the bladder in workers exposed to benzidine alone.

Maltoni and Ghetti (1964) reported the occurrence of four cases of upper urinary-tract tumours in workers engaged in the production of benzidine in an Italian dye factory.

Goldwater *et al.* (1965) examined the occurrence of bladder tumours among workers employed from 1912-1962 in a British company where coal-tar dyes were made. Among 76 workers exposed to benzidine alone, 17 developed bladder malignancies (total

incidence, 21.3%). Mean age at diagnosis of tumour was 49.7 years, and the mean induction period was 18.7 years (range, 5-33 years).

Ferber *et al.* (1976) reviewed the incidence of bladder tumours between 1930 and 1975 in workers at a dye plant in the USA. Thirty-six cases of bladder tumour occurred in workers exposed to benzidine alone.

Primary tumours at sites other than the bladder have been noted in several of the series of workers exposed to benzidine (Uebelin and Pletscher, 1954; Mancuso and El-Attar, 1966; Reinl, 1967). Three historical reviews (Hueper, 1942, 1969; Haley, 1975) have reported that the worldwide spread of cancer of the bladder in people exposed to benzidine has followed the international spread of the dyestuffs industry.

Several epidemiological studies of bladder cancer incidence and mortality in people exposed to benzidine have been conducted. An analysis of mortality in a cohort of British chemical workers exposed to benzidine showed 10 deaths certified as being due to bladder tumour; only 0.72 such deaths would have been expected on the basis of rates for the whole male population of England and Wales (standardized mortality ratio, SMR = 1390; $p < 0.001$). An additional 24 nonfatal cases of bladder tumours were noted to have occurred in members of this cohort exposed to benzidine (Case *et al.*, 1954). In a cohort study of a factory population in the US (Ohio) exposed to benzidine, 16 cases of bladder cancer were observed, and the cumulative incidence rate of bladder cancer in workers exposed to benzidine was reported to be 237 per 100 000. The authors calculated that mortality from cancer of the bladder in the exposed workers was 30 times higher than that expected on the basis of Ohio male mortality rates (Mancuso and El-Attar, 1966, 1967).

Zavon *et al.* (1973) conducted an analysis of bladder cancer incidence in 25 workers in the US engaged in benzidine manufacture. Thirteen of them were found to have developed tumours of the bladder (total incidence, 52%), including all 5 workers with more than 15 years' exposure. Mean duration of exposure in workers who developed a tumour was 13.6 years; mean time from onset of exposure to first appearance of tumour was 16.6 years; mean age at first diagnosis of tumour was 45.6 years. Concentrations of airborne benzidine in the plant ranged from less than 0.005 to 17.6 mg/m^3. Three of the original cohort had had about one year of exposure to 2-naphthylamine, and three had had exposure to *ortho*-toluidine. [The Working Group considered that the high incidence of bladder tumour in this cohort was striking evidence of the carcinogenic potency of benzidine.]

An epidemiological analysis of Japanese dyestuffs workers engaged in the production or use of benzidine (Tsuchiya *et al.*, 1975) showed that 72 had developed bladder tumours, 21 of which were fatal. Of these cases, 61 (17.6%) occurred in 346 production workers, and 11 (1.6%) in 669 workers who used benzidine. Mean time from first occupational exposure to benzidine to appearance of tumour was 16.2 years. Mean age at diagnosis of bladder cancer in workers exposed to benzidine was 43.2 years.

The incidence of bladder cancer in workers decreased after a reduction in industrial exposure (Ferber *et al.*, 1976). [The evidence on which this statement is based is incomplete in that no information is provided on the number of workers first exposed after a reduction in the benzidine in the plant.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzidine and its dihydrochloride were tested in mice, rats and hamsters by oral administration, in mice and rats by subcutaneous administration and in rats by inhalation and intraperitoneally. Following its oral administration to mice of different strains, both sexes, newborn and adult, and following its subcutaneous administration, it significantly increased the incidence of liver-cell tumours (benign and malignant). In female rats, it markedly increased the incidence of mammary tumours; and in male and female hamsters, it increased the incidence of liver tumours following its oral administration. The subcutaneous administration of benzidine or its sulphate to rats produced a high incidence of Zymbal-gland tumours; colonic tumours were also reported. The results of the inhalation study in rats could not be interpreted. The intraperitoneal administration of benzidine to rats resulted in a marked increase in the incidence of mammary and Zymbal-gland tumours. It was also tested in dogs by oral administration, producing bladder carcinomas. Studies in fish, rabbits and frogs could not be evaluated.

The metabolites of benzidine, *N,N'*-diacetylbenzidine and *N*-hydroxy-*N,N'*-diacetylbenzidine, produced mammary and Zymbal-gland tumours in rats following their intraperitoneal injection.

Benzidine and urine from rats fed benzidine are mutagenic to *Salmonella typhimurium* with metabolic activation. Benzidine is mutagenic to *Drosophila melanogaster*. It inhibits DNA synthesis in HeLa cells and in renal and hepatic cells in mice *in vivo*. It induces unscheduled DNA synthesis in HeLa cells and in rat hepatocytes. Benzidine transformed Syrian hamster embryo cells and was positive in the BHK21 clone-13 cell system.

The data were inadequate to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzidine or its dihydrochloride has and probably still does occur during their manufacture and conversion to derived dyes and during the use of those dyes. When benzidine is used for blood testing or to enhance fingerprints, laboratory or field workers may be exposed. Environmental exposure can occur under certain conditions, when benzidine-based dyes are converted to benzidine in streams into which dye-containing wastes have been discharged.

No data were available to assess the mutagenicity or teratogenicity of benzidine to man.

Occupational exposure to benzidine has been strongly associated with bladder cancer in numerous case reports from many countries. The association has also been observed in several epidemiological studies. In one extreme instance, all five of a group of workers continuously employed in benzidine manufacture for 15 years or more developed bladder cancer. [See also Annex: Some Aspects of Quantitative Cancer Risk Estimation.]

4.3 Evaluation[1]

There is *sufficient evidence* that benzidine is carcinogenic to mice, rats, hamsters and dogs.

There is *sufficient evidence* that benzidine is carcinogenic to man.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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para-BENZOQUINONE DIOXIME

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 105-11-3

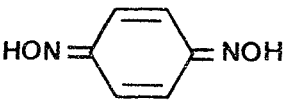
Chem. Abstr. Name: 2,5-Cyclohexadiene-1,4-dione, dioxime

IUPAC Systematic Name: *p*-Benzoquinone, dioxime

Synonyms: 1,4-Benzòquinone dioxine; *para*-quinone dioxime; *para*-quinone oxime

Trade Names: Actor Q; Dibenzo PQD; G-M-F; PQD; QDO

1.2 Structural and molecular formulae and molecular weight



$C_6H_6N_2O_2$ Mol. wt: 138.1

1.3 Chemical and physical properties of the pure substance

From Weast (1979)

(*a*) *Description*: Pale-yellow needles (from water)

(*b*) *Melting-point*: Decomposes at 240°C

(*c*) *Solubility*: Soluble in hot water

(*d*) *Conversion factor*: ppm = 0.177 x mg/m^3

1.4 Technical products and impurities

para-Benzoquinone dioxime is available in the US as a grey to dark-brown aqueous press-cake, with the following specifications: 93% minimal purity; 55% minimal solids; 7% maximal quinone monoxime; 5% maximal acetone insolubles; 1% maximal ash; and minimal decomposition point, 223°C. It is also available in an oiled grade containing 2-4% oil (Martin Marietta Chemicals, 1973).

In Japan, *para*-benzoquinone dioxime is available as a dark-brown powder which decomposes at above 215°C.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

para-Benzoquinone dioxime was first prepared in 1887 by the reaction of hydroxylamine hydrochloride with *para*-benzoquinone. It can be prepared by the reaction of hydroxylamine hydrochloride with *para*-benzoquinone monoxime (*para*-nitrosophenol), *para*-benzoquinone imide oxime (*para*-nitrosoaniline), *para*-nitrosodiphenylamine, or *para*-nitroso-*N*-benzylaniline (Prager *et al.*, 1925). *para*-Benzoquinone dioxime is made commercially in the US and Japan by the reaction of hydroxylamine hydrochloride or sulphate with *para*-benzoquinone monoxime (*para*-nitrosophenol). It was first produced in Japan in 1935 and in the US in 1941-1943 (US Tariff Commission, 1945).

Although commercial production of *para*-benzoquinone dioxime in the US was last reported by one company in 1977 (US International Trade Commission, 1978), two companies are believed currently to produce commercial quantities of the chemical. US imports of *para*-benzoquinone dioxime through the principal customs districts were last reported in 1975, when they amounted to 68 kg (US International Trade Commission, 1977).

This compound is believed to be produced commercially by one company each in France and the UK.

An estimated 50 thousand kg of *para*-benzoquinone dioxime were made in 1980 by the two Japanese producing companies. Japanese exports of this chemical are believed to be negligible.

(*b*) *Use*

para-Benzoquinone dioxime is believed to be used principally as a vulcanizing agent [see also IARC, 1982] and, to a lesser degree, as a chemical intermediate.

The major use appears to be in the curing of butyl rubber (a copolymer of isobutylene and isoprene), although it is also used in the curing of EPDM elastomers (terpolymers of ethyl, propylene and a small amount of nonconjugated diolefin) (Lord Corporation, 1979). It has been reported to be useful for the curing of polysulphide elastomers made from the reaction of an organic dihalide, such as ethylene dichloride, with sodium polysulphide (Bill Communications, Inc., 1981).

para-Benzoquinone dioxime is used as a chemical intermediate for the commercial production of at least two chemicals: poly-*para*-dinitrosobenzene, which is used as a rubber conditioner, particularly in butyl rubber; and dibenzoyl-*para*-quinone dioxime, which is used as a vulcanizing agent in applications similar to those of *para*-benzoquinone dioxime when a delayed reaction is required (Bill Communications, Inc., 1981).

In Japan, *para*-benzoquinone dioxime is used as a vulcanizing agent, primarily for butyl rubber.

para-Benzoquinone dioxime is approved by the US Food and Drug Administration (1980) for use as a vulcanizing agent in rubber articles intended for repeated use with food products.

2.2 Occurrence

para-Benzoquinone dioxime is not known to occur as such in Nature. No data on its occurrence in the environment were available to the Working Group.

2.3 Analysis

No information on methods for the analysis of *para*-benzoquinone dioxime were available to the Working Group.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks old, were fed diets containing 1500 or 750 mg/kg [ppm] *para*-benzoquinone dioxime. (The compound was obtained in two batches, which exhibited greatly different ultra-violet absorption spectra,

for which specific identification was not achieved; chromatographic analyses showed the presence of several substances, and a maximum possible purity of 90% was established for one batch by elemental analysis.) Groups of 18 male and 20 female control animals were fed the same diet without *para*-benzoquinone dioxime. The compound was administered for 104 weeks. Animals were sacrificed one week after cessation of treatment. By the end of the experiment, 86% of males fed the high dose, 88% fed the low dose and 67% of the controls and 78% of females fed the high dose, 64% fed the low dose and 80% of the controls were still alive. No significant increase in the occurrence of neoplasms over that seen in the control groups was noted in the test groups (National Cancer Institute, 1979).

Rat: Groups of 50 male and 50 female Fischer 344 rats, 6 weeks old, were fed diets containing 750 or 375 mg/kg [ppm] *para*-benzoquinone dioxime (purity as specified above) for 104 weeks, followed by one week of diet minus the test agent. Twenty control animals of each sex were fed the same diet without *para*-benzoquinone dioxime. By the end of the experiment, 70% of males fed the high dose, 78% fed the low dose and 85% of the controls, and 78% of females fed the high dose, 78% fed the low dose and 90% of the controls were still alive. Of female rats fed the higher dose, 4/44 had transitional-cell papillomas of the bladder and 7/44 had transitional-cell carcinomas of that organ ($p = 0.012$ for the two combined). Although not considered to be statistically significant, 2/50 of the males fed the higher dose demonstrated tubular-cell adenomas of the kidney and 1/50 had tubular-cell adenocarcinomas of the kidney. No papilloma or neoplasm of the bladder was seen in concurrent controls (National Cancer Institute, 1979). [The Working Group noted that the report makes no mention of whether bladder or renal calculi were looked for.]

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The oral LD_{50} of *para*-benzoquinone dioxime in mice was reported to be 1542 mg/kg bw (experimental conditions were not described) (Vorobieva and Mezentseva, 1964). The oral LD_{50} in rats was 464 mg/kg bw (National Cancer Institute, 1979).

In a 15-day inhalation study in rats exposed to 340-400 mg/m^3 [60-71 ppm; particle size not given] for two hours/day, neither local nor systemic effects were produced. An experiment with rabbits given 20 mg/kg bw every second day for two months and every day for two further months by gavage revealed a decrease in prothrombin activity and an increase in serum alkaline phosphatase and aldolase, without gross signs of toxicity (Vorobieva and Mezentseva, 1964).

In seven-week subchronic feeding studies, male and female Fischer 344 rats and $B6C3F_1$ mice were fed diets containing up to 10 000 and 14 700 mg/kg [ppm] *para*-benzoquinone dioxime, respectively. In rats, there was an increase in mortality in the groups fed 10 000 ppm and 6800 ppm and a dose-dependent decrease in body weight gain at doses down to 680 ppm, but no other clinical abnormalities (National Cancer Institute, 1979).

In chronic feeding studies with dietary levels of 375 and 750 mg/kg [ppm] in rats and 750 and 1500 mg/kg in mice, there was a slight increase in chronic inflammation and epithelial hyperplasia in the kidney in mice and rats, haemosiderosis of the spleen in rats of both sexes, and minimal inflammatory responses of the lungs of mice (National Cancer Institute, 1979). [The site of epithelial hyperplasia in the kidney was not specified.]

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

No data were available to the Working Group.

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

para-Benzoquinone dioxime was tested for carcinogenicity at two dose levels in mice and rats by oral administration. No significant increase in the number of neoplasms was observed in male rats; but in females given the high dose, a significant increase in the number of transitional-cell papillomas and carcinomas of the urinary bladder occurred. In mice, no carcinogenic effect was observed.

No data were available to assess the mutagenicity or teratogenicity of this compound.

4.2 Human data

Occupational exposure to *para*-benzoquinone dioxime probably occurs during its manufacture, its use as a rubber vulcanizing agent and its conversion to chemical derivatives.

No data were available to assess the mutagenicity or teratogenicity of this compund to man.

No case report or epidemiological study was available to the Working Group.

4.3 Evaluation[1]

There is *limited evidence* that *para*-benzoquinone dioxime is carcinogenic to rats.

No case report or epidemiological study was available.

No evaluation could be made of the carcinogenicity of *para*-benzoquinone dioxime to man.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

5. References

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BUTYL BENZYL PHTHALATE

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 85-68-7

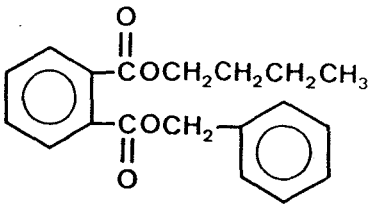
Chem. Abstr. Name: 1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester

IUPAC Systematic Name: Benzyl butyl phthalate

Synonyms: BBP; benzyl *n*-butyl phthalate; butylbenzyl phthalate; *n*-butyl benzyl phthalate; butyl phenylmethyl 1,2-benzenedicarboxylate; phthalic acid, benzyl butyl ester

Trade Names: Palatinol BB; Santicizer 160; Sicol 160; Unimoll BB

1.2 Structural and molecular formulae and molecular weight



$C_{19}H_{20}O_4$ Mol. wt: 312.4

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Clear, oily liquid (Hawley, 1981)

(*b*) *Boiling-point*: 377°C (Versar, Inc. and SRI International, Inc., 1979)

(*c*) *Melting-point*: -35°C (Versar Inc. and SRI International, Inc., 1979)

(*d*) *Density*: d_{25}^{25} 1.113-1.121 (Hawley, 1981)

(*e*) *Solubility*: 2.9 mg/l in water (Versar, Inc. and SRI International, Inc., 1979)

(*f*) *Conversion factor*: ppm = 0.782 x mg/m^3

1.4 Technical products and impurities

Butyl benzyl phthalate is available in the US as a clear, oily liquid with the following specifications: refractive index (25°C), 1.535-1.540; specific gravity (25°/25°C), 1.115-1.123; maximal acidity, 0.37 mEq/100 g; and maximal moisture, 0.15% (Monsanto Co., undated).

In Japan, butyl benzyl phthalate has the following specifications: specific gravity (20°/20°C), 1.116-1.122; maximal acid value, 0.2; and maximal weight loss on heating (3 h at 125°C), 0.2%.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Butyl benzyl phthalate was first synthesized by the reaction of the monobutyl ester of phthalic acid with benzyl chloride in neutral aqueous or alcoholic solution (Reid, 1925). Commercial production in the US is based on the same process with the monobutyl ester produced by the reaction of phthalic anhydride with *n*-butyl alcohol in the presence of an acidic catalyst. In Japan, the monobutyl ester is treated with benzyl alcohol in the presence of an acid catalyst.

Butyl benzyl phthalate has been produced commercially in the US since 1946 (US Tariff Commission, 1948) and in Japan since about 1955. It is currently produced commercially by only one US company, the production of which in 1979 is estimated to have been 68 million kg.

Data on imports of butyl benzyl phthalate through the principal US customs districts were last reported in 1974, when 661 thousand kg were imported (US International Trade Commission, 1976). US exports of this chemical in 1978 are estimated to have been approximately 9 million kg.

Butyl benzyl phthalate is believed to be produced by two companies each in the Federal Republic of Germany and the UK and by one company in Belgium.

About 3 million kg of this compound were produced by the two Japanese producing companies in 1979.

A factory manufacturing butyl benzyl phthalate is believed to have started production in Brazil in 1980.

(*b*) *Use*

Butyl benzyl phthalate is used exclusively as a plasticizer (generally at levels of 50-75 parts per 100 parts of resin). Over half of that used goes into polyvinyl chloride from

which vinyl floor tiles are made. The next most important use is in polyvinyl acetate emulsions used as adhesives, e.g., in the packaging industry. Other polymers that can be plasticized with butyl benzyl phthalate include acrylic resins, ethyl cellulose, polyvinyl formal and polyvinyl butyral (Monsanto Co., undated).

Virtually all of the butyl benzyl phthalate used in western Europe and Japan is in vinyl floor tile manufacture. Minor quantities have been used in Japan in coatings.

The US Environmental Protection Agency (EPA) (1980) has identified butyl benzyl phthalate as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme.

As part of the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of butyl benzyl phthalate are subject to a variety of labelling, packaging, quantity and shipping regulations consistent with its designation as a hazardous material.

Butyl benzyl phthalate has been approved by the US Food and Drug Administration (1980) as a component of the following materials used in contact with food products: adhesives in articles used in packaging, transporting or holding food; paper and paperboard used in contact with dry food; cross-linked polyester resins used in articles intended for repeated use; polymeric substances used in the manufacture of articles for producing, manufacturing, packing, processing, preparing, treating, packaging, transporting or holding food.

The US Environmental Protection Agency (1981; Anon., 1981) has exempted butyl benzyl phthalate from the requirement of a tolerance when it is used as an inert ingredient in laminated dispensers for controlled release of gossyplure (a synthetic pheromone) on cotton, but only when used to disrupt the mating of the pink bollworm.

The Japanese Ministry of Health and Welfare has established three separate regulations to limit the migration of phthalate esters (such as butyl benzyl phthalate) and other substances from fats, oils and fatty foodstuffs; liquors, wines and alcoholic beverages; and other foodstuffs (Omori, 1976).

2.2 Occurrence

(*a*) *Natural occurrence*

Butyl benzyl phthalate has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

It has been estimated that over 66 000 workers in the US are potentially exposed to butyl benzyl phthalate (National Institute for Occupational Safety and Health, 1980).

(*c*) *Water and sediments*

Butyl benzyl phthalate has been identified in US drinking-water, surface water (Table 1), well and ground water and industrial effluents (Shackelford and Keith, 1976).

Table 1. Concentrations of butyl benzyl phthalate found in US drinking- and surface water

Concentration (mg/l)	Type of water	Location	Reference
0.08-1.8	Drinking	New Orleans, Louisiana	Keith *et al.* (1976)
0.3-1	Delaware River	New Jersey and Pennsylvania	Sheldon and Hites (1978)
0.3-0.6	Delaware River	Philadelphia, Pennsylvania	Sheldon and Hites (1978)
0.1	Drinking		
0.25	Waukegan Harbor	Illinois	Gledhill *et al.* (1980)
0.23	Waukegan Creek		
0.47	Illinois River		
0.43	Saginaw River	Michigan	
0.38	Meramec River	Missouri	
0.2-0.25	Missouri River		
0.3-2.4	Mississippi River		

Butyl benzyl phthalate has also been identified in industrial effluents. A concentration of 40 μg/l was found in industrial effluents near Philadelphia, Pennsylvania. These effluents and others are treated at a sewage treatment plant which discharges into the Delaware River; and effluents from the treatment plant were found to contain 100 μg/l butyl benzyl phthalate (Sheldon and Hites, 1979).

This compound has also been reported in sediment samples taken from the Saginaw River in Michigan at concentrations of 400-567 ng/g. Missouri River sediments were found to contain 100 ng/g (Gledhill *et al.*, 1980).

2.3 Analysis

Typical methods for the analysis of butyl benzyl phthalate are summarized in Table 2.

Table 2. Analytical methods for the analysis of butyl benzyl phthalate

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Water and sediment	Extract with dichloromethane; pass extract through layers of sodium chloride and sodium sulphate; concentrate	GC/MS	water, 0.2 μg/l sediment, 100 ng/kg	Sheldon and Hites (1978)
Water	Adsorb onto carbon; extract with chloroform; concentrate under vacuum	GC/MS	not given	Keith *et al.* (1976)
	Add dichloromethane; acidify with hydrochloric acid; extract with dichloromethane	GC/MS	not given	Sheldon and Hites (1978)

a Abbreviation: GC/MS, gas chromatography/mass spectrometry

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Three groups of 50 male and 50 female B6C3F$_1$ mice, 5-6 weeks old at the start of the experiment, were fed diets containing 0, 6000 or 12 000 mg/kg [ppm] butyl benzyl phthalate (maximum purity, 97.2%; with at least 10 unidentified impurities found by gas-liquid chromatography) for 103 weeks. The experiment ended at 105-106 weeks, at which time 84-88% of males and 70-72% of females were still alive. The incidences and types of tumours observed were comparable in treated and control animals (National Toxicology Program, 1981).

Rat: Two groups of 50 male and 50 female 5-week-old Fischer 344 rats were fed diets containing 6000 or 12 000 mg/kg [ppm] butyl benzyl phthalate (same sample as above) for 28 weeks (males) and 103 weeks (females). Another group of 50 animals per sex were used as matched controls. Male rats in both dosed groups began to die at 14 weeks, and by 29-30 weeks all of them were killed without further histological examination. Surviving female rats (31/50 controls and 29/50 and 32/50 treated animals) were killed at 105-106 weeks. An increased incidence of myelomonocytic leukaemia was observed in high-dose females (7/49 controls, 7/49 low-dose and 18/50 high-dose). The incidences and types of other tumours were comparable in treated and control groups (National Toxicology Program, 1981). [The Working Group noted that the mean incidence of these tumours in historical controls was 11% (range 8-15%) in females and 17% (range 9-24%) in males (Goodman *et al.*, 1979; National Toxicology Program, 1981; Tarone *et al.*, 1981).]

(*b*) *Intraperitoneal administration*

Mouse: Three groups of 20 male A/St mice, 6-8 weeks old, were given 24 i.p. injections (three times weekly during six weeks) of 160, 400 or 800 mg/kg bw butyl benzyl phthalate (reagent grade, >95% pure, with unspecified purities) in tricaprylin. An appropriate control group was injected with tricaprylin. Mice were killed 18 weeks after the last injection and analysed for the presence of lung tumours: 0.10 to 0.25 lung tumours/mouse were found in treated animals and 0.39 in vehicle controls. These differences were not significant, and no dose-response relationship was seen (Theiss *et al.*, 1977). [The Working Group noted that a negative result in this experimental model cannot be taken as evidence of the non-carcinogenicity of the compound.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The LD_{50} values for a single oral dose of butyl benzyl phthalate in corn oil have been reported as 2.33 g/kg bw for Fischer 344 rats of both sexes and 4.17 for female and 6.16 g/kg bw for male B6C3F$_1$ mice (National Toxicology Program, 1981).

It was reported in an abstract that dogs (sex and number unspecified) ingesting diets containing 1, 2 or 5% (w/w) butyl benzyl phthalate for 90 days exhibited no alterations in urinary or haematological parameters and no gross or histopathological effects (Hammond, 1981).

Fischer 344 rats fed diets containing 0-100 000 mg/kg [ppm] and B6C3F_1 mice fed 0-25 000 mg/kg [ppm] butyl benzyl phthalate for 14 days or 0-25 000 mg/kg for 90 days showed no evidence of compound-related mortality. In the 14-day studies, body weight gain of male and female rats was depressed with doses of 25 000 mg/kg and more. Testicular degeneration was observed in male rats fed 50 000 or 100 000 mg/kg; and thymic atrophy was observed in all rats given 100 000 mg/kg. In the 90-day studies, male rats fed 25 000 mg/kg had depressed body weight gain and testicular degeneration, but female rats were unaffected. Neither gross not histopathological effects were observed in mice in either study (National Toxicology Program, 1981).

A concentration of 1.8×10^{-4}M butyl benzyl phthalate inhibited the growth of cultured neonatal rat cerebellar cells *in vitro*. Frank cellular toxicity was observed with concentrations of 7×10^{-4}M and more (Kasuya, 1980; Teranishi and Kasuya, 1980).

Effects on reproduction and prenatal toxicity

In the one available study, 0.05 ml undiluted butyl benzyl phthalate (0.05 g) produced no malformation in 17 chicks when injected into 32 fertilized hens' eggs (Bower *et al.*, 1970).

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

Butyl benzyl phthalate (30 mg/plate) was reported to be non-mutagenic in *Escherichia coli*, and negative in recombination repair assays with *E. coli* and *Bacillus subtilis* (Omori, 1976). Doses of up to 10 μl/plate butyl benzyl phthalate (Santicizer 160) were not mutagenic to *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100, nor to *Saccharomyces cerevisiae* strain D4, in the presence or absence of a rat liver microsomal preparation (Anon., 1976). Santicizer 160 did not induce forward mutations at the TK locus of L5178Y mouse lymphoma cells (Anon., 1977).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Butyl benzyl phthalate was tested in mice and female rats by oral administration and in male mice by intraperitoneal injection. A somewhat higher incidence of monocytic leukaemias was observed in female rats. In mice, no increased incidence of tumours was observed.

Butyl benzyl phthalate was not mutagenic for *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium* or *Saccharomyces cerevisiae* or in the mouse lymphoma assay.

No data were available to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to butyl benzyl phthalate has and probably still does occur during its manufacture, its use as a plasticizer and in the further processing or use of plasticized products containing it.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study was available to the Working Group.

4.3 Evaluation

The available studies were inadequate to evaluate the carcinogenicity of butyl benzyl phthalate to mice and rats.

No case report or epidemiological study was available.

No evaluation could be made of the carcinogenic risk to man of butyl benzyl phthalate.

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4,4′-DIAMINODIPHENYL ETHER

This substance was considered by a previous Working Group, in June 1977 (IARC, 1978). Since that time, new data have become available, and these have been incorporated into the monograph and have been taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

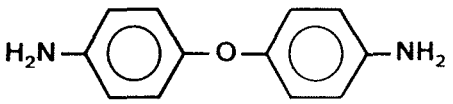
Chem. Abstr. Services Reg. No.: 101-80-4

Chem. Abstr. Name: Benzenamine, 4,4′-oxybis-

IUPAC Systematic Name: Aniline, 4.4′-oxydi-

Synonyms: 4-Aminophenyl ether; *para*-aminophenyl ether; bis(4-aminophenyl) ether; bis(*para*-aminophenyl)ether; 4,4′-diaminobiphenyl ether; diaminodiphenyl ether; *para*,*para*′-diaminodiphenyl ether; 4,4′-diaminodiphenyl oxide; 4,4′-diaminophenyl ether; 4,4′-diaminophenyl oxide; oxybis(4-aminobenzene); 4,4′-oxybis(aniline); *para*,*para*′-oxybis(aniline); 4,4′-oxybis(benzenamine); *para*,*para*′-oxydianiline; oxydianiline; 4,4′-oxydiphenylamine; oxydi-*para*-phenylenediamine

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2O$ Mol. wt: 200.2

1.3 Chemical and physical properties of the pure substance

From Dean (1973), unless otherwise specified

(*a*) *Description*: Colourless crystals

(*b*) *Boiling-point*: >300°C (Kazinik *et al.*, 1971a)

(*c*) *Melting-point*: 186-187°C

(*d*) *Solubility*: Insoluble in water, benzene, carbon tetrachloride and ethanol; soluble in acetone

(*e*) *Conversion factor*: ppm = 0.122 x mg/m^3

1.4 Technical products and impurities

4,4′-Diaminodiphenyl ether is available in the US as a beige to dark-brown powder, crystals or granules, with the following specifications: minimal purity, 98%; 0.1% maximal loss on drying; and 0.1% maximal residue on ignition (Mallinckrodt, Inc., 1980). It is also available as a light-pink to white powder, with the following specifications: minimal melting-point: 180.0°C; 0.25% maximal volatile matter; 0.1% maximal acid-insoluble impurities; and a maximum of 50 mg/kg iron (E.I. du Pont de Nemours and Co., Inc., 1978).

In Japan, 4,4′-diaminodiphenyl ether is available as a white or colourless powder.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

4,4′-Diaminodiphenyl ether was first prepared in 1896 by the reduction of 4,4′-dinitrodiphenyl ether with tin and hydrochloric acid (Prager *et al.*, 1930). Other methods are variations of the hydrogenation of 4,4′-dinitrodiphenyl ether, using various catalytic systems (Jamieson *et al.*, 1973; Spiegler, 1965); such catalytic hydrogenations are believed to be used for commercial production.

4,4′-Diaminodiphenyl ether has been produced commercially in the US since 1959 (US Tariff Commission, 1960) and in Japan since 1967. Two US companies now produce it, in undisclosed quantities (see preamble, section 8(*b*)(ii)). Imports through the principal US customs districts in 1980 amounted to 22 thousand kg (US International Trade Commission, 1981). The single Japanese company making 4,4′-diaminodiphenyl ether is believed to have produced approximately 20 thousand kg in 1979.

(*b*) *Use*

4,4′-Diaminodiphenyl ether is one of several aromatic diamines that can be used to produce straight polyimide resins. The composition of these special resins and the identity of the raw materials used to make the various commercial products are not readily available. However, 4,4′-diaminodiphenyl ether appears to be one of the few aromatic diamines that produce outstanding high-temperature resistance in such products and is

believed to be used widely. For production of a typical straight polyimide resin, the diamine is reacted with an aromatic dianhydride, e.g., pyromellitic dianhydride, to form a poly(amide-acid), which is soluble in highly polar solvents such as dimethyl formamide. Evaporation of the solvent and dehydration at high temperature are then carried out to produce the desired insoluble, infusible polyimide resin. Such straight polyimide resins are used in a variety of products designed to resist high temperatures, such as wire enamels, coatings, film, adhesives, insulating varnishes, coated fabrics and machined parts.

4,4′-Diaminodiphenyl ether may also be used as a raw material in the production of two other, closely related high-temperature resins: poly(amide-imide) resins and poly(esterimide) resins. The heat-resistance of these resins is generally not as good as that of the straight polyimide resins, but they are usually easier to process. The poly(amide-imide) resins find application in such products as wire enamels and coatings, and the poly(esterimide) resins are used in wire enamels.

The total amount of these high-temperature polyimide-type resins used in the US in 1979 is estimated to have been 2.3 million kg, with polyimide resins (both straight and unsaturated) representing approximately 40% of the total.

4,4′-Diaminodiphenyl ether can also be used as an intermediate in the manufacture of epoxy resins and adhesives (Mallinckrodt, Inc., 1980), but no evidence was found that it is so used commercially.

Although polyimide-type resins are produced commercially in western Europe, it is not known whether 4,4′-diaminodiphenyl ether is used in their manufacture. In Japan, 4,4′-diaminodiphenyl ether is used to produce polyimide-type resins.

2.2 Occurrence

4,4′-Diaminodiphenyl ether is not known to occur as such in Nature. No data on its occurrence in the environment were available to the Working Group.

2.3 Analysis

A method for determining the purity of 4,4′-diaminodiphenyl ether by thin-layer chromatography has been described (Eulenhoefer and Bauer, 1969). Thin-layer chromatography using four different solvent systems has been used to separate and identify a group of aromatic diamines, including 4,4′-diaminodiphenyl ether, and *N*-benzamides (products of thermal degradation of polyamindes *in vacuo*) (Krasnov *et al.*, 1970).

A colorimetric method for detecting as little as 0.2 mg 4,4′-diaminodiphenyl ether involves reaction with formaldehyde in formic acid azomethine (Shemyakin and Zelenina, 1968).

Paper chromatography has been used to separate and identify a group of aromatic 4,4′-diamines, including 4,4′-diaminodiphenyl ether (Gasparic and Snobl, 1971).

Gas chromatography with flame ionization detection has been used to separate and identify one group of high-boiling aromatic amines, including 4,4′-diaminodiphenyl ether, with an error of not more than 10-12% (Kazinik *et al.*, 1971a) and another group with an error of not more than 5.8% (Kazinik *et al.*, 1971b).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Three groups of 50 male and 50 female B6C3F$_1$ mice, 6 weeks of age, were fed diets containing 150, 300 or 800 mg/kg [ppm] 4,4′-diaminodiphenyl ether (minimum purity, 98.9%, with three trace impurities) for 103 weeks. An equal number of untreated mice of both sexes were used as controls. All surviving animals (70% or more) were killed at 104 to 105 weeks. Tumours that appeared statistically to be related to treatment were adenomas of the Harderian gland in animals of both sexes: females, 2/50 controls, and 15/50, 14/50 and 12/50 given the low, mid and high doses, respectively; males, 1/50 controls and 17/50, 13/49 and 17/50 given the low, mid and high doses, respectively ($p < 0.001$). Hepatocellular adenomas or carcinomas occurred in 40/50 low-dose males (29/50 controls) ($p = 0.015$) and in 29/50 high-dose female mice (8/50 controls; $p < 0.001$). Follicular-cell adenomas of the thyroid occurred in 7/48 of the high-dose females ($p = 0.007$). Other tumours, including adenomas of the pituitary and haemangiomas of the circulatory system, occurred more frequently in high-dose males than in controls, but the increased incidences were not statistically significant (National Cancer Institute, 1980).

In a less well controlled study, 16 male and 24 female CC57W mice (age unspecified) received diets containing 5 mg/animal 4,4′-diaminodiphenyl ether in 0.2 ml sunflower oil on five days per week for six weeks, and later by oral gavage (total dose, 440 mg/animal); animals were observed up to 472 days. Among 14 mice still alive at the appearance of the first tumour (212 days), 8 had developed 10 tumours (6 lymphomas, 2 haemangiomas of the ovaries and 2 lung adenomas). The historical incidence of lymphomas in untreated mice of that strain was 6.8% (Dzhioev, 1975). [The Working Group noted that only historical controls were used.]

Rat: 4,4′-Diaminodiphenyl ether (purity and impurities unspecified) was administered by stomach tube to 20 female 40-day-old Sprague-Dawley rats; 10 equal doses of 40 mg/rat, which was the maximum tolerated dose, were given at three-day intervals. The experiment was terminated at nine months. No increase in tumour incidence was observed in the 11 animals that were autopsied (Griswold *et al.*, 1968). [The Working Group noted the short duration of the study.]

A group of 15 male and 33 female colony-bred rats received diets containing 25 mg/animal 4,4′-diaminodiphenyl ether in 0.5 ml sunflower oil five times per week for 1.5-9 months and later by oral gavage (total dose, 4.12 g/animal) for up to 826 days. Of 16 rats

still alive at the appearance of the first tumour (540 days), 7 had developed 9 tumours (1 kidney carcinoma, 3 reticulum-cell sarcomas, 1 liver fibrosarcoma, 1 neurogenic sarcoma, 2 seminomas and 1 mammary gland fibroadenoma). The authors reported that testicular and kidney tumours were not observed in control rats of this stock (Dzhioev, 1975).

Three groups of 50 male and 50 female five-week-old Fischer 344 rats were given diets containing 200, 400 or 500 mg/kg [ppm] 4,4′-diaminodiphenyl ether (minimum purity, 98.9%, with three trace impurities) for 103 weeks. The controls consisted of 50 male and 50 female untreated rats. Surviving animals were killed at 105-106 weeks. Significant increases in the incidences of hepatocellular carcinomas were seen in treated rats: males: 0/50, 4/50, 23/50 and 22/50 in the control, low-, mid- and high-dose groups (positive trend: $p < 0.001$); females: 0/50, 0/49, 4/50 and 6/50 (positive trend: $p = 0.002$), respectively. Neoplastic nodules of the liver occurred in males: 1/50, 9/50, 18/50 and 17/50 (positive trend: $p < 0.001$); females: 3/50, 0/49, 4/50 and 6/50 (positive trend: $p = 0.002$), respectively. Neoplastic nodules of the liver occurred in males: 1/50, 9/50, 18/50 and 17/50 (positive trend: $p < 0.001$); females: 3/50, 0/49, 20/50 and 11/50 (positive trend: $p < 0.001$). An increased incidence of thyroid follicular-cell adenomas or carcinomas also occurred: males: 1/46, 6/47, 17/46 and 28/50 (positive trend: $p < 0.001$); females: 0/49, 4/48, 29/48 and 23/50 (positive trend: $p < 0.001$). The incidences of tumours at other sites were not increased (National Cancer Institute, 1980).

In a preliminary report available as an abstract, male and female Sprague-Dawley rats [number and age at start not specified] were fed a diet containing 200 or 400 mg/kg [ppm] 4,4′-diaminodiphenyl ether [purity and impurities unspecified] for two years. A control group of rats was available. Statistically significant increased incidences of uterine carcinomas and interstitial-cell testicular tumours were found in females receiving the higher level and in all treated males, respectively (Kaplan *et al.*, 1980).

(*b*) *Subcutaneous and/or intramuscular administration*

Mouse: A group of 15 male and 18 female CC57W mice received s.c. injections of 5 mg/animal 4,4′-diaminodiphenyl ether in 0.2 ml sunflower oil weekly (total dose, 175 mg/animal); animals were observed for up to 316 days. Of 9 mice alive at the appearance of the first tumour (271 days), 3 developed 3 tumours (2 lymphomas and 1 lung adenoma). The incidence of lymphomas in untreated mice of this strain was 6.8% (Dzhioev, 1975). [The Working Group noted that only historical controls were used.]

Rat: A group of 30 male and 32 female, white, colony-bred rats received s.c. injections of 25 mg/animal 4,4′-diaminodiphenyl ether in 0.5 ml sunflower oil weekly (total dose, 2 g/animal); animals were observed for up to 949 days. Among 39 rats alive at the appearance of the first tumour (529 days), 7 developed 7 tumours (2 lymphomas, 1 reticulum-cell sarcoma, 1 liver fibrosarcoma, 1 carcinoma of the kidney and 2 mammary gland fibroadenomas) (Dzhioev, 1975). [The Working Group noted that no controls were used.]

A group of 20 male and 20 female Wistar rats [age at start not specified] were given s.c. injections of 4,4′-diaminodiphenyl ether [purity and impurities unspecified] as single weekly doses of 100-300 mg/kg bw for up to 670 days and observed for life. The dose was reduced when signs of toxicity occurred. The total dose administered was 14.4 g/kg bw. A matched control group of 25 male and 25 female rats was treated with saline and observed for life. The median survival time was 860 days for treated and 907 days for control animals. Of 40 treated rats, 10 developed malignant liver tumours and 12, benign

liver tumours. [The sex of the animals was not specified and no details of histological types were given.] None of 50 control animals had liver tumours (Steinhoff, 1977). [The Working Group noted that no details of tumours other than those in the liver were given and that the exact dosage schedule was unclear.]

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The i.p. LD_{50} values were reported to be 300 $\pm$ 25 mg/kg bw in mice and 365 $\pm$ 25 mg/kg bw in rats; intragastric LD_{50} values of 685 $\pm$ 42 mg/kg bw were reported in mice and 725 $\pm$ 50 mg/kg bw in rats. The animals were observed for 15 days after treatment (Lapik *et al.*, 1968).

After administration of 3000 mg/kg [ppm] 4,4′-diaminodiphenyl ether in the diet for 14 days, 1/5 male and 0/5 female rats and 2/5 male and 4/5 female mice were still alive (National Cancer Institute, 1980).

In mice treated with 5 mg and rats given 25 mg 4,4′-diaminodiphenyl ether orally or subcutaneously 1-5 times weekly for several weeks, both liver and kidney injuries were produced. The nephrotoxic effect was more prevalent in animals treated subcutaneously than in those treated orally (Dzhioev, 1975).

Administration of 72.5 mg/kg bw per day 4,4′-diaminodiphenyl ether orally to rats for 15 days was reported to decrease the haemoglobin concentration of blood and to cause an increase in splenic and adrenal weight. Exposure of rats to 4.2-4.8 mg/m^3 of 4,4′-diaminodiphenyl 'dust' (particle size, 1-3 μ) [no analytical methodology was reported] for four hours per day for four months produced a decrease in the haemoglobin concentration of the blood, but no changes in the weights or histological appearance of individual organs (Lapik *et al.*, 1968).

Oral administration of a dose of 0.25 of the LD_{50} (12 doses of 5 mg/mouse over 14 days) inhibited the growth of spontaneous mammary tumours and of transplanted tumours in mice (Boyland, 1946).

A 90-day study was conducted in male and female Fischer 344 rats and B6C3F$_1$ mice using dietary concentrations of 0-2000 mg/kg [ppm] 4,4′-diaminodiphenyl ether. Concentration-dependent retardations in body weight gain were observed in rats and mice of both sexes fed dietary levels of $\geqslant$600 mg/kg; compound-related deaths occurred in the rats given 1000 or 2000 mg/kg, but not in mice. Diffuse parenchymatous goitre, pituitary hyperplasia, seminiferous tubular degeneration and atrophy of the prostate and seminal vesicles occurred in rats and mice fed dietary concentrations of 600 (rats) or 1000 mg/kg (mice) and above. Bone-marrow hypoplasia and renal microlithiasis were also observed in rats, but the latter lesion was not concentration-dependent (Hayden *et al.*, 1978; National Cancer Institute, 1980).

A two-year feeding study was conducted in male and female Fischer 344 rats using dietary concentrations of 0, 200, 400 or 500 mg/kg [ppm] 4,4′-diaminodiphenyl ether, and

in male and female B6C3F$_1$ mice using 0, 150, 300 or 800 mg/kg. All treated rats and mice exhibited diminutions in body weight gain, but survival was reduced only among high-dose female rats. Thyroid follicular-cell hyperplasia, pituitary hyperplasia (female rats only), mineralization in the kidney, and epithelial hyperplasia in the renal pelvis occurred in rats of both sexes fed 400 or 500 mg/kg. Thyroid follicular-cell hyperplasia was also seen in male and female mice fed 800 mg/kg, while ovarian cysts were observed in female mice fed either 300 or 800 mg/kg (National Cancer Institute, 1980).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Mutagenicity and other short-term tests

4,4′-Diaminodiphenyl ether in concentrations of 50-500 μg/plate was mutagenic to *Salmonella typhimurium* strains TA98 and TA100 when tested in the presence of an exogenous metabolic activation system from Aroclor 1254-treated rats (Takemura and Shimizu, 1978; Lavoie *et al.*, 1979).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

4,4′-Diaminodiphenyl ether was tested in mice and rats by oral administration and by subcutaneous injection. In two studies in rats, it produced benign and malignant liver-cell tumours following oral or subcutaneous administration; and following its oral administration in one study, benign and malignant follicular-cell tumours of the thyroid were produced. Other studies in rats by oral and subcutaneous administration were not adequate for evaluation. In a study in mice by oral administration it produced benign and malignant liver-cell tumours in females given the high dose and in males given the low dose and adenomas of the Harderian gland in animals of both sexes.

4,4′-Diaminodiphenyl ether is mutagenic to *Salmonella typhimurium* with metabolic activation.

No data were available to assess its teratogenicity to experimental animals.

4.2 Human data

Occupational exposure to 4,4′-diaminodiphenyl ether probably occurs during its manufacture and its conversion to polyimide-type resins.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study was available to the Working Group.

4.3 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of 4,4′-diaminodiphenyl ether in mice and rats.

No case report or epidemiological study was available.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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ortho- and *para*-DICHLOROBENZENES

These compounds were considered by a previous Working Group, in June 1974 (IARC, 1974). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

ortho-*Dichlorobenzene*

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No., 95-50-1

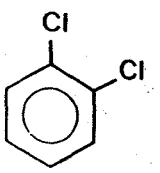
Chem. Abstr. Name.: Benzene, 1,2-dichloro-

IUPAC Systematic Name: *o*-Dichlorobenzene

Synonyms: DCB; 1,2-dichlorobenzene; ODB; ODCB; orthodichlorobenzene

Trade Names: Chloroben; Chloroden; Cloroben; Dilatin DB; Dizene; Dowtherm E; Termitkil

1.2 Structural and molecular formulae and molecular weight



$C_6H_4Cl_2$ Mol. wt: 147.0

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Colourless liquid (Verschueren, 1977)

(*b*) *Boiling-point*: 180.5°C

(*c*) *Melting-point*: -17.0°C

(*d*) *Density*: d_4^{20} 1.3048

(*e*) *Refractive index*: n_D^{20} 1.5515

(*f*) *Spectroscopy data*: λ_{max} 250, 256, 263, 270 and 277 nm (in ethanol); mass spectra and carbon-13 nuclear magnetic resonance spectra have been presented (NIH/EPA Chemical Information System, 1980).

(*g*) *Solubility*: Practically insoluble in water (145 mg/l at 25°C) (Verschueren, 1977); soluble in diethyl ether and ethanol; miscible with acetone, benzene, carbon tetrachloride and ligroin

(*h*) *Viscosity*: 1.3018 cP (Kao and Poffenberger, 1979)

(*i*) *Volatility*: Vapour pressure, 1.5 mm at 25°C (Verschueren, 1977)

(*j*) *Stability*: Stable; combustible (flash-point, 71°C) (Kao and Poffenberger, 1979)

(*k*) *Conversion factor*: ppm = 0.166 x mg/m^3

1.4 Technical products and impurities

ortho-Dichlorobenzene is available in the US as a high-purity grade, a technical grade and a special grade with additives to make the product emulsifiable. Specifications for the high-purity grade are: 98.0% minimum active ingredient; maximal distillation range, 2.5°C (including 180°C); and pH, 6.0-8.0. Specifications for the technical grade are: 80-90% active ingredient; maximal distillation range, 5°C (including 180°C); pH, 6.0-8.0; and 20% maxima of *para*- plus *meta*-dichlorobenzene (PPG Industries, Inc., undated a). The following compositions have been reported: high-purity grade, 98.0% *ortho*-dichlorobenzene, <0.2% 1,2,4-trichlorobenzene and <0.05% monochlorobenzene; technical grade, 80.0% *ortho*-dichlorobenzene, <19.0% other dichlorobenzene isomers, <1.0% trichlorobenzenes and <0.05% monochlorobenzene (Kao and Poffenberger, 1979).

In 1937, commercial *ortho*-dichlorobenzene was described as the liquor remaining after the separation of crystalline *para*-dichlorobenzene and containing: *ortho*-dichlorobenzene, 48.8%; *para*-dichlorobenzene, 28%; trichlorobenzene, 15%; monochlorobenzene, 6%; tetrachlorobenzene, 2%; and benzene, 0.2% (Cameron *et al.*, 1937).

In western Europe, *ortho*-dichlorobenzene is available from one manufacturer as a

technical product with a density of 1.248-1.257 at 20°C and containing 85% *ortho*-dichlorobenzene and approximately 15% of the *meta* and *para* isomers.

ortho-Dichlorobenzene is available in Japan as a colourless, transparent liquid with a distillation range (96% minimum) of 170-190°C.

para-*Dichlorobenzene*

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 106-46-7

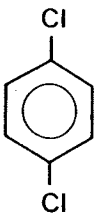
Chem. Abstr. Name: Benzene, 1,4-dichloro-

IUPAC Systematic Name: *p*-Dichlorobenzene

Synonyms: *para*-Chlorophenyl chloride; 1,4-dichlorobenzene; paradichlorobenzene; PDB; PDCB

Trade Names: Di-chloricide; Di-cloricide; Evola; Paracide; Paradi; Paradow; Para-moth; Parazene; Persia-Perazol; Santochlor

1.2 Structural and molecular formulae and molecular weight



$C_6H_4Cl_2$ Mol. wt: 147.0

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Monoclinic prisms or leaflets (from acetone)

(*b*) *Boiling-point*: 174°C

(*c*) *Melting-point*: 53.1°C

(*d*) *Density*: d_4^{20} 1.2475

(*e*) *Refractive index*: n_D^{20} 1.5285

(*f*) *Spectroscopy data*: λ_{max} 258, 266, 273 and 280 nm (in ethanol); mass spectra and carbon-13 nuclear magnetic resonance spectra have been presented (NIH/EPA Chemical Information System, 1980).

(*g*) *Solubility*: Practically insoluble in water (79 mg/l at 25°C) (Verschueren, 1977); soluble in benzene, carbon disulphide, chloroform and diethyl ether; miscible with acetone and ethanol

(*h*) *Volatility*: Vapour pressure, 1.8 mm at 30°C (Verschueren, 1977)

(*i*) *Stability*: Stable; combustible (flash-point, 67°C) (Kao and Poffenberger, 1979)

(*j*) *Conversion factor*: ppm = 0.166 x mg/m^3

1.4 Technical products and impurities

para-Dichlorobenzene is available in the US as technical grades of unusually high purity: 100% in crystalline form and 99.92% in liquid form (on an anhydrous basis). Typical properties are: freezing-point: crystals, 53.00°C, liquid, 52.93°C; residue: crystals, 8 mg/kg, liquid, 10 mg/kg; and both forms are neutral (PPG Industries, Inc., undated b). The following typical composition for *para*-dichlorobenzene has been reported: *ortho*-dichlorobenzene, <0.5%; *meta*-dichlorobenzene, <0.5% and monochlorobenzene and trichlorobenzenes, <0.1% (Kao and Poffenberger, 1979).

In western Europe, *para*-dichlorobenzene is available with a minimum purity of 99%, a maximal *ortho*-dichlorobenzene content of 1% and a minimal fusion-point of 50°C.

para-Dichlorobenzene is available in Japan as a white, flaked product, with a minimal freezing-point of 52.9°C.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

ortho-*Dichlorobenzene*

(*a*) *Production*

Chlorobenzenes, including *ortho*-dichlorobenzene, were first synthesized in the mid-1800s; direct chlorination of benzene was reported in 1905, and commercial production was started in England in 1909 and in the US in 1915. *ortho*-Dichlorobenzene is now

produced commercially by the direct chlorination of benzene in the liquid phase in the presence of a Friedel-Crafts catalyst (usually ferric chloride) and fractionation of the resulting mixture of chlorinated benzenes (Kao and Poffenberger, 1979).

Total US production of *ortho*-dichlorobenzene by four producers in 1979 amounted to 26 million kg. Another company reported production of an undisclosed amount of a mixture of *ortho*- and *para*-dichlorobenzenes (US International Trade Commission, 1980a). Separate production data for this mixture were last reported in 1964, when three companies reported a total production of 5.9 million kg (US Tariff Commission, 1965).

In 1979, imports of *ortho*-dichlorobenzene through the principal US customs districts totalled only 913 kg (US International Trade Commission, 1980b), down dramatically from the 403 thousand kg imported in 1976 (US International Trade Commission, 1977a). Imports of '*ortho*-dichlorobenzene, mixture' through the principal US customs districts were last reported in 1978, when 22.7 thousand kg were imported (US International Trade Commission, 1979), down sharply from the 1184 thousand kg imported in 1975 (US International Trade Commission, 1977b). Separate data on US exports of *ortho*-dichlorobenzene are not reported; however, in 1980, total US exports of all dichlorobenzenes amounted to 17.3 million kg (US Department of Commerce, 1981).

Total production capacity for *all* chlorobenzenes in western Europe is estimated to have been more than 208 million kg in 1980. Approximately half of this capacity was located in the Federal Republic of Germany, where two companies produce chlorobenzenes. Chlorobenzenes, including *ortho*-dichlorobenzene, are also produced in France (two producing companies), Italy (two) and Spain (two). Total production of *ortho*-dichlorobenzene by members of the European Economic Communities in 1979 is estimated to have been 23 million kg. Of the total production in 1978 of 179 million kg of chlorobenzenes in western Europe, approximately 29 million kg were accounted for by *ortho*-dichlorobenzene.

An estimated 13 million kg of *ortho*-dichlorobenzene were produced in 1979 by the six Japanese producing companies. Japanese exports of this chemical are believed to be negligible.

(*b*) *Use*

Use of *ortho*-dichlorobenzene in the US in 1978 is estimated to have been as follows: organic synthesis (mainly for production of 3,4-dichloroaniline), 70%; solvents for toluene diisocyanate production, 15%; miscellaneous solvent usage, 8%; dye manufacture, 4%; and other applications, 3%.

The principal derivative of *ortho*-dichlorobenzene is 3,4-dichloroaniline, a key intermediate in the production of the following herbicides: propanil (3′,4′-dichloropropionanilide), diuron [3(3,4-dichlorophenyl)-1,1-dimethylurea] and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea]. The most important of these is propanil, 4.6 million kg of which were used to control weeds in rice in the US in 1978.

Toluene diisocyanates [see IARC, 1979] are produced by phosgenation of toluenediamines in *ortho*-dichlorobenzene or monochlorobenzene. These diisocyanates are important raw materials for the manufacture of flexible foams and other polyurethane resin and elastomer products.

ortho-Dichlorobenzene is used as a solvent in the following applications: motor-oil additive formulations; paints; formulations for removing paints, greases, gums, tars, etc; engine cleaning compounds (to remove lead and carbon deposits); shoe, metal and other polishes; firearm cleaners; rust preventatives; dissolution of pitch on papermaking felts; removal of grease from leather hides and woollen pelts; and as a carrier for wood preservatives and repellents (PPG Industries, Inc., undated a). The technical-grade product is reported to be used in: emulsion combinations with cresylic acid for degreasing; shoe dyes as a penetrant; and upper cylinder lubricants (Monsanto Co., 1978). It can also be used as a reaction medium for the manufacture of three dyes (The Society of Dyers and Colourists, 1971a).

A specially purified form of *ortho*-dichlorobenzene has been used to a limited extent as a heat transfer fluid (Kao and Poffenberger, 1979). Older sources indicate that *ortho*-dichlorobenzene has been used in a variety of additional applications: as a magnetic coil coolant (Hardie, 1964); as an insecticide against termites and locust borers and for the desulphurization of illuminating gas (Windholz, 1976); and for deodorizing garbage and sewage (PPG Industries, Inc., 1970).

The principal use of *ortho*-dichlorobenzene in western Europe is as a chemical intermediate in the production of 3,4-dichloroaniline.

The estimated 13 million kg of *ortho*-dichlorobenzene used in Japan in 1979 was distributed as follows: disinfectant, 40%; pesticide intermediate, 30%; toluene diisocyanate process solvent, 23%; and other uses, 7%.

Seventeen countries have been reported to limit occupational exposure to *ortho*-dichlorobenzene by regulation or recommended guideline. These standards are listed in Table 1.

The US Environmental Protection Agency (EPA) (1979) requires that notification be given whenever discharges containing 45.4 kg or more of dichlorobenzenes are made into waterways. The EPA has also identified *ortho*-dichlorobenzene as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. Spent *ortho*-dichlorobenzene, still bottoms from the recovery of *ortho*-dichlorobenzene, the heavy ends or distillation residues from the distillation of tetrachlorobenzene in the production of 2,4,5-T, distillation or fractionating column bottoms from the production of chlorobenzenes and separated aqueous stream from the reactor product washing step in the production of chlorobenzenes are included in a list of hazardous wastes from specific sources, and dichlorobenzenes are identified among the hazardous constituents present in these wastes (US Environmental Protection Agency, 1980a,b, 1981).

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of *ortho*-dichlorobenzene are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

Table 1. National occupational exposure limits for *ortho*-dichlorobenzene[a]

Country	Year	Concentration mg/m³	ppm	Interpretation[b]	Status
Australia	1978	300	50	Ceiling	Guideline
Belgium	1978	300	50	Ceiling	Regulation
Bulgaria	1971	20	—	Ceiling	Regulation
Finland	1975	300	50	TWA	Regulation
German	1979	300	—	Maximum (30 min)	Regulation
Democratic Republic		150	—	TWA	
Federal Republic of Germany	1979	300	50	TWA	Guideline
Hungary	1974	20	—	TWA[c]	Regulation
Italy	1978	240	40	TWA	Guideline
Japan	1978	300	50	Ceiling	Guideline
The Netherlands	1978	300	50	Ceiling	Guideline
Poland	1976	20	—	Ceiling[d]	Regulation
Romania	1975	75	—	TWA	Regulation
		100	—	Maximum	
Sweden	1978	300	50	Maximum (15 min)	Guideline
Switzerland	1978	300	50	TWA	Regulation
USA					
OSHA	1980	300	50	Ceiling	Regulation
ACGIH	1981	300	50	Ceiling	Guideline
USSR	1977	20	—	Maximum[d] (30 min)	Regulation
Yugoslavia	1971	150	25	TWA	Regulation

a From American Conference of Governmental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980); US Occupational Safety and Health Administration (OSHA) (1980)
b TWA, time-weighted average
c May be exceeded five times per shift as long as average does not exceed value
d Skin irritant notation added

para-*Dichlorobenzene*

(*a*) *Production*

Chlorobenzenes, including *para*-dichlorobenzene were first synthesized in the mid-1800s; direct chlorination of benzene was reported in 1905, and commercial production was started in England in 1909 and in the US in 1915. *para*-Dichlorobenzene is now produced commercially by the direct chlorination of benzene in the liquid phase in the presence of a Friedel-Crafts catalyst (usually ferric oxide) and fractionation of the resulting mixture of chlorinated benzenes (Kao and Poffenberger, 1979).

Total US production of *para*-dichlorobenzene by five producers in 1979 amounted to 37.9 million kg. Another company reported production of an undisclosed amount of a mixture of *ortho*- and *para*-dichlorobenzenes (US International Trade Commission, 1980a). Separate production data for this mixture were last reported in 1964, when three companies reported a total production of 5.9 million kg (US Tariff Commission, 1965).

Imports of *para*-dichlorobenzene through the principal US customs districts were last reported in 1978, when they totalled 10.9 thousand kg (US International Trade Commission, 1979). Separate data on US exports of *para*-dichlorobenzene are not reported; however, in 1980, total US exports of all dichlorobenzenes amounted to 17.3 million kg (US Department of Commerce, 1981).

Total production capacity for *all* chlorobenzenes in western Europe is estimated to have been more than 208 million kg in 1980. Approximately half of this capacity was located in the Federal Republic of Germany, where two companies produce chlorobenzenes. Chlorobenzenes, including *para*-dichlorobenzene, are also produced in France (two producing companies), Italy (two) and Spain (two). Total production of *para*-dichlorobenzene by members of the European Economic Communities in 1979 is estimated to have been 28 million kg. Of the total production in 1978 of 179 million kg of chlorobenzenes in western Europe, approximately 43 million kg were accounted for by *para*-dichlorobenzene.

An estimated 27.5 million kg of *para*-dichlorobenzene were produced in 1979 by the six Japanese producing companies. Imports in 1980 totalled 8.0 million kg (mostly from the US); exports of this chemical are believed to be negligible.

(*b*) *Use*

Use of *para*-dichlorobenzene in the US in 1978 is estimated to have been as follows: space deodorant, 55%; moth control, 35%; and other applications, 10%.

Blocks of *para*-dichlorobenzene, with or without perfumes, are used for masking odours in toilets. Crystals can be used to counteract odours in garbage and other refuse. When used as a moth repellent on clothes, a level of 0.16 g/l of space has been recommended. It can also be used to control mildew and other fungi (PPG Industries, Inc., undated b). *para*-Dichlorobenzene is registered as an animal repellent and fumigant in the US, for use in combination with other materials (US Environmental Protection Agency, 1972).

para-Dichlorobenzene is used as a chemical intermediate for dyes, insecticides, pharmaceuticals and other organic chemicals. The dye intermediate, 2,5-dichloroaniline, is an important derivative (PPG Industries, Inc., undated b). Separate data for the production of the salt of 2,5-dichloroaniline (known as Azoic Diazo Component 3, Salt) were last reported in the US in 1975, when five producers had a combined production of 109 thousand kg (US International Trade Commission, 1977c). Although it was reported in 1971 that 19 dyes and pigments can be prepared from 2,5-dichloroaniline (The Society of Dyers and Colourists, 1971b,c), only seven of these have been produced in commercial quantities in the US in recent years. That most widely produced is believed to be Pigment Red 2, of which total production by seven companies in 1979 was 30 thousand kg (US International Trade Commission, 1980a).

In recent years, the production of polyphenylene sulphide resins (trade name, Ryton^{R})

has become an increasingly important application for *para*-dichlorobenzene. Because of their resistance to high temperatures, these resins are used in the electrical and electronics industries. Total US consumption in 1978 is estimated to have been 1.5 million kg (an amount that would require use of about 2 million kg of *para*-dichlorobenzene).

para-Dichlorobenzene may have had minor application as an extreme-pressure lubricant (Hardie, 1964).

The estimated 35.5 million kg of *para*-dichlorobenzene used in Japan in 1979 was distributed as follows: moth control and space doedorant, 94%; other applications, 6%.

Thirteen countries have been reported to limit occupational exposure to *para*-dichlorobenzene by regulation or recommended guideline. These standards are listed in Table 2.

Table 2. National occupational exposure limits for *para*-dichlorobenzene[a]

Country	Year	Concentration mg/m³	Concentration ppm	Interpretation[b]	Status
Australia	1978	450	75	TWA	Guideline
Belgium	1978	450	75	TWA	Regulation
Finland	1975	450	75	TWA	Regulation
German Democratic Republic	1979	600	—	Maximum (30 min)	Regulation
		200	—	TWA	
Federal Republic of Germany	1979	450	75	TWA	Guideline
Italy	1978	600[c]	100[c]	TWA	Guideline
Japan	1978	300	50	TWA	Guideline
The Netherlands	1978	450	75	TWA	Guideline
Romania	1975	75	—	TWA	Regulation
		100	—	Maximum	
Sweden	1978	450	75	Maximum (15 min)	Guideline
Switzerland	1978	450	75	TWA	Regulation
USA					
OSHA	1980	450	75	TWA	Regulation
ACGIH	1981	450	75	TWA	Guideline
		675	110	STEL	
Yugoslavia	1971	450	75	TWA	Regulation

a From American Conference of Governmental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980); US Occupational Safety and Health Administration (OSHA) (1980)
b TWA, time-weighted average; STEL, short-term exposure limit
c May be reduced to 40 ppm [240 mg/m³]

The US Environmental Protection Agency (EPA) (1979) requires that notification be given whenever discharges containing 45.4 kg or more of dichlorobenzene are made into waterways. The EPA has also identified *para*-dichlorobenzene as a toxic waste and

requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. Distillation or fractionating column bottoms from the production of chlorobenzenes and the separated aqueous stream from the reactor product washing step in the production of chlorobenzenes are included in a list of hazardous wastes from specific sources, and dichlorobenzenes are identified as among the hazardous constituents present in these wastes (US Environmental Protection Agency, 1980a,b, 1981).

As part of the US Department of Transportation (1980) Hazardous Material Regulations, shipments of *para*-dichlorobenzene are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

The US Food and Drug Administration (1980) has approved the use of polyphenylene sulphide resins as coatings or components of coatings of articles intended for repeated use in contact with food, provided the resin has a maximum residual content of 0.8 mg/kg *para*-dichlorobenzene.

2.2 Occurrence

(*a*) *Natural occurrence*

Dichlorobenzenes are not known to occur as such in Nature.

(*b*) *Occupational exposure*

The number of employees potentially exposed to dichlorobenzenes has been estimated to be as follows: *ortho*-dichlorobenzene, 10 000 (during production, processing and industrial solvent use) and 2 000 000 (for all occupational activities); and *para*-dichlorobenzene, 1 000 000 (during production, processing into space deodorants and use as an intermediate) (US Environmental Protection Agency, 1980c).

The following levels of dichlorobenzenes have been found in factories where mono- and dichlorobenzenes are manufactured: up to 4350 mg/m^3 of *para*-dichlorobenzene in a factory where it was being made or handled; 42-288 mg/m^3 near shovelling and centrifuging operations; and 108-204 mg/m^3 during pulverizing and packaging operations (US Environmental Protection Agency, 1980d). In a monochlorobenzene manufacturing plant, *para*-dichlorobenzene was detected in workplace air at levels of 32.5-52.1 mg/m^3 (Albrecht, 1980). The air in a chlorobenzene factory was found to contain 24-34 ppm [144-204 mg/m^3] *para*-dichlorobenzene; levels of *ortho*-dichlorobenzene were up to 25% of those for the *para* isomer. The air in a factory where moth cakes were made contained 9-25 ppm [54-150 mg/m^3] of *para*-dichlorobenzene; and that in an abrasive-wheel facility using *para*-dichlorobenzene in the manufacturing process was 8-11.5 ppm [48-99 mg/m^3]. Concentrations ranging from 12-550 ppm [72-3300 mg/m^3] of unspecified airborne dichlorobenzenes were found in 1940 in work areas of a *para*-dichlorobenzene plant (US Environmental Protection Agency, 1980c).

Occupational exposure to *ortho*-dichlorobenzene can occur during its use as an industrial cleaner in the automotive, trucking and aircraft industries. It has been estimated that 200 workers may be exposed to fumes in transmission shops alone (US Environmental Protection Agency, 1980c).

It has been reported that *ortho*-dichlorobenzene is a component of the gaseous emissions from the production of silicone medical tubing; concentrations in the workplace air were less than 20 mg/m^3 (US Environmental Protection Agency, 1980d).

A survey in the late 1930s of three woollen mills where *ortho*-dichlorobenzene was used as a solvent reported vapour concentrations in workplace air to be 60-1620 mg/m^3 (US Environmental Protection Agency, 1980d).

(*c*) *Air*

An estimated 5-10% of the annual US production of *ortho*-dichlorobenzene has been reported to be released into the air. Johnston *et al.* (1979) estimated that 2000 kg of *ortho*-dichlorobenzene were released into the air in 1977 by venting during its use in the US as a process solvent in the manufacture of toluene diisocyanates.

As estimated 70-90% of the annual US production of *para*-dichlorobenzene has been reported to be released into the air. In 1972, loss of *para*-dichlorobenzene to the US environment during manufacture was estimated to have been 540 thousand kg (US Environmental Protection Agency, 1980c). About 1700 thousand kg of *para*-dichlorobenzene were estimated to be released into US air from its use as a toilet bowl deodorant; 3700 thousand kg from its use as a garbage deodorant; and 7800 thousand kg from its use for moth control (Johnston *et al.*, 1979).

In 1978, air samples taken five miles from a waste disposal site in New Jersey were found to contain 0-10 μg/m^3 [0-2 ppb] *ortho*-dichlorobenzene and 0-0.5 μg/m^3 [0-0.08 ppb] *para*-dichlorobenzene. At the site of a New Jersey chemical plant, *ortho*-dichlorobenzene was detected in air at a level of 1.3 μg/m^3 [0.22 ppb]. Air samples in Birmingham, Alabama, were found to contain 0.35 μg/m^3 [0.06 ppb] *ortho*-dichlorobenzene; and an industrial cloud over Henderson, Nevada, reportedly contained a total of 1.6-33 μg/m^3 [0.3-5.5 ppb] dichlorobenzene isomers, in comparison with 0.6-5 μg/m^3 [0-1.1 ppb] in nearby Las Vegas (US Environmental Protection Agency, 1980c). Air samples taken in five cities in New Jersey in 1978 were found to contain low average concentrations of *ortho*- and *para*-dichlorobenzenes: the highest were found in Newark - 1.0 ppb [6 μg/m^3] *ortho*-dichlorobenzene and 0.66 ppb [3.96 μg/m^3] *para*-dichlorobenzene; in Rutherford, New Jersey, concentrations of the *ortho* isomer averaged 0.08 ppb [0.48 μg/m^3] in a residential area and 0.48 ppb [2.9 μg/m^3] in an industrial area; and corresponding values for the *para* isomer were 0.1 ppb [0.6 μg/m^3] and 0.41 ppb [2.4 μg/m^3] (Bozzelli and Kebbekus, 1979). Levels of *ortho*-dichlorobenzene found in aerial fallout samples taken in 1977 along the southern California coast were less than 53 ng/m^2 (US Environmental Protection Agency, 1980d).

The ambient air of central Tokyo was reported in 1975 to contain 2.7-4.2 μg/m^3 [0.9-9.4 ppb] *para*-dichlorobenzene, and air in suburban Tokyo contained 1.5-2.4 μg/m^3 [0.5-0.8 ppb] (US Environmental Protection Agency, 1980d).

The following concentrations of *para*-dichlorobenzene have been measured indoors as a result of its use as a space deodorizer or moth repellent: 315 and 1700 μg/m^3 [52 and 283 ppb] (inside a wardrobe) and 105 μg/m^3 [18 ppb] (in a bedroom) (US Environmental Protection Agency, 1980c).

(d) Water and sediments

Both *ortho-* and *para*-dichlorobenzenes have been detected in raw and finished drinking-water (Shackelford and Keith, 1976). Levels of the *ortho* isomer were 1 μg/l prior to and including 1975 and 2.5 μg/l between 1976-1977. The *para* isomer was found at a concentration of I μg/l prior to 1975, 0.5 μg/l in 1975 and 0.07-2.0 μg/l in 1976-1977 (US Environmental Protection Agency, 1980d).

Ground-water in Miami, Florida, has been found to contain 1 μg/l *ortho-* and 0.5 μg/l *para*-dichlorobenzene (US Environmental Protection Agency, 1980d). Ground-water in The Netherlands contained 0.3-3 μg/l *para*-dichlorobenzene (Zoeteman *et al.*, 1980).

Water from the Rhine River in the Federal Republic of Germany, sampled during 1976-1977, contained *ortho*-dichlorobenzene at levels in the range of 0.2-10 μg/l (Borneff *et al.*, 1979). Zoeteman *et al.* (1980) reported average concentrations of 0.8-1.1 μg/l *ortho*-dichlorobenzene in the Rhine River when measured 1-12 months after infiltration of the chemical into the river and at levels in the range of 0.9-1.9 μg/l before infiltration.

Unspecified dichlorobenzenes have been reported at a level of 0.4 μg/l in Delaware River water samples taken in New Jersey and Pennsylvania (Sheldon and Hites, 1978).

Both *ortho-* and *para*-dichlorobenzene were detected in samples of effluent water from southern California sewage plants. Levels were generally in the range of 2-12 μg/l, although as much as 435 μg/l *ortho*-dichlorobenzene and 230 μg/l *para*-dichlorobenzene were found. Samples collected from wastewater treatment plants in Georgia contained 4.0-268 μg/l of unspecified dichlorobenzenes (US Environmental Protection Agency, 1980c). *ortho*-Dichlorobenzene was found in industrial discharge waters at levels of 15-690 μg/l and *para*-dichlorobenzene at a concentration of 58 μg/l. *ortho*-Dichlorobenzene has been reported to enter water systems at an average level of 2 mg/l as a result of its use in industrial wastewater treatment plants for odour control (US Environmental Protection Agency, 1980d).

The combination of venting and scrubbing washings during *ortho*-dichlorobenzene manufacture reportedly contributed 405 thousand kg to the US environment in 1972 (US Environmental Protection Agency, 1980c).

During 1977, as much as 2.18 million kg *ortho*-dichlorobenzene were estimated to have been released to water as a result of its use as a process solvent in the production of toluene diisocyanates; and as much as 4.1 million kg of *para*-dichlorobenzene were released resulting from its use as a deodorant in toilet bowls and garbage (Johnston *et al.*, 1979). *para*-Dichlorobenzene has also been detected in the effluents of textile finishing plants. Up to 10 μg/l of an unspecified isomer of dichlorobenzene was detected in the cooling water and seepage lagoons of a US detergent manufacturing plant (US Environmental Protection Agency, 1980c).

Sediments collected in the vicinity of sewage plants in Los Angeles, California, contained detectable levels of both *ortho-* and *para*-dichlorobenzene (US Environmental Protection Agency, 1980c).

(e) Soil and plants

Both *ortho-* and *para*-dichlorobenzene have been reported to occur in soils as a

product of lindane degradation. They have also been detected in the roots of wheat plants grown from lindane-treated seeds (US Environmental Protection Agency, 1980d).

(*f*) *Food, beverages and animal feeds*

Dichlorobenzenes may be present in foods as a result of contamination. Pork meat has reportedly been tainted with a disagreeable odour and taste as a result of the use in pig stalls of an odour-control agent containing *para*-dichlorobenzene. Eggs have also been similarly tainted after hens were exposed to 20-30 mg/m^3 [3.3-5 ppm] *para*-dichlorobenzene (US Environmental Protection Agency, 1980d).

(*g*) *Animals*

Bovine tissue with an unusual smell was reported to contain the following concentrations of *para*-dichlorobenzene: muscle, 4.4-55.9 mg/kg [ppm]; perirenal fat, 165 mg/kg; pancreas, 11.3 mg/kg; lung, 1.9 mg/kg; liver, 3.4 mg/kg; and spleen, 2.8 mg/kg (Yoneda and Tsuda, 1979).

Fish collected from the Great Lakes drainage system and other fresh water sources in the US have been found to contain detectable amounts of dichlorobenzene in their tissue (US Environmental Protection Agency, 1980c). A species of mackerel from Japanese coastal water contained *para*-dichlorobenzene at a level of 0.05 mg/kg wet weight (US Environmental Protection Agency, 1980d). Fish and mussels taken from rivers in Slovenia and the Gulf of Trieste were found to contain traces to 1.2 µg/g *ortho*-dichlorobenzene (on a fat basis) and traces to 0.45 µg/g *para*-dichlorobenzene (Jan and Malnersic, 1980).

Samples of adipose tissue from pigeons captured in central and suburban Tokyo contained mean concentrations of 1.35-2.43 mg/kg *para*-dichlorobenzene (Morita and Ohi, 1978).

(*h*) *Human tissues and secretions*

Some human adipose tissue samples collected at a university hospital and at a medical examiner's office in central Tokyo were reported to contain levels of less than 10 mg/kg *para*-dichlorobenzene (Morita and Ohi, 1978).

2.3 Analysis

Several methods for the separation and detection of dichlorobenzenes by gas chromatography have been described (Cowan and Hartwell, 1961; Nadeau and Oaks, 1961; Habboush and Tameesh, 1970; Karasek and Fong, 1971). Its determination in chemical plant effluent was described by Sprowl *et al.* (1962). Typical methods for the analysis of *ortho*- and *para*-dichlorobenzene in various matrices are summarized in Table 3.

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Draw through XAD-2 resin	GC/PID	0.7 ppb (4.2 μg/m³)	Langhorst and Nestrick (1979)
	Draw air through charcoal adsorption tube	GC/FID	useful range, 30-900 mg/m³ (5-150 ppm)[b]	SRI (1976a)
			useful range 75-1350 mg/m³ (12-225 ppm)[c]	SRI (1976b)
Water	Extract with diethyl ether/ hexane; dry by passage through anhydrous crystalline sodium sulphate; concentrate	GC/E	0.005 μg/1	Dressman *et al.* (1977)
	Direct aqueous injection through a permaselective membrane[b]	GC/ECD	35 μg/l	Simmonds and Kerns (1979)
	Pre-concentrate on Chromosorb 102 resin; elute with pentane; concentrate. Or liquid/liquid extraction with pentane	GC/ECD	0.001 μg/l	Oliver and Bothen (1980)
Blood	Dilute with water; extract with carbon tetrachloride	GC/PID	3.60 μg/kg[b] 3.00 μg/kg[c]	Langhorst and Nestrick (1979)
	Centrifuge; extract plasma with benzene; dry over anhydrous sodium sulphate[c]	GC/ECD	not given	McKinney *et al.* (1970)
	Extract (toluene); inject[c]	GC/ECD	50 μg/l	Schmidt (1977)
Urine	Extract with carbon tetra-chloride	GC/PID	0.9 μg/kg[b] 0.75 μg/kg[c]	Langhorst and Nestrick (1979)
	Acidify with hydrochloric acid; extract with benzene; dry over anhydrous sodium sulphate[c]	GC/ECD	not given	McKinney *et al.* (1970)
	Extract (toluene); inject[c]	GC/ECD	50 μg/l	Schmidt (1977)
Tissue	Homogenize (toluene, then sodium chloride solution)[c]; steam distill; extract toluene; clean (sulphuric acid)	GC/ECD	0.1 μg/g	Schmidt (1977)

a Abbreviations: GC/PID, gas chromotography/photo-ionization detection; GC/FID, gas chromatography/flame ionization detection; GC/E, gas chromatography/electrolytic conductivity detection; GC/ECD, gas chromatography/electron capture detection

b *ortho*-Dichlorobenzene only

c *para*-Dichlorobenzene only

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

Inhalation

ortho-*Dichlorobenzene*

A study in which *ortho*-dichlorobenzene vapour was administered by inhalation to various animal species was available to the Working Group (Hollingsworth *et al.*, 1958). [This study was too short in duration and involved too few animals to have any significance for the evaluation of the possible carcinogenicity of this compound.]

para-*Dichlorobenzene*

No data were available to the Working Group.

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Quantitative lethality data for both *ortho* and *para*-dichlorobenzene are shown in Table 4.

The administration of *para*-dichlorobenzene vapour by inhalation to rabbits produced signs of central nervous depression, irritation of mucous membranes, granulocytopenia and degeneration of renal tubules (Zupko and Edwards, 1949). Feeding *para*-dichloroben-

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[1] The Working Group was aware of studies in progress in which *ortho*- and *para*-dichlorobenzene were being administered orally to mice and rats, and of a completed but unpublished study in which *para*-dichlorobenzene was administered orally to mice (IARC, 1981). The Working Group was also aware of a review paper including data on carcinogenicity and mutagenicity studies on *para*-dichlorobenzene, which has been submitted for publication.

Table 4. Quantitative lethality data for *ortho* and *para*-dichlorobenzenes

Route	Species	Measurement	Value	Reference
ortho-Dichlorobenzene				
oral	rat	LD_{50}	500 mg/kg bw	Jones *et al.* (1968)
inhalation	rat	LCLo[a]	4946 mg/m^3 (821 ppm)/7 h	Hollingsworth *et al.* (1958)
intravenous	mouse	LDLo[b]	0.4 ml/kg bw(~ 520 mg/kg bw)	Cameron *et al.* (1937)
oral	rabbit	LD_{50}	500 mg/kg bw	Thomson (1978-1979)
intravenous	rabbit	LDLo	0.5 ml/kg bw (~ 650 mg/kg bw)	Cameron *et al.* (1937)
oral	guinea-pig	LD_{100}	2000 mg/kg bw	Hollingsworth *et al.* (1958)
inhalation	guinea-pig	LCLo	4819 mg/m^3 (800 ppm)/24 h	Cameron *et al.* (1937)
para-Dichlorobenzene				
oral	rat	LD_{50}	500->1000 mg/kg bw	Ben-Dyke *et al.* (1970)
intraperitoneal	rat	LD_{50}	2562 mg/kg bw	Zupko and Edwards (1949)
oral	mouse	LD_{50}	2950 mg/kg bw	Spencer (1968)
subcutaneous	mouse	LD_{50}	5145 mg/kg bw	Irie *et al.* (1973)
oral	guinea-pig	LD_{100}	2800 mg/kg bw	Hollingsworth *et al.* (1956)

a LCLo, lowest reported lethal concentration
b LDLo, lowest reported lethal dose

zene to guinea-pigs results in hepatic steatosis (Frada and Cali, 1958) accompanied by elevated serum glutamic-oxaloacetic transaminase (Totaro, 1961) and clotting deficiencies (Coppola *et al.*, 1963).

Rats given 770 mg/kg bw *para*-dichlorobenzene intragastrically for five days developed signs of hepatic porphyria, i.e., increases in urinary coproporphyrins, and increases in porphobilinogen and δ-aminolaevulinic acid (Rimington and Ziegler, 1963). However, in another study, no porphyria occurred in female rats fed 200 mg/kg bw per day in corn oil for 120 days (Carlson, 1977).

The maximum tolerated dose of *ortho*-dichlorobenzene for rats fed by gavage on five days a week for about 28 weeks was 19-190 mg/kg bw per day. Minimal liver and kidney damage occurred at higher dosage levels. Inhalation of *ortho*-dichlorobenzene vapour was also reported to cause liver and kidney damage (Hollingsworth *et al.*, 1958).

The more pronounced toxicity to the liver of the *ortho* isomer has been associated with a more pronounced binding of the compound or its intermediate metabolites to liver proteins (Reid and Krishna, 1973).

Twenty-four hours after an i.p. dose of 5 mmol/kg bw [735 mg/kg bw] *ortho*-dichlorobenzene, rats displayed an increase in bile-duct-pancreatic fluid flow, and the protein concentration of these secretions was decreased. *para*-Dichlorobenzene failed to produce these effects (Yang *et al.*, 1979).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

The serum concentration of *para*-dichlorobenzene in male 6dd-Y mice was 6.5 ppm [mg/l] following continuous inhalation exposure to 150 ppm [904 mg/m^3] *para*-dichlorobenzene vapour for eight hours per day for 14 days (Irie *et al.*, 1973).

Hawkins *et al.* (1980) studied the disposition of ^{14}C-labelled *para*-dichlorobenzene given orally, subcutaneously or by inhalation of a vapour in the rat. Ethereal sulphate and glucuronide conjugates of 2,5-dichlorophenol were the major urinary metabolites, but small amounts of 2,5-dichlorohydroquinone were detected. They also observed that the rat, unlike the rabbit (Azouz *et al.*, 1955), excretes a mercapturic acid derivative of 2,5-dichlorophenol. Recently, Kimura *et al.* (1979) detected appreciable levels of two metabolites of *para*-dichlorobenzene, 2,5-dichlorophenyl methyl sulphoxide and 2,5-dichlorophenyl methyl sulphone, in the blood, kidney, adipose tissue, liver, urine and faeces of rats fed *para*-dichlorobenzene.

After its oral administration to rabbits, *ortho*-dichlorobenzene is metabolized mainly to 3,4-dichlorophenol; but 2,3-dichlorophenol, 3,4-dichlorophenylmercapturic acid and 3,4- and 4,5-dichlorocatechol are also formed. *para*-Dichlorobenzene is converted to 2,5-dichlorophenol and 2,5-dichlorohydroquinone conjugated with glucuronic or sulphuric acid (Azouz *et al.*, 1955; McKinney *et al.*, 1970).

The effect of inducers and inhibitors of microsomal mixed-function oxidases on the rate of metabolism and the extent of binding of *ortho*- and *para*-dichlorobenzene to

cellular constituents suggests that arene oxides (epoxides) may be precursors of the excreted metabolites, and that these arene oxides may be responsible for the different biological properties of the parent compounds (Reid and Krishna, 1973).

Mutagenicity and other short-term tests

ortho-Dichlorobenzene showed no mutagenic activity in a number of *Salmonella typhimurium* strains when tested with or without metabolic activation (Andersen *et al.*, 1972; Lawlor *et al.*, 1979). It was very weakly mutagenic at the $meth_3$ locus in *Aspergillus nidulans* (Prasad, 1970).

In a study reported in an abstract, *para*-dichlorobenzene showed no mutagenic activity for *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100, with or without metabolic activation by Aroclor 1254-treated rat liver microsomes (Lawlor *et al.*, 1979). *para*-Dichlorobenzene was reported to induce back-mutations of a methionine-requiring auxotroph of *Aspergillus nidulans* (Prasad, 1970). [The Working Group considered that the results of this study needed to be confirmed.]

para-Dichlorobenzene has been reported to induce chromosomal abnormalities and breakage in root tips of *Vicia faba* and other plant species (Sharma and Bhattacharyya, 1956; Srivastava, 1966).

(*b*) *Humans*

Toxic effects

Inhaled or ingested *para*-dichlorobenzene is toxic in man. One case of pulmonary granulocytosis (Weller and Crellin, 1953) and two cases of haemolytic anaemia (Hallowell, 1959; Campbell and Davidson, 1970) have been reported. A case of allergic purpura after exposure to *para*-dichlorobenzene has also been described (Nalbandian and Pearce, 1965). No evidence of organic injury or of untoward haematological effect has been found in workers that was attributable to exposure to air containing *ortho*-dichlorobenzene at concentrations ranging from 1-44 ppm [6-265 mg/m^3] (average, 15 ppm [90 mg/m^3]) for many years (Hollingsworth *et al.*, 1958).

Absorption, distribution, excretion and metabolism

Some adipose tissue samples collected at a university hospital and at a medical examiner's office in central Tokyo were reported to contain levels of less than 10 mg/kg *para*-dichlorobenzene (Morita and Ohi, 1978).

para-Dichlorobenzene is converted to 2,5-dichlorophenol and 2,5-dichlorohydroquinone in man; the amount of 2,5-dichlorophenol present in urine can serve as an indication of exposure (Hallowell, 1959; Pagnotto and Walkley, 1965). The phenolic metabolites are excreted as conjugates of glucuronic or sulphuric acids (Hallowell, 1959).

3.3 Case reports and epidemiological studies of carcinogenicity in humans

Girard *et al.* (1969) reported five cases of blood disorders occurring in subjects exposed to *ortho*- or *para*-dichlorobenzene as a solvent for other chemicals or in

chlorinated benzene mixtures. No evidence of exposure to benzene was found. Two of the cases were chronic lymphoid leukaemia, two were acute myeloblastic leukaemia and one was a myeloproliferative syndrome. The two patients with chronic lymphoid leukaemia were occupationally exposed: one had been exposed to a glue containing 2% *ortho*-dichlorobenzene from 1945-1961, and the other had been exposed from 1940-1950 to a solvent containing *ortho*- (80%), *meta*- (2%) and *para*- (15%) dichlorobenzene, which was used for cleaning electrical parts. One of the two patients with acute myeloblastic leukaemia had been exposed non-professionally to the same mixture of *ortho*-, *meta*- and *para*-dichlorobenzene which she used for cleaning clothes (1-2 litres per year for many years); and the other case was a 15-year old girl who had for an undefined time removed stains from her own clothes with a product containing 37% *ortho*-dichlorobenzene.

No epidemiological study was available to the Working Group.

[The Working Group was aware of epidemiological studies and case reports that indicate a relationship between exposure to technical-grade chlorophenols and to chlorinated phenoxy acids and soft-tissue sarcomas and lymphomas (see Hardell, 1981, for review).]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

No adequate study on *ortho*-dichlorobenzene was available to the Working Group, and no data were available on *para*-dichlorobenzene.

No data were available to evaluate the teratogenicity of these compounds to experimental animals.

Neither *ortho*- nor *para*-dichlorobenzene was mutagenic to *Salmonella typhimurium*. Mutagenic or clastogenic activity in other cell systems has not been substantiated.

4.2 Human data

Occupational exposure to *ortho*-dichlorobenzene occurs during its manufacture, its conversion to 3,4-dichloroaniline and other derivatives and its use as a solvent in toluene diisocyanate production and for other purposes. Its use in manufacturing and solvents may also be significant sources of discharges into water.

Occupational exposure to *para*-dichlorobenzene occurs during its manufacture, its conversion to polyphenylene sulphide resins and its use as an air deodorant and moth control agent. These two uses are also potential sources of exposure for the general population.

One case report has suggested an association between leukaemia and exposure to dichlorobenzenes.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

4.3 Evaluation

No adequate data were available to evaluate the carcinogenicity of *ortho-* or *para-*dichlorobenzene to experimental animals.

The epidemiological data were inadequate to evaluate the carcinogenicity of dichlorobenzenes.

No evaluation could be made of the carcinogenicity of *para-* or *ortho*-dichlorobenzene to man.

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3,3′-DICHLOROBENZIDINE AND ITS DIHYDROCHLORIDE

This substance was considered by a previous Working Group, in June 1973 (IARC, 1974a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

3,3′-Dichlorobenzidine

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 91-94-1

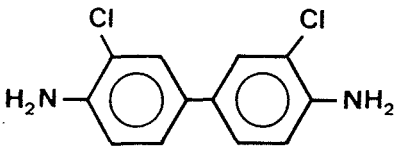
Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine, 3,3′-dichloro-

IUPAC Name: 3,3′-Dichlorobenzidine

Synonyms: C. I. 23060; DCB; 4,4′-diamino-3,3′-dichlorobiphenyl; 4,4′-diamino-3,3′-dichlorodiphenyl; dichlorobenzidine; *ortho*,*ortho*′-dichlorobenzidine; dichlorobenzidine base; 3,3′-dichlorobenzidine base; 3,3′-dichlorobiphenyl-4,4′-diamine; 3,3′-dichloro-4,4′-biphenyldiamine; 3,3′-dichloro-4,4′-diaminobiphenyl; 3,3′-dichloro-4,4′-diamino(1,1-biphenyl)

Trade name: Curithane C126

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{10}Cl_2H_2$ Mol. wt: 253.1

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Grey to purple crystalline solid (Hawley, 1981)

(*b*) *Melting-point*: 132-133°C (Windholz, 1976); 133°C (Ferber, 1978); 165°C (Hawley, 1981)

(*c*) *Spectroscopy data*: Mass spectral data have been reported (NIH/EPA Chemical Information System, 1980).

(*d*) *Solubility*: Almost insoluble in water; readily soluble in benzene, diethyl ether, ethanol and glacial acetic acid (Windholz, 1976; Hawley, 1981)

(*e*) *Reacticity*: Undergoes usual reactions of benzidine derivatives, e.g., formation of diazonium salts and acyl and alkyl derivatives (Ferber, 1978)

(*f*) *Conversion factor*: ppm = 0.0966 x mg/m^3

1.4 Technical products and impurities

3,3′-Dichlorobenzidine is sold in the US as the dihydrochloride salt (Ferber, 1978).

3,3′-Dichlorobenzidine dihydrochloride

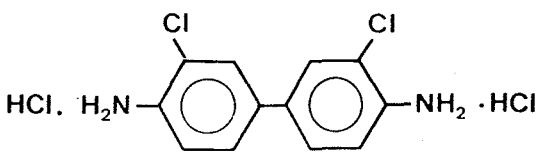
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 612-83-9

Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine, 3,3′-dichloro-, dihydrochloride

IUPAC Systematic Name: 3,3′-Dichlorobenzidine, dihydrochloride

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{10}Cl_2N_2.2HCl$ Mol. wt: 326.1

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Needles (Windholz, 1976)

(*b*) *Spectroscopy data*: Mass spectral data have been reported (NIH/EPA Chemical Information System, 1980).

(*c*) *Solubility*: Slightly soluble in water (4 mg/l at 22°C) (US Environmental Protection Agency, 1979); readily soluble in ethanol (Windholz, 1976)

1.4 Technical products and impurities

3,3′-Dichlorobenzidine dihydrochloride is available in the US as moist white crystals with the following specifications: 67% minimal purity (as 3,3′-dichlorobenzidine); 11% maximal moisture; 1.9-2.1 mol hydrochloric acid per mol 3,3′-dichlorobenzidine; and no sulphate content (The Upjohn Company, 1978). A typical sample contains 10 trace metals in concentrations ranging from <0.001 to 14 mg/kg each (The Upjohn Company, undated). It is available from another company as a white to light-grey powder with the following specifications: 60% minimal purity (as 3,3′-dichlorobenzidine); melting-point, 130-134°C; and 0.2% maximal insolubles in hydrochloric acid (Bofors Lakeway, Inc., undated).

3,3′-Dichlorobenzidine dihydrochloride is available in Japan as a white to light-grey powder, with the following specifications: 60.6% minimal purity (as 3,3′-dichlorobenzidine); 97.5% minimal purity (dried basis); and 22% maximal moisture content.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

3,3′-Dichlorobenzidine was first synthesized from *ortho*-chloronitrobenzene in 1900 (Windholz, 1976). It is produced commercially by alkaline reduction of *ortho*-chloronitrobenzene and rearrangement of the resulting hydrazo compound with hydrochloric acid to produce the dihydrochloride salt, which is the commercially available form (Ferber, 1978).

3,3′-Dichlorobenzidine has been produced commercially in western Europe since 1932 (Schwenecke, 1980); in the US since at least 1937 (US Tariff Commission, 1938); and in Japan since about 1955. At the present time, two US companies produce commercial quantities of 3,3′-dichlorobenzidine dihydrochloride. The production of one of these companies in 1977 was 0.454-4.54 million kg (US Environmental Protection Agency, 1981). Combined US production of 3,3′-dichlorobenzidine and its salts in 1971 by three US

companies amounted to 1.6 million kg (US Tariff Commission, 1973). US imports of 3,3′-dichlorobenzidine and its salts through the principal US customs districts totalled 147 thousand kg in 1980 (US International Trade Commission, 1981), down sharply from the 658 thousand kg imported in 1971 (US Tariff Commission, 1972).

3,3′-Dichlorobenzidine and its salts are believed to be produced by one company each in the Federal Republic of Germany, France, Italy and the UK. Total production in 1979 is estimated to have been 100 thousand to 500 thousand kg.

Commercial production of 3,3′-dichlorobenzidine dihydrochloride by the two producing companies in Japan in 1980 was about 1.8 million kg; in 1975, production was only about 870 thousand kg, and about 100 thousand kg were exported.

(b) Use

The principal use of 3,3′-dichlorobenzidine (in the form of its dihydrochloride) is as an intermediate for organic pigments. The Society of Dyers and Colourists (1971, 1975) indicated that 15 organic dyes and pigments can be prepared from this chemical. Only four 3,3′-dichlorobenzidine-based dyes were, but are no longer, produced commercially in the US; eight 3,3′-dichlorobenzidine-based pigments were produced in the US, but one, Pigment Yellow 63, is no longer being produced (JRB Associates Inc., 1979). Available data on the US production (or sales), imports and principal uses of these eight pigments during the period 1971-1979 are given in an appendix to the 'General Remarks on the Substances Considered' of this volume. These data illustrate that, in general, the 3,3′-dichlorobenzidine-based pigments have maintained a fairly steady production/sales level over that period and in some cases (e.g., Pigment Yellow 12 and Pigment Yellow 13) have shown considerable growth.

3,3′-Dichlorobenzidine has been used alone, or in blends with 4,4′-methylenebis(2-chloroaniline) [see IARC, 1974b], as a curing agent for liquid-castable polyurethane elastomers. 3,3′-Dichlorobenzidine or its salts has also been reported to be used in a colour test for the presence of gold (Lurie, 1964).

In Japan, essentially all of the estimated 1.5 million kg of 3,3′-dichlorobenzidine dihydrochloride used in 1980 went into the production of pigments.

Regulations in the US concerning 3,3′-dichlorobenzidine and its salts designate strict procedures to avoid worker contact: Mixtures containing 1.0% or more 3,3′-dichlorobenzidine and its salts must be maintained in isolated or closed systems, employees must observe special personal hygiene rules, and certain procedures must be followed for movement of the material and in case of accidental spills and emergencies (US Occupational Safety and Health Administration, 1980).

The following five countries have designated, by regulation or guidelines, 3,3′-dichlorobenzidine as a skin irritant and as a carcinogen: Australia, Belgium, the Federal Republic of Germany, Switzerland and the USA. Finland, Italy and Sweden have designated it as a carcinogen. In the UK, 3,3′-dichlorobenzidine and its salts are 'controlled substances', subject to special preventive measures, including prescribed medical examinations. A license is required for the production of 3,3′-dichlorobenzidine and its salts in Japan (International Labour Office, 1980).

The US Environmental Protection Agency (EPA) (1980a) has identified 3,3′-dichlorobenzidine as a toxic waste and requires that persons who generate, transport, treat, store

or dispose of it comply with the regulations of a Federal hazardous waste management programme. The EPA also requires notification whenever discharges containing 4.54 kg or more of 3,3′-dichlorobenzidine are made into waterways (US Environmental Protection Agency, 1980b).

As part of the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of 3,3′-dichlorobenzidine are subject to a variety of labelling, packaging, quantity and shipping regulations consistent with its designation as a hazardous material.

2.2 Occurrence

(*a*) *Natural occurrence*

3,3′-Dichlorobenzidine has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

In 1973, 18 US companies had been confirmed to be using 3,3′-dichlorobenzidine, and 166 employees were potentially exposed. A possible total of 250 employees were suspected of being exposed (Bell, 1973).

A Japanese study in 1970 of workers exposure to 3,3′-dichlorobenzidine in a pigment manufacturing plant showed that concentrations of 3,3′-dichlorobenzidine in the air reached a level of 25 μg/m^3 [2 ppb] within 10 min of charging reaction vessels and dropped to 2 μg/m^3 [0.2 ppb] within 20 min (US Environmental Protection Agency, 1980c).

(*c*) *Water and sediments*

Analysis of purge wells and seepage water near a waste disposal lagoon receiving wastes from the manufacture of 3,3′-dichlorobenzidine were reported to contain 0.13-0.27 mg/l 3,3′-dichlorobenzidine. In Japan, the Sumida River, which receives waste effluents from several dye and pigment factories, was also found to contain 3,3′-dichlorobenzidine (US Environmental Protection Agency, 1980c).

3,3′-Dichlorobenzidine has been detected in oil refinery effluents, industrial effluents and surface water (Hushon *et al.*, 1980).

2.3 Analysis

An IARC Manual (Egan *et al.*, 1981) gives selected methods for the analysis of aromatic amines and azo dyes, including 3,3′-dichlorobenzidine. Typical methods for the analysis of 3,3-dichlorobenzidine in various matrices are summarized in Table 1.

Table 1. Methods for the analysis of 3,3'-dichlorobenzidine

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect sample on glass filter and silica gel; desorb (triethylamine in methanol); elute (70:30 acetonitrile:water)	HPLC/UV	3 μg/m^3	Morales *et al.* (1979)
Water	Filter aqueous sample; elute (70:30 acetonitrile:5 % acetic acid in water)	HPLC/UV	5 ng	Sikka *et al.* (1978)
	Three alternative procedures: (1) direct injection; (2) extract (chloroform), wash and concentrate, dilute (acetate buffer); or (3) adjust pH (phosphate buffer), clean by column chromatography, wash and concentrate, dilute (acetate buffer), concentrate	HPLC/E	(1) 1 μg/l (2) 0.05 μg/l (3) 0.1 μg/l	Riggin and Howard (1979)
Wastewater	Clean by column chromatography; elute (acetone); add hydrochloric acid and shake; concentrate; extract (benzene); add sodium hydroxide to aqueous layer and extract with benzene; clean benzene extract by column chromatography; elute (benzene); evaporate; dissolve (benzene); analyse directly or synthesize derivatives with pentafluoropropionic anhydride	GC/ECD or GC/NPD	0.018 μg/l 0.60 μg/l	Bowman and Rushing (1977)
Fish tissue	Digest ground tissue and extract (benzene); wash (sulphuric acid); dry (sodium sulphate) and concentrate; clean up by gel permeation chromatography	GC/MS	not given	Diachenko (1979)

Animal chow	Add sodium hydroxide; extract (benzene); centrifuge; evaporate; dissolve (benzene); add hydrochloric acide; extract (benzene); add benzene and sodium hydroxide to aqueous layer and extract; clean benzene layer by column chromatography; elute (benzene); evaporate; dissolve (benzene); analyse directly or synthesize derivatives with pentafluoro-propionic anhydride	GC/ECD or GC/NPD	3 µg/kg 40 µg/kg	Bowman and Rushing (1977)
Municipal sludge	Dilute (phosphate buffer); extract (chloroform); extract (sulphuric acid); neutralize (sodium phosphate); add methanol and concentrate; dilute (acetate buffer)	HPLC/E	10 µg/kg	Warner *et al.* (1980)
Biological samples				
Human urine	Clean with column chromatography; elute (acetone); concentrate; extract (benzene); add sodium hydroxide and benzene to aqueous layer and extract; clean benzene layer by column chromatography; elute (benzene); evaporate; dissolve (benzene); analyse directly or synthesize derivatives with pentafluoro-propionic anhydride	GC/ECD or GC/NPD	0.06 µg/l 1.8 µg/l	Bowman and Rushing (1977)
Hamster urine	Dissolve (methanol); evaporate; (1:1 methanol:potassium phosphate)	HPLC/UV	1 µg/l	Nony and Bowman (1980)

a Abbreviations: HPLC/UV, high-performance liquid chromatography/ultra-violet spectrometry; HPLC/E, high performance liquid chromatography/electrochemical detection; GC/ECD, gas chromatography/electron capture detection; GC/NPD, gas chromatography/nitrogen phosphorus detection; GC/MS, gas chromatography/mass spectrometry

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Twenty-six male ICR/JCL mice [age at start not specified] were fed a diet containing 0.1% 3,3′-dichlorobenzidine [purity unspecified] for up to 12 months. The animals were killed after 6 or 12 months of treatment. All (100%) had hepatomas, with mean numbers of 8 and 18 hepatomas/mouse, respectively. Of 39 control mice maintained on a normal diet and killed at 6, 12 and 18 months, 0, 9.5 and 38.5% had hepatomas, with mean numbers of 0, 2 and 5 hepatomas/mouse, respectively (Osanai, 1976). [The Working Group noted the absence of information on survival of treated and control animals.]

Rat: A group of 15 female and 35 male outbred Rappolovo rats (110-130 g) received food containing 3,3′-dichlorobenzidine in the form of a paste (45.3% 3,3′-dichlorobenzidine, 50% water and 4.7% unspecified impurities) at a dose of 10-20 mg/day, which was administered on six days a week for 12 months (total dose, 4.5 g/rat). The numbers of animals that survived were: 34 at six months, 27 at 12 months and 29 rats at the time of the appearance of the first tumour (11 months). The animals were observed for life. Twenty-three rats developed tumours, including 7 tumours of the Zymbal gland, 3 skin tumours, 7 mammary gland tumours, 2 adenocarcinomas of the ileum, 3 bladder tumours, 3 tumours of the haematopoietic system, 2 tumours of connective tissue, 2 tumours of the salivary gland, 1 tumour of the liver and 1 of the thyroid. Among a group of 130 controls injected with octadecylamine and methylstearylamine, no tumours were found within 23 months (Pliss, 1959). [The Working Group noted the absence of adequate controls.]

A group of 20 female Sprague-Dawley rats (40 days of age) were given 10 doses of 3,3′-dichlorobenzidine dihydrochloride [purity and impurities unspecified] every three days by gastric intubation (total dose, 300 mg/rat, which was the maximum tolerated dose). The observation period was nine months, when the surviving animals (14) were killed. No mammary tumours were observed in 15 rats autopsied, while 100% of the positive control group (treated with 7,12-dimethylbenz[*a*]anthracene) and 3% of animals treated with sesame oil only had mammary tumours (Griswold *et al.*, 1968).

A group of 50 male and 50 female 5-week-old ChR-CD rats were given a diet containing 1000 mg/kg [ppm] 3,3′-dichlorobenzidine [purity and impurities unspecified] for 349-353 days, and an equal number of animals were maintained on a control diet for a period of 12 months. Six rats per group and per sex were killed at 12 months. Of the remaining treated rats, six survived up to 15 months, at which time they were killed; controls were maintained under observation up to approximately two years. A statistically significant ($p < 0.05$) increase in the incidence of tumours was observed in treated compared with control animals, for the following target sites: in male treated rats, 9/44 granulocytic leukaemias, 7/44 mammary adenocarcinomas and 8/44 Zymbal gland carcinomas; the corresponding incidences in control rats were 2/44, 0/44 and 0/44. Of female rats, 26/44 treated animals developed mammary adenocarcinomas *versus* 3/44 in control rats (Stula *et al.*, 1975).

Hamster: In lifetime studies in this species, dietary levels of 0.1% 3,3′-dichlorobenzidine [purity and impurities unspecified] did not induce tumours in 30 male or 30 female Syrian golden hamsters, when compared with a similar number of untreated animals. However, in later studies in similar groups of animals, 0.3% 3,3′-dichlorobenzidine in the diet produced four transitional-cell carcinomas of the bladder and some liver-cell and cholangiomatous tumours (Sellakumar *et al.*, 1969). These tumours were not found in the control animals (Saffiotti *et al.*, 1967).

Dog: Six female one-year-old beagle dogs were each given 100 mg 3,3′-dichlorobenzidine (reported to be 100% pure) in a gelatin capsule three times per week for six weeks, then five times per week continuously for periods up to 7.1 years. The intake of 3,3′-dichlorobenzidine was between 9.1 and 12.8 mg/kg bw per dose. One dog sacrificed after 3.5 years on test had no tumour. Another sacrificed after 6.6 years on test (total intake, 164 g) had an undifferentiated carcinoma of the urinary bladder. Of the dogs killed at 7.1 years (total intake, 176 g/dog), 4/4 had papillary transitional-cell carcinomas of the urinary bladder and 3/4 had hepatocellular carcinomas. None of the six control dogs had these tumours. However, 4/6 control animals killed at 8-9 years of age had major tumours of the mammary gland (adenocarcinomas and carcinosarcoma) (Stula *et al.*, 1978).

(*b*) *Subcutaneous and/or intramuscular administration*

Rat: A group of 25 female and 36 male outbred rats received weekly s.c. injections of an 8.8% suspension of 3,3′-dichlorobenzidine paste (45.3% 3,3′-dichlorobenzidine, 50% water and 4.7% unspecified impurities) in glycerol at a dose of 120 mg/rat. Because of toxic effects, beginning at the sixth month, the dose was reduced to 20 mg/rat. The total dose was 1.62-3 g/rat over 10-11 months. The numbers of rats that survived were: 40 at six months, 23 at 12 months, and 35 at the time (seven months) of appearance of the first tumour. The animals were observed for life. Twenty-six (74.3%) had tumours at different sites: 10 had tumours of the Zymbal gland, 5, skin tumours, 6, tumours of the mammary gland, 7, local subcutaneous sarcomas, 2, remote tumours of connective tissue, 2, tumours of the haematopoietic system and 1, a tumour of the salivary gland. No tumour occurred within 23 months in 130 controls injected with octadecylamine or methylstearylamine (Pliss, 1959). [The Working Group noted the absence of adequate controls.]

A group of rats received s.c. injections of 15-60 mg/rat 3,3′-dichlorobenzidine [purity and impurities unspecified] in sunflower seed oil or glycerol and water at unspecified intervals for 10 to 13 months. Tumours occurred in 74% of animals. Skin, sebaceous and mammary gland tumours were observed most frequently, and there were also intestinal, urinary bladder and bone tumours. Among 50 control rats injected with the vehicle alone, or left untreated, one tumour was reported (Pliss, 1963). [The Working Group noted the inadequate reporting of the experiment.]

(*c*) *Other experimental systems*

Administration in conjunction with other chemicals: Nine groups of 22 (and one of 96) male Wistar *rats* received the following compounds alone or in sequence for a period of four weeks per compound: *ortho-N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (0.01% in drinking-water), *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (0.15% in the diet), *N*-fluorenylacetamide (0.025% in the diet) and 3,3′-dichlorobenzidine (0.3% in the diet). An untreated control group consisted of 12 rats. The animals were killed when 40 weeks old. 3,3′-Dichlorobenzidine given in sequence with one or more of the other compounds induced histological changes of the liver (cystic change of bile ducts and oval cell proliferation)

in 44-60% of animals ($p < 0.05$ when compared with groups that did not receive 3,3′-dichlorobenzidine). No change was seen in the liver when 3,3′-dichlorobenzidine was given alone. The incidence of urinary bladder tumours was not significantly increased when 3,3′-dichlorobenzidine was added to the diet (Tatematsu *et al.*, 1977).

Transplacental exposure: During the last week of pregnancy, a group of BALB/c *mice* [number and age not specified] were treated with 5 s.c. injections of 2 mg/injection 3,3′-dichlorobenzidine (total dose, 10 mg/mouse). Another group was treated with 0.1 ml sesame oil. Of the offspring that lived 12-20 months, 13/24 had tumours, compared with 6/30 of the control progeny. A significant increase in the incidence of lymphoid leukaemias (7/24 in treated and 0/30 in control animals [sex unspecified]) was observed in the offspring. Tumours were also observed at other sites, but there was no statistically significant increase over the incidence in controls (5/24 lung adenomas *versus* 3/30; 4/24 mammary tumours *versus* 3/30) (Golub *et al.*, 1974).

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The oral LD_{50} of 3,3′-dichlorobenzidine in olive oil has been reported to be 7.07 g/kg bw in albino rats [sex and strain not specified]. The oral LD_{50} for the dihydrochloride as a 20% suspension in corn oil was 3.82 g/kg bw in Sprague-Dawley rats of both sexes (Gerarde and Gerarde, 1974).

Female beagle dogs were given 100 mg 3,3′-dichlorobenzidine (100% pure) in capsular form three times weekly for six weeks, and five times weekly thereafter for approximately seven years. Serum glutamic pyruvic transaminase activities were elevated in 6/6 treated dogs over the initial three years of the study, indicating the presence of liver injury. The transaminase activity remained elevated in 2/4 surviving treated dogs throughout the seven-year study (Stula *et al.*, 1978).

Effects on reproduction and prenatal toxicity

3,3′-Dichlorobenzidine crosses the placenta of mice, as demonstrated by growth changes in explanted kidneys of embryos from Balb/c mice treated during pregnancy with the compound (Golub, 1969; Shabad *et al.*, 1972; Kolesnichenko and Shabad, 1979) and by increased tumour incidences in offspring of treated Balb/c mice (Golub *et al.*, 1974).

Absorption, distribution, metabolism and excretion

No data were available on the extent of intestinal or dermal absorption of 3,3′-dichlorobenzidine in experimental animals; however, the appearance of systemic toxicity [see section on toxic effects] following oral administration or dermal application indicates some degree of absorption *via* these routes.

Mongrel dogs [sex unspecified] given 1 g 3,3′-dichlorobenzidine intraperitoneally as a suspension in gum tragacanth excreted less than 2% in faeces and less than 0.2% in

urine as the parent compound (Sciarini and Meigs, 1961). Thus, 3,3′-dichlorobenzidine is probably degraded rapidly *in vivo*.

Male Wistar rats and male beagles given 0.2 mg/kg bw ^{14}C-3,3′-dichlorobenzidine intravenously (dissolved in 0.5% Tween 20^{R} in water) displayed multiphasic blood clearances. The final phase, predominant by 24 hours after treatment, had a half-life of 68 hours in rats and 86 hours in dogs. Faecal excretion was the major route of elimination in rats, dogs and rhesus monkey, accounting for 30-85% of the administered dose within seven days; 10-40% was eliminated in urine. Urinary excretion was delayed for 3-5 hours after treatment, and most of the urinary products were 3,3′-dichlorobenzidine metabolites. One metabolite obtained from monkey urine co-chromatographed with monoacetyl benzidine, a urinary metabolite of benzidine in monkeys. Some parent compound was eliminated in the urine during the first few hours after treatment. The majority of the administered dose could be recovered from bile, intestine and liver within 14 hours of treatment, indicating the importance of hepato-biliary excretion in the elimination of this compound. By 7 or 14 days after treatment, residual radioactivity was recovered primarily from the excretory organs (kidney, bladder, liver, bile) or their products, but also from the adrenals (rat) and the lungs (dog) (Kellner *et al.*, 1973).

Mutagenicity and other short-term tests

3,3′-Dichlorobenzidine was mutagenic to *Salmonella typhimurium* strains TA1538, TA98 and TA100, in the presence and (for some strains) absence of a liver activation system; activity was enhanced by the presence of an activation system (Garner *et al.*, 1975; Lazear and Louie, 1977; Anderson and Styles, 1978).

3,3′-Dichlorobenzidine (10^{-7}-10^{-4} M) induced unscheduled DNA synthesis in HeLa cells in the presence of a phenobarbital-induced rat liver activation system (Martin *et al.*, 1978). At a concentration of 5 μg/ml, it enhanced transformation of high-passage cells containing Rauscher leukaemia virus in the Fischer rat embryo cell system (Freeman *et al.*, 1973). It induced cell transformation in the BHK test (Styles, 1978).

(*b*) *Humans*

Toxic effects

Dermal exposure to 3,3′-dichlorobenzidine base has caused dermatitis in dye workers (Gerarde and Gerarde, 1974).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, metabolism and excretion

Constituents that co-chromatographed with 3,3′-dichlorobenzidine were isolated from the urine of workers occupationally exposed to 3,3′-dichlorobenzidine. In one worker drenched with a slurry of this compound in water, urinary excretion was increased approximately 10-fold over the rate observed in other workers (Meigs *et al.*, 1954).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No case report is known in which 3,3′-dichlorobenzidine has been associated with the occurrence of cancer in man. [3,3′-Dichlorobenzidine may, however, have contributed to cases of bladder cancer attributed to benzidine, as both substances may be prepared in the same plant.]

No bladder tumour was found in three retrospective epidemiological studies of workers exposed to 3,3′-dichlorobenzidine (Gerarde and Gerarde, 1974; Gadian, 1975; MacIntyre, 1975). [However, all of these studies examined relatively small cohorts of workers - 207, 35 and 225, respectively - and their statistical power to detect a two-fold increase in bladder cancer mortality based on the number of workers was correspondingly limited (powers of 23%, 10% and 25%, respectively). Also, time since first exposure to 3,3′-dichlorobenzidine was 20 or fewer years for over two-thirds of the workers included in these three studies.] In the study by Gerarde and Gerarde (1974), follow-up of exposed workers was less than 85% complete. A variety of other tumours were noted by those authors, including six lipomata. [The significance of these findings is uncertain.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

3,3′-Dichlorobenzidine was tested in mice, rats, hamster and dogs by oral administration, in rats by subcutaneous administration and in mice by transplacental exposure. Following its oral administration, it produced liver-cell tumours in mice, hepatocellular carcinomas in dogs, mammary and Zymbal-gland tumours in rats and carcinomas of the urinary bladder in hamsters and dogs. Increased incidences of leukaemias were observed in rats following oral administration and in mice following transplacental exposure.

3,3′-Dichlorobenzidine is mutagenic to *Salmonella typhimurium* with or without metabolic activation and induces unscheduled DNA synthesis in HeLa cells.

No data were available on the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to 3,3′-dichlorobenzidine dihydrochloride has and probably still does occur during its manufacture and conversion to derived pigments. Rubber workers were formerly and may still be exposed to 3,3′-dichlorobenzidine used for curing polyurethane elastomers.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report on exposure to 3,3′-dichlorobenzidine was available to the Working Group. Although three retrospective epidemiological studies of workers exposed to 3,3′-dichlorobenzidine gave no evidence for carcinogenicity, the studies were of insufficient quality or statistical power to permit confident exclusion of that possibility. Because 3,3′-dichlorobenzidine and benzidine may be made in the same plant, the possibility cannot be excluded that dichlorobenzidine has contributed to the incidence of human bladder cancer attributed to benzidine.

4.3 Evaluation[1]

There is *sufficient evidence* that 3,3′-dichlorobenzidine is carcinogenic in mice, rats, hamsters and dogs.

The epidemiological data are inadequate to evaluate the carcinogenicity of 3,3′-dichlorobenzidine to man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DI(2-ETHYLHEXYL) ADIPATE

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 103-23-1

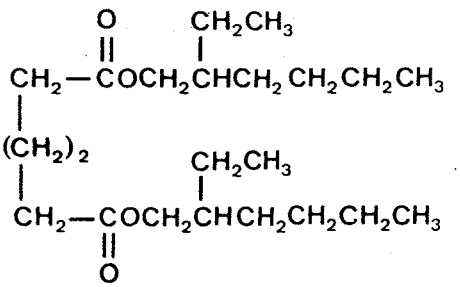
Chem. Abstr. Name: Hexanedioic acid, bis(2-ethylhexyl) ester

IUPAC Systematic Name: Adipic acid, bis(2-ethylhexyl) ester

Synonyms: BEHA; bis(2-ethylhexyl) adipate; DEHA; di-2-ethyl hexyl adipate; dioctyl adipate; DOA; hexanedioic acid, dioctyl ester; octyl adipate

Trade Names: Adipol 2EH; Bisoflex DOA; Effomoll DOA; Ergoplast AdDO; Flexol A26; Kodaflex DOA; Mollan S; Monoplex DOA; Plastomoll DDA; PX-238; Reomol DOA; Rucoflex Plasticizer DOA; Sicol 250; Staflex DOA; Truflex DOA; Uniflex DOA; Vestinol OA; Wickenol 158; Witamol 320

1.2 Structural and molecular formulae and molecular weight



$C_{22}H_{42}O_4$ Mol. wt: 370.6

1.3 Chemical and physical properties of the pure substance

From Weast (1979), unless otherwise specified

(*a*) *Description*: Light-coloured oily liquid (Hawley, 1981)

(*b*) *Boiling-point*: 214°C at 5 mm

(*c*) *Melting-point*: -67.8°C

(*d*) *Density*: d_4^{25} 0.922

(*e*) *Refractive index*: n_D^{20} 1.4474

(*f*) *Spectroscopy data*: Mass spectral data have been reported (NIH/EPA Chemical Information System, 1980).

(*g*) *Solubility*: Insoluble in water; soluble in acetic acid, acetone, diethyl ether and ethanol

(*h*) *Viscosity*: 13.7 cP at 20°C (Hawley, 1981)

(*i*) *Volatility*: Vapour pressure, <0.01 mm at 20°C (Mitre Corp., 1976) and 2.60 mm at 200°C (Hawley, 1981)

(*j*) *Conversion factor*: ppm = 0.0659 x mg/m^3

1.4 Technical products and impurities

Di(2-ethylhexyl) adipate is available in the US from numerous suppliers. The following specifications for the products offered by two companies are typical: a clear oily liquid with 0.02% maximal acidity (as adipic acid); 0.1% maximal moisture; specific gravity, 0.921-0.927 (25°/25°C); and refractive index, 1.444-1.448 (25°C) (Monsanto Co., 1973); and a clear liquid with 99% minimal ester content; 0.01% maximal acidity (as adipic acid); 0.05% maximal moisture; and specific gravity, 0.925-0.929 (20/20°C) (Hooker Chemical Corp., undated).

Di(2-ethylhexyl) adipate available in western Europe has a minimal purity of 99%. That available in Japan has the following specifications: maximal acid value, 0.07; maximal weight loss on heating at 125°C for 3 h, 0.1%; and specific gravity, 0.924-0.930 (20/20°C).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Di(2-ethylhexyl) adipate is produced commercially by the reaction of excess 2-ethylhexanol with adipic acid in the presence of an acid catalyst such as sulphuric acid or *para*-toluene sulphonic acid. It was first produced in commercial quantities in western

Europe in the 1940s, in the US in 1945 (US Tariff Commission, 1947) and in Japan in about 1956. Thirteen US companies presently produce di(2-ethylhexyl) adipate; in 1979, 12 companies produced nearly 21.2 million kg (US International Trade Commission, 1980a), up dramatically from the 13.8 million kg produced by 11 US companies in 1975 (US International Trade Commission, 1977). US imports of di(2-ethylhexyl) adipate through the principal customs districts amounted to only 289 thousand kg in 1979 (US International Trade Commission, 1981), down sharply from the 559 thousand kg imported in 1979 (US International Trade Commission, 1980b). Separate data on US exports of di(2-ethylhexyl) adipate are not published, but total US exports of all adipic acid esters of monohydric alcohols in 1980 amounted to 721 thousand kg, almost two-thirds of which was exported to Japan (US Department of Commerce, 1981).

Di(2-ethylhexyl) adipate is believed to be produced by five companies in the Federal Republic of Germany, three in the UK, two in Spain, and one in each of Austria, Belgium, Denmark, Italy, Sweden and Switzerland.

An estimated 19 million kg di(2-ethylhexyl) adipate were made in 1980 by the four Japanese producing companies, down from an estimated 22 million kg produced in 1979.

(*b*) *Use*

Di(2-ethylhexyl) adipate is used primarily as a plasticizer, but significant amounts are also used as a lubricant and functional (hydraulic) fluid.

It is an effective plasticizer and imparts low-temperature flexibility. Because it is more volatile than the corresponding phthalate [see monograph in this volume], it is frequently used in blends with the phthalate in products intended for low-temperature uses. It is used with polyvinyl chloride resins in film, sheeting, extrusions and plastisols; the single most important market is stretchable polyvinyl chloride film used for wrapping meat. Such film may contain 20-30% of the plasticizer (Boettner and Ball, 1980). This chemical is also used in synthetic rubber, nitrocellulose, ethyl cellulose, vinyl copolymers and synthetic rubber. It has also been reported to be useful in plasticizing polyvinyl butyral, cellulose acetate butyrate, polystyrene and dammar wax (Monsanto Co., 1973). A blend of plasticizers including di(2-ethylhexyl) adipate can be used to plasticize an acrylamide-ethylene-vinyl chloride copolymer used as a binder for nonwoven cellulose-nylon blend fibres (Danly and Campbell, 1978).

Di(2-ethylhexyl) adipate was used in polypropylene food wrap (Darby and Sears, 1969), but no recent evidence of this application was found.

This compound has been used as a plasticizer or solvent in the following cosmetics: bath oils, eye shadow, cologne, foundations, rouge, blusher, nail-polish remover, moisturizers and indoor tanning preparations (US Food and Drug Administration, 1978).

Dibasic acid esters of C_8-C_{13}-branched-chain alcohols are used as hydraulic fluids and lubricants and are the bases of all aircraft jet-engine lubricants (Wills, 1980). Although di(2-ethylhexyl) sebacate has been widely used for this purpose (Booser, 1981), the adipate is believed to be used in increasing quantities because it is cheaper.

In western Europe, as much as 70% of the di(2-ethylhexyl) adipate used may be in making polyvinyl chloride food-packaging film. In Japan, also, di(2-ethylhexyl) adipate is used primarily as a plasticizer in flexible polyvinyl chloride products, particularly in stretch film for food packaging.

Di(2-ethylhexyl) adipate is approved for use by the US Food and Drug Administration (1980) as a plasticizer in polymeric substances used in the manufacture of articles which are used in contact with food, and up to 50% may occur as a component of the following products when used in contact with food: adhesives; cellophane; closures with sealing gaskets for food containers; water-insoluble hydroxyethyl cellulose film; and rubber articles intended for repeated use.

2.2 Occurrence

(a) Natural occurrence

Di(2-ethylhexyl) adipate is not known to occur as such in Nature.

(b) Occupational exposure

In 1980, the number of US workers potentially exposed to di(2-ethylhexyl) adipate was estimated to be approximately 11 000 (National Institute for Occupational Safety and Health, 1980). The 1974 National Occupational Hazard Survey estimated that workers primarily exposed to di(2-ethylhexyl) adipate are those involved in the manufacture of plastic materials and resins, rubber products and nonferrous wire (National Institute for Occupational Safety and Health, 1977).

The average concentration of di(2-ethylhexyl) adipate in the air of a meat-wrapping department of a supermarket, as a result of heating polyvinyl chloride film during meat packaging operations, was estimated to be 0.014 ppm [0.2 mg/m^3] (Cook, 1980).

(c) Water and sediments

Di(2-ethylhexyl) adipate has been found in the waste waters from an oil-sands plant in Alberta, Canada (Hrudey *et al.*, 1976).

It has been detected in the Delaware River, at levels of 0.08-0.3 μg/l during winter and 0.02-0.3 μg/l in summer (Sheldon and Hites, 1978). In a study of the transportation of di(2-ethylhexyl) adipate in river water, an initial level of 2000 μg/l from a chemical plant effluent was traced six miles along the Delaware River (through various water-treatment facilities) and into 'finished' drinking-water at Philadelphia, Pennsylvania, where it occurred at a level of 0.002 μg/l (Sheldon and Hites, 1979).

Di(2-ethylhexyl) adipate has been detected in the Monatiquot River in Massachusetts at a concentration of 30 μg/l (Hites, 1973) and in the Great Lakes at levels of 0.01-7.0 μg/l (Hrudey *et al.*, 1976). It was detected in 'finished' drinking-water in New Orleans, Louisiana, at an average concentration of 0.10 μg/l, but not in drinking-water in two smaller, nearby cities. It was found in surface water from Lake Tahoe, California (NIH/EPA Chemical Information System, 1981).

(d) Food, beverages and animal foods

Di(2-ethylhexyl) adipate has been found to migrate to food from polyvinyl chloride packaging film: Levels of up to 2350 mg/m^2 were shown to migrate to meat with a high

fat content (Daun and Gilbert, 1977). In a Japanese study, 0-1.4 mg/kg was found in pineapple, 25.3-29.5 mg/kg in beef and 4.3-25.3 mg/kg in tuna fish (Amano *et al.*, 1978).

(*e*) *Other*

The migration of di(2-ethylhexyl) adipate from plastic packs into blood has been demonstrated, but no levels were reported (Rubin, 1973).

2.3 Analysis

Methods used for the analysis of di(2-ethylhexyl) adipate in various matrices are listed in Table 1.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Two groups of 50 male and 50 female B6C3F_1 mice, six weeks old, were fed diets containing 12 000 or 25 000 mg/kg [ppm] di(2-ethylhexyl) adipate (two batches, 98.1 and 99.7% pure, the former containing at least seven unidentified organic impurities and the second, five, as found by gas-liquid chromatography) for 103 weeks. A control group of 50 male and 50 female mice was available. Survival rates at the end of the study (105-106 weeks) ranged from 72 to 84%. The incidences of hepatocellular adenomas in control, low- and high-dose groups were as follows: males - 6/50, 8/49 and 15/49 ($p = 0.021$); females - 2/50, 5/50 and 6/49, respectively. The incidences of hepatocellular carcinomas in control, low- and high-dose groups were as follows: males - 7/50, 12/49 and 12/49; females - 1/50, 14/50 ($p < 0.001$) and 12/49 ($p = 0.001$), respectively. In females, times to observation of the first hepatocellular tumour were 79, 85 and 106 weeks in the high- and low-dose and in the control group, respectively. No other tumour was related to treatment (National Toxicology Program, 1981).

Rat: Three groups of five-week-old F344 rats, 50 males and 50 females per group, were exposed to diets containing 0 (control), 12 000 or 25 000 mg/kg [ppm] di(2-ethylhexyl) adipate (same samples as used above) for 103 weeks. Survival at 105-107 weeks was 68% in control and low-dose males, and 80% in high-dose males, and 58% in control females, 78% in low-dose females and 88% in high-dose females. There was no difference in the incidence or type of tumours between treated and control animals. The most common tumours were myelomonocytic leukaemia, lymphoma, pituitary tumours and interstitial-cell tumours of the testis (National Toxicology Program, 1981).

Table 1. Methods for the analysis of di(2-ethylhexyl) adipate

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Meat and meat/fat mixtures	Add anhydrous sodium sulphate; extract with hexane; evaporate; saponify with potassium hydroxide in methanol. Evaporate; steam distill residue; extract distillate with diethyl ether. Dry with anhydrous sodium sulphate; evaporate to small volume	GC/FID	not given	Daun and Gilbert (1977)
Polyvinyl chloride thermal degradation products	Rinse sample hood with ethanol	GC/FID	not given	Boettner and Ball (1980)
Water				
River water and sediments	Extract with diethyl ether or hexane	GC/MS	not given	Ogino *et al.* (1979)
River water	Strip volatile organic compounds; add hydrochloric acid; extract with dichloro-methane; transfer to silica gel column; fractionate by successive elution with hexane, benzene, methanol; dissolve fractions in dichloromethane; extract with sodium hydroxide	GC/MS	not given	Sheldon and Hites (1978)
Industrial wastewater, municipal sewage effluent, river water, finished drinking-water	Add hydrochloric acid; extract with dichloromethane; fractionate by liquid chromatography	GC/MS	not given	Sheldon and Hites (1979)
Wastewater streams	Extract with diethyl ether; extract with sodium bicarbonate; elute the diethyl ether-soluble extract on an alumina column successively with hexane, benzene, diethyl ether chloroform and methanol	GC/FID ; GC/MS	not given	Hrudey *et al.* (1976)

a Abbreviations: GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

Single i.p. doses of 100 ml [92 g]/kg bw and 50 ml [46 g]/kg bw di(2-ethylhexyl) adipate to male ICR/Harlan albino Swiss mice and male Sprague-Dawley rats, respectively, were reported to be nonlethal (Singh *et al.*, 1973, 1975). The LD_{50} of a single oral dose in male Carworth-Wistar rats was reported to be 9.11 g/kg bw; the dermal LD_{50} in New Zealand white rabbits was 16.3 ml [15.9 g]/kg bw; and the maximum no-effect level in a 90-day feeding study in rats, 0.16 g/kg bw per day (Smyth *et al.*, 1951).

A 13-week subchronic study was conducted in male and female Fischer 344 rats and $B6C3F_1$ mice using dietary concentrations of 0 to 25 000 mg/kg [ppm]. No compound-related death was observed, but weight gain in male rats was slightly reduced with levels of 12 500 and 25 000 mg/kg. Neither gross nor histopathological changes were observed in any of the treated animals (National Toxicology Program, 1981).

Dietary administration of di(2-ethylhexyl) adipate at a concentration of 2% [20 000 mg/kg] increased liver size, induced hypolipidaemia and hepatic peroxisomal proliferation, and increased the activities of peroxisomal enzymes in the livers of male Fischer 344 rats. These effects on the liver are similar to those of di(2-ethylhexyl) phthalate, clofibrate and related hypolipidaemic drugs (Moody and Reddy, 1978; Reddy, 1981).

Effects on reproduction and prenatal toxicity

Groups of five Sprague-Dawley rats were given i.p. injections of 1, 5 or 10 ml [0.9, 4.5 or 9.2 g]/kg bw di(2-ethylhexyl) adipate on days 5, 10 and 15 of pregnancy. No embryolethality occurred, but reduced foetal weight was seen with the two highest doses (Singh *et al.*, 1973).

Male ICR mice given a single i.p. dose of 0.5-10 ml [0.45-9.2 g]/kg bw and mated for eight successive weeks showed evidence of reduced fertility when given the highest dose (Singh *et al.*, 1975).

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

Di(2-ethylhexyl) adipate did not induce reverse mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100, with or without metabolic activation (Simmon *et al.*, 1977). Single i.p. doses of 0.5-10 ml [0.45-9.2 g]/kg bw to male mice produced dominant lethal mutagenic effects at pre- and post-meiotic stages of spermatogenesis (Singh *et al.*, 1975).

(b) Humans

No data were available to the Working Gorup.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Di(2-ethylhexyl) adipate was tested in mice and rats by oral administration. It induced significant increases in the incidence of liver-cell tumours in male and female mice. No increase in tumour incidence was observed in rats.

The data were inadequate to assess the teratogenicity of this compound to experimental animals.

Di(2-ethylhexyl) adipate induces dominant lethal mutations and reduces fertility in mice. Mutagenicity tests in *Salmonella typhimurium* were negative.

4.2 Human data

Occupational exposure to di(2-ethylhexyl) adipate occurs during its production, its use as a plasticizer and its use as a lubricant and functional fluid. Its presence as a plasticizer in polyvinyl chloride film used for wrapping food may result in exposure of the general public.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No case report or epidemiological study was available to the Working Group.

4.3 Evaluation[1]

There is *limited evidence* that di(2-ethylhexyl) adipate is carcinogenic in mice.

No case report or epidemiological study on this compound was available.

No evaluation could be made of the carcinogenicity of di(2-ethylhexyl) adipate to man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DI(2-ETHYLHEXYL) PHTHALATE

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 117-82-7

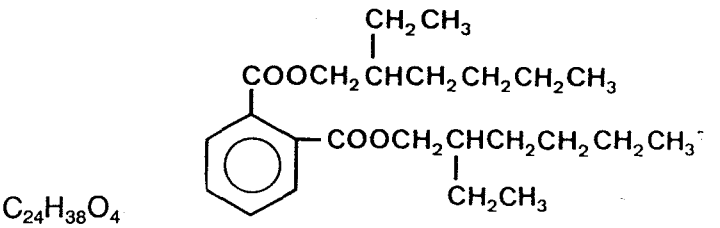
Chem. Abstr. Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester

IUPAC Systematic Name: Phthalic acid, bis(2-ethylhexyl) ester

Synonyms: BEHP; 1,2-benzenedicarboxylic acid, bis(ethylhexyl) ester; bis(2-ethylhexyl) 1,2-benzenedicarboxylate; bis(2-ethylhexyl) ester of phthalic acid; bis(2-ethylhexyl) phthalate; DEHP; di(2-ethylhexyl) *ortho*-phthalate; di(ethylhexyl) phthalate; dioctyl phthalate; DOP; ethylhexyl phthalate; 2-ethylhexyl phthalate; octyl phthalate; phthalic acid dioctyl ester

Trade Names: Bisoflex 81; Bisoflex DOP; Compound 889; DAF 68; Ergoplast FDO; Eviplast 80; Eviplast 81; Fleximel; Flexol DOP; Good-Rite GP 264; Hatcol DOP; Kodaflex DOP; Mollan O; Nuoplaz DOP; Octoil; Platinol AH; Platinol DOP; Pittsburgh; PX-138; Reomol DOP; Reomol D 79P; Sicol 150; Staflex DOP; Truflex DOP; Vestinol AH; Vinicizer 80; Witcizer 312

1.2 Structural and molecular formulae and molecular weight



$C_{24}H_{38}O_4$ Mol. wt: 390.5

1.3 Chemical and physical properties of the pure substance

From Hawley (1981), unless otherwise specified

(*a*) *Description*: Light-coloured liquid

(*b*) *Boiling-point*: 231°C at 5 mm

(*c*) *Melting-point*: -46°C (pour-point)

(*d*) *Density*: d_{20}^{20} 0.9861

(*e*) *Refractive index*: n_D^{20} 1.4836

(*f*) *Spectroscopy data*: Mass spectral data have been reported (NIH/EPA Chemical Information System, 1980).

(*g*) *Solubility*: Insoluble in water; miscible with mineral oil

(*h*) *Viscosity*: 81.4 cP at 20°C

(*i*) *Volatility*: Vapour pressure, 1.32 mm at 200°C

(*j*) *Stability*: Stable (flash-point, 281°C)

(*k*) *Conversion factor*: ppm = 0.626 x mg/m^3

1.4 Technical products and impurities

Di(2-ethylhexyl) phthalate is available in the US in a variety of technical grades. Typical product specifications are: 99.0-99.6% minimal ester content; 0.1% maximal moisture content; 0.007-0.01% acidity (as acetic acid or phthalic acid); specific gravity, 0.980-0.985 (25°/25°C); refractive index, 1.4850-1.4870 (23°C); and minimal flash-point, 216°C (BASF Wyandotte Corp., 1976; Reichhold Chemicals, Inc., undated; Tenneco Chemicals, Inc., undated; US Steel Corp., undated).

In western Europe, di(2-ethylhexyl) phthalate is available with the following specifications: maximal acid value, 0.06; maximal weight loss on heating at 140°C for 3 h, 1%; and saponification value, 284-290 mg KOH/g.

In Japan, di(2-ethylhexyl) phthalate must fulfill the following specifications: maximal acid value, 0.05; maximal weight loss on heating at 125°C for 3 h, 0.10%; and specific gravity, 0.983-0.989 (20°/20°C).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Di(2-ethylhexyl) phthalate is produced commercially by the reaction of excess 2-ethylhexanol with phthalic anhydride in the presence of an acid catalyst such as sulphuric

acid or *para*-toluene sulphonic acid. It was first produced in commercial quantities in Japan in about 1933 and in the US in 1939 (US Tariff Commission, 1940). Nine US companies currently produce it. In 1979, 10 US companies produced about 136 million kg (US International Trade Commission, 1980a), down considerably from the highest production, in 1972, when 14 companies produced 197.5 million kg (US Tariff Commission, 1974). Preliminary data for the production in 1980 of all dioctyl phthalates indicate that only 123.9 million kg were produced (US International Trade Commission, 1981a).

US imports of di(2-ethylhexyl) phthalate are not reported separately; imports of 'dioctyl pthalates, all other' through the principal customs districts in 1980 were 352 thousand kg (US International Trade Commission, 1981b), down sharply from the 1.47 million kg imported in 1979 (US International Trade Commission, 1980b). Total US exports of all dioctyl phthalates in 1980 amounted to 4.45 million kg, mainly to Mexico and the Union of South Africa (US Department of Commerce, 1981).

Di(2-ethylhexyl) phthalate is believed to be produced by seven companies in the Federal Republic of Germany, four companies each in Spain and the UK, three companies in Italy, two companies each in France and The Netherlands, and one company each in Austria, Belgium, Denmark, Finland and Switzerland. The Federal Republic of Germany probably produced the largest volume. Total production of all phthalate plasticizers in western Europe in 1979 is estimated to have been nearly 1000 million kg.

An estimated 240 million kg of di(2-ethylhexyl) phthalate were manufactured in 1980 by the eight Japanese producing companies, down from an estimated 260 million kg produced in 1979. Japanese imports amounted to approximately 5 million kg in 1980, and exports are estimated to have been 18 million kg.

(*b*) *Use*

Di(2-ethylhexyl) phthalate is used primarily as a plasticizer; only a very small percentage is used in other applications, such as dielectric fluids.

It is the most widely used plasticizer. In 1979, use of all phthalate plasticizers in the US amounted to an estimated 533 million kg, equal to approximately 76% of total US plasticizer consumption; the next largest class, epoxy esters, accounted for only about 7% of the total. The estimated 138 million kg of di(2-ethylhexyl) phthalate used in the US in 1979 represented nearly 26% of total US phthalate plasticizer consumption.

Addition to polyvinyl chloride (PVC) and vinyl chloride copolymer resins [see IARC, 1979] (at typical levels of 50%) is the dominant market for all plasticizers (representing about 84% of total US use in 1979), for phthalate plasticizers (about 90% of the total), and even more so for di(2-ethylhexyl) phthalate. Di(2-ethylhexyl) phthalate is the plasticizer used preferentially in many flexible polyvinyl chloride products (e.g., calendered film, sheeting and coated fabrics) because of its properties: low extraction by oil and water, good stability to heat and light and fast production rates. It is frequently used in blends with other plasticizers (e.g., adipates) when special properties are needed.

The relatively small amount of di(2-ethylhexyl) phthalate used otherwise than in polyvinyl chloride is believed to be in plasticizing a variety of polymeric materials: natural rubber, synthetic rubber (e.g., acrylonitrile-butadiene types and chlorinated rubber), cellulose acetate butyrate, ethyl cellulose, nitrocellulose, polymethyl methacrylate, polyvinyl butyral, polystyrene, and polyvinylidine chloride.

The only significant non-plasticizer use for di(2-ethylhexyl) phthalate is as a replacement for polychlorinated biphenyl in dielectric fluids for electrical capacitors. Up to 7-9 million kg of di(2-ethylhexyl) phthalate may have been used in this way annually in recent years.

The following miscellaneous uses for di(2-ethylhexyl) phthalate have been reported: as a solvent in eraseable ink; as an acaricide for use in orchards; as an inert ingredient in pesticides; as a component of cosmetic products; as a vacuum-pump oil; in detecting leaks in respirators; and in the testing of air filtration systems. Several of these reported applications are believed to be no longer practised or never to have been carried out on a commercial scale.

In Japan, the estimated 227 million kg of di(2-ethylhexyl) phthalate used in 1980 was almost exclusively in the production of flexible polyvinyl chloride plastics.

Eight countries have been reported to limit occupational exposure to di(2-ethylhexyl) phthalate by regulation or recommended guideline. The standards are listed in Table 1.

Table 1. National occupational exposure limits for di(2-ethylhexyl) phthalate[a]

Country	Year	Concentration mg/m^3	Interpretation[b]	Status
Australia	1978	5	TWA	Guideline
Czechoslovakia	1976	5	TWA	Regulation
		10	Ceiling	
Federal Republic of Germany	1979	10	TWA	Guideline
The Netherlands	1978	5	TWA	Guideline
Romania	1975	2	TWA	Regulation
		5	Maximum	
Switzerland	1978	5	TWA	Regulation
USA				
OSHA	1980	5	TWA	Regulation
ACGIH	1981	5	TWA	Guideline
		10	STEL	
Yugoslavia	1971	5	TWA	Regulation

a From American Conference of Govermental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980)

b TWA, time-weighted average; STEL, short-term exposure limit; OSHA, Occupational Safety and Health Administration

The US Environmental Protection Agency (1980a) has identified di(2-ethylhexyl) phthalate as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. The EPA has also identified this compound as a hazardous constituent of water or caustic cleaning wastes from equipment and tank cleaning from paint manufac-

turing and emission control dust or sludge from paint manufacturing (US Environmental Protection Agency, 1980b).

Under the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of di(2-ethylhexyl) phthalate are subject to a variety of labelling, packaging, quantity and shipping regulations consistent with its designation as a hazardous material.

Because di(2-ethylhexyl) phthalate had been approved for certain uses in the US before 6 September 1958, it was designated a 'prior-sanctioned' food ingredient; and it is thus exempt from classification as a food additive by the US Food and Drug Administration when used as a plasticizer which may migrate to foods of high water content from food packaging materials. It is approved for use, subject to certain limitations, as a component of the following products when used in contact with food: adhesives; coatings; defoaming agents used in the manufacture of paper and paperboard; flow promoters in acrylic and modified acrylic plastics; cellophane; and lubricants for rolling metallic foil or sheet stock (US Food and Drug Administration, 1980).

The Japanese Ministry of Health and Welfare has established three separate regulations with regard to di(2-ethylhexyl) phthalate to limit its migration into fats, oils and fatty foodstuffs; liquors, wines and alcoholic beverages; and other foodstuffs (Omori, 1976).

2.2 Occurrence

The Working Group noted that the occurrence of di(2-ethylhexyl) phthalate in some matrices has been reported, but was subsequently found to have resulted from contamination of the matrix by plasticizer extracted from plastic tubing or other equipment.

(*a*) *Natural occurrence*

Di(2-ethylhexyl) phthalate has been reported to be a possible natural product in animals and plants (Mathur, 1974; Peakall, 1975).

(*b*) *Occupational exposure*

In 1980, the number of US workers potentially exposed to di(2-ethylhexyl) phthalate was estimated to be about 625 000 (National Institute for Occupational Safety and Health, 1980). Major industries in which exposure was judged to be highest were plastics and rubber products manufacture, blast-furnace and steel-mill operations, nonferrous wire manufacture and motor vehicle and aircraft manufacture (National Institute for Occupational Safety and Health, 1977a).

Di(2-ethylhexyl) phthalate was detected at levels of 0.4-3.2 mg/m^3 [0.25-2 ppm] in the air of a factory in which coatings were applied to a variety of products (Ruhe and Donohue, 1979).

(*c*) *Air*

Di(2-ethylhexyl) phthalate has been detected in air over ocean water at levels ranging from 0.4 ng/m^3 [0.25 ppt] over the Gulf of Mexico to 2.9 ng/m^3 [1.8 ppt] over the North

Atlantic (Giam *et al.*, 1978). A later study showed the average level over the Gulf of Mexico to be 1.16 ng/m^3 [0.73 ppt] (Giam *et al.*, 1980).

(*d*) *Water and sediments*

Di(2-ethylhexyl) phthalate has been detected in the Missouri River (5 μg/l), in a rural industrial bay of Lake Superior (300 μg/l), and in the Charles River in Boston, Massachusetts (0.9-1.9 μg/l) (Hrudey *et al.*, 1976).

Di(2-ethylhexyl) phthalate was found in surface water at average levels of 80 ng/l in open water in the Gulf of Mexico, 130 ng/l near the coast and 5 ng/l in the North Atlantic, compared with an average level of 70 ng/l found in the Mississippi delta. Ocean sediments from the Gulf of Mexico contained an average level of 2 ng/g, whereas 69 ng/g were found in the Mississippi delta (Giam *et al.*, 1978).

In the vicinity of a US specialty chemicals plant, di(2-ethylhexyl) phthalate was detected in river water at levels ranging from 0.001-0.05 mg/l, and in river sediment at levels ranging from 0.2-56 mg/l (Hites *et al.*, 1979).

Dioctyl phthalates (believed to include di(2-ethylhexyl) phthalate) were identified in the Delaware River at levels of 3-5 μg/l during winter and 0.06-2 μg/l during summer (Sheldon and Hites, 1978).

In Japan, di(2-ethylhexyl) phthalate was detected in stream waters in the Miyashiro Prefecture at levels of up to 7.6 μg/l (Takahashi *et al.*, 1976), and in river water, river water sediment and drinking-water in the Shizuoka Prefecture (Shigeo, 1979). Rivers, lakes and harbours in Hokkaido, Japan, were found to contain levels ranging from 0.3-8.7 μg/l (Satoh *et al.*, 1977). This compound was detected in the Tama River in Tokyo at a concentration of 2-5 μg/l (Hrudey *et al.*, 1976) and in another river at a level of 10 μg/l (Mori, 1976).

Water samples from the Rhine, Ijssel and Meuse rivers in The Netherlands were found to contain di(2-ethylhexyl) phthalate at an average concentration of 1 μg/l (Schouten *et al.*, 1979). Sediments from the Rhine and Ijssel rivers contained 1-70 mg/l, and those from the Meuse river, 1-17 mg/l (Schwartz *et al.*, 1979).

Di(2-ethylhexyl) phthalate has been detected in the waste waters from an oil-sands plant in Alberta, Canada (Hrudey *et al.*, 1976). It was also identified in waste waters generated from US water-base paint manufacturing plants at an average concentration of 400 μg/l (US Environmental Protection Agency, 1980c), and from ink manufacturing plants at an average concentration of 12 500 μg/l (US Environmental Protection Agency, 1980d).

In a study of the transportation of di(2-ethylhexyl) phthalate in river water, an initial level of 200 μg/l from industrial effluents was traced six miles along the Delaware River (through various water-treatment facilities) and into 'finished' drinking-water in Philadelphia, Pennsylvania, where it occurred at a level of 0.6 μg/l (Sheldon and Hites, 1979).

Di(2-ethylhexyl) phthalate was deteced in tap-water in Chicago, Illinois, at an average level of approximately 1 μg/l and in Tokyo, Japan, at levels in the range of 1.2-1.8 μg/l. It was identified in 'finished' drinking-water in New Orleans, Louisiana, at average concentrations of 0.05-11 μg/l, in two nearby smaller cities at levels of 0.16-1.2 μg/l, and

in several major eastern US cities at an average level of approximately 1 μg/l (NIH/EPA Chemical Information System, 1981). Two surveys by the US Environmental Protection Agency in 1975 indicated that the highest concentrations of di(2-ethylhexyl) phthalate found in drinking-water were 30 μg/l in Miami, Florida, and 17 μg/l in Cincinnati, Ohio (National Research Council, 1977).

Di(2-ethylhexyl) phthalate was detected in well waters in Japan at levels of 3.85-4.82 μg/l, and in deionized water at a level of 1.31 μg/l (Ishida *et al.*, 1980).

(*e*) *Soil and plants*

Di(2-ethylhexyl) phthalate has been detected in soils under native range and crested wheatgrass in Alberta, Canada (Dormaar *et al.*, 1980).

(*f*) *Food, beverages and animals feeds*

Levels of di(2-ethylhexyl) phthalate not exceeding 1 mg/kg were detected in fish from Long Island Sound, New York, San Francisco Bay, California, and Lake Michigan; most fish were found to contain less than 0.2 mg/kg. In a sampling of a wide variety of foods, the highest levels of di(2-ethylhexyl) phthalate were found in milk (31.4 mg/l, fat basis) and in cheese (35 mg/kg, fat basis) (Hartung, 1976).

Di(2-ethylhexyl) phthalate was found in rice, beef, pork, chicken and some vegetables in Japan (Kato *et al.*, 1979).

In a study of migration of di(2-ethylhexyl) phthalate from plastic packaging films, such as polyvinyl chloride film, in Japan, 0.11-68 mg/kg were found in tempura (frying) powder, 0.04-3 mg/kg in instant cream soup, 0.05-9.1 mg/kg in fried potato cake and 0.05 mg/kg in orange juice (US Environmental Protection Agency, 1980e).

(*g*) *Animals*

Di(2-ethylhexyl) phthalate was detected in biota, including fish, crabs, shrimps, eels and rays, in the open ocean at an average concentration of 4.5 ng/g (Giam *et al.*, 1978).

Residues of di(2-ethylhexyl) phthalate are commonly found in fresh-water fish and especially in those from industrialized areas (Stalling *et al.*, 1973). It was found in siskiwit trout from an island lake on Lake Superior at levels of 0.2-0.7 μg/g wet weight (Swain, 1978). Fathead minnows exposed to 4.6 μg/l ^{14}C-di(2-ethylhexyl) phthalate in water for 56 days concentrated the material 886-fold (Mehrle and Mayer, 1976).

This compound was identified as a component of liquid swine manure at a level of 0.24 ng/l (raw manure) (Yasuhara and Fuwa, 1979).

(*h*) *Human tissues and secretions*

Di(2-ethylhexyl) phthalate was detected in whole blood that had been stored in polyvinyl chloride blood bags at levels ranging from 75-109 mg/l (Pik *et al.*, 1979), and in plasma stored in such bags at levels up to 250 mg/l. Another study showed concentrations of 66 mg/l in blood stored in these bags (National Toxicology Program, 1982). Blood stored at 4°C in such bags leached di(2-ethylhexyl) phthalate from the plastic at a linear rate of approximately 2.5 $\pm$ 0.3 mg/l per day (Jaeger and Rubin, 1972).

Table 2. Methods for the analysis of di(2-ethylhexyl) phthalate

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect on cellulose membrane filter; extract (carbon disulphide)	GC/FID	range: 2.03-10.9 mg/m^3 [1.27-6.82 ppm] for a 32-litre sample at 23°C	National Institute for Occupation al Safety and Health (1977b)
	Absorb on Florisil or glass-fibre filters; desord	GC/FID	10 μg/Florisil tube; < 20 μg/ filter	Ruhe and Donohue (1979)
Marine air	Trap on glass-fibre filters with foam plugs; Soxhlet extract (petroleum ether); concentrate extracts; clean-up on deactivated Florisil columns	GC/ECD	0.1 ng/m^3	Giam *et al.* (1980)
Airborne parti-culate matter	Soxhlet extract (methanol); concentrate; centrifuge	GC/MS	not given	Karasek *et al.* (1978)
Water				
River water	Extract (hexane); filter	HPLC/UV (normal and reversed-phase adsorption chromatography and gel chromatography)	2 ng at 224 nm	Mori (1976)
Industrial waste water; municipal sewage effluent, river water, finished drinking-water	Add hydrochloric acid; extract (dichloromethane); clean-up by liquid chroma-tographic fractionation	GC/MS (EI and CI modes); with SIM	not given	Sheldon and Hites (1979)
River sediment	Freeze-dry; homogenize; extract (hexane: acetone: methanol); evaporate; dissolve (hexane); filter	HPLC/UV (233 nm)	10 ng	Schwartz *et al.* (1979)

Biological				
Human serum	Centrifuge; extract (chloroform: methanol); evaporate; dissolve (ethyl acetate); treat with alumina; decant; rinse; filter; evaporate; dissolve residue (hexane-containing butyl benzyl phthalate as an internal standard)	GC/FID; GC/MS	50 µg/l	Lewis *et al.* (1977)
Stored blood: (1) platelet-rich or -poor plasma; washed red-blood cells; (2) washed platelets	Lyophilize (1) with chloroform: methanol mixture; filter; wash residue (chloroform: methanol); mix with distilled water; centrifuge; add silicic acid to chloroform phase; mix; centrifuge; decant; evaporate; dissolve (methanol); centrifuge. Extract (2) as above without lyophilization	GC/FID	not given	Contreras *et al.* (1974)
Human and animal tissue and urine	Grind wet tissue samples in saline; extract homogenate or urine; dilute with chloroform: methanol	GC/FID	0.3 µg/g (wet tissue): 15 ng/ml (urine)	Chen *et al.* (1979a,b)
Laboratory supplies				
Water	Wash (hexane); evaporate; dissolve (diethyl ether)	GC/FID	not given	Ishida *et al.* (1980)
Organic solvents (e.g., benzene)	Evaporate; dissolve (diethyl ether)			
Solid reagents (e.g., Florisil)	Immerse in 1:1 chloroform: methanol; filter; rinse; evaporate			
Aluminium foil and other materials (e.g., rubber tubing)	Cut into small pieces; immerse in 1:1 chloroform: methanol; extract			
Intravenous solutions	Add hydrochloric acid; extract (dichloromethane) ; redissolve	GC/ECD (simultaneous determinations of the mono- and di-ester)	4 µg/l	Albin and Ostelius (1980)

a Abbreviations: GC/FID, gas chromatography/flame-ionization detection; GC/ECD, gas chromatography/electron capture detection; GC/MS, gas chromatography/mass spectrometry; HPLC/UV, high-performance liquid chromatography/ultraviolet spectroscopy; EI, electron impact; CI, chemical ionization; SIM, selected ion monitoring

In one study of subjects who received haemodialysis, blood transfusions or blood that had previously been in contact with polyvinyl chloride medical products, di(2-ethylhexyl) phthalate was found at the following levels (μg/g wet tissue): brain (1.9), heart (0.5), kidney (1.2-2.2), liver (1.5-4.6), lung (1.4-2.2) and spleen (2.2-4.7) (Chen *et al.*, 1979a).

The levels of di(2-ethylhexyl) phthalate in neonatal heart tissue from infants who had undergone umbilical catheterization, either alone or with the administration of blood products, were reported to be higher than those in similar tissue from untreated infants (Hillman *et al.*, 1975).

(*i*) *Other*

Di(2-ethylhexyl) phthalate was detected in particulate and gaseous hydrocarbons from diesel-fuel exhaust gases (Cuthbertson *et al.*, 1979).

The compound has been detected in commercial organic solvents: diethyl ether (43.6 μg/l), methane (78.7 μg/l), ethanol (61.7 mg/l), acetonitrile (180 μg/l) and benzene (1960 μg/l) (Ishida *et al.*, 1980).

2.3 Analysis

Methods used for the analysis of di(2-ethylhexyl) phthalate in environmental samples are listed in Table 2.

A method has been described for identifying phthalate esters, including di(2-ethylhexyl) phthalate, in mixtures by gas chromatography-chemical ionization-mass spectrometry, which could be used to identify components of a mixture of phthalate esters separated by gel filtration from lipids or biological and environmental materials (Addison, 1979).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Groups of 50 male and 50 female, 6-week-old $B6C3F_1$ mice were given diets containing 0 (control), 3000 or 6000 mg/kg [ppm] di(2-ethylhexyl) phthalate (>99.5% pure, with two impurities found by gas-liquid chromatography) for 103 weeks. At the end of the study (105 weeks), an adequate number of animals were still alive in which late-appearing tumours could be observed. A significant incidence of liver-cell tumours was observed in the treated compared with control animals. The incidences of hepatocellular adenomas in control, low- and high-dose groups were as follows: males - 6/50, 11/48 and 10/50; females - 1/50, 5/50 and 1/50, respectively. The incidences of hepatocellular

carcinomas in control, low- and high-dose groups were as follows: males - 9/50, 14/48 and 19/50 ($p = 0.022$) (positive trend: $p = 0.018$); females - 0/50, 7/50 ($p = 0.006$) and 17/50 ($p < 0.001$) (positive trend: $p < 0.001$), respectively. Metastases of hepatocellular carcinomas were found in the lungs of 7 low-dose and 5 high-dose males and of 1 low-dose and 7 high-dose females. No metastatic hepatocellular carcinomas occurred in the lungs of control mice of either sex. No other neoplastic lesion was found to be associated with the treatment (National Toxicology Program, 1982).

Rat: Two groups of 50 male and 50 female, 5-6 week old Fischer 344 rats were maintained on a diet containing 6000 to 12 000 mg/kg [ppm] di(2-ethylhexyl) phthalate (same sample as above) for 103 weeks. Another group of 50 males and 50 females served as controls. The animals were killed at 104-105 weeks; no difference in survival was observed between treated and control rats. Chronic exposure to the compound was associated with the appearance of liver-cell tumours: The incidences of neoplastic nodules in controls, low- and high-dose groups were as follows: males - 2/50, 5/49 and 7/49; females - 0/50, 4/49 and 5/50 ($p = 0.028$) (positive trend: $p = 0.03$), respectively. The incidences of hepatocellular carcinomas in controls, low- and high-dose groups were as follows: males - 1/50, 1/49 and 5/49 (positive trend: $p = 0.047$); females - 0/50, 2/49 and 8/50 ($p = 0.003$) (positive trend: $p = 0.002$), respectively. No other neoplastic lesion or tumour was found to be positively associated with the treatment (National Toxicology Program, 1982).

[The Working Group was aware of two other studies by oral administration (Carpenter *et al.*, 1953; Harris *et al.*, 1956), but these were considered inadequate for an evaluation of carcinogenicity due to the small numbers of animals tested.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Acute, single-dose LD_{50} values for di(2-ethylhexyl) phthalate are listed in Table 3.

Table 3. LD_{50} values for di(2-ethylhexyl) phthalate in several species

Species	Sex	Route	LD_{50}	Reference
Rat, Wistar	male	oral	30.6 g/kg bw	Shaffer *et al.* (1945)
Rat, Wistar	male	i.p.	30.7 g/kg bw	Shaffer *el al.* (1945)
Mouse	(unspecified)	oral	33.5 g/kg bw	Krauskopf (1973)
Mouse, ICR	male	i.p.	37.8 g/kg bw	Lawrence *et al.* (1975)
Rabbit, albino	male	oral	33.9 g/kg bw	Shaffer *et al.* (1945)
Guinea-pig	(unspecified)	oral	26.3 g/kg bw	Krauskopf (1973)

The animals in the studies by Shaffer *et al.* (1945) were observed for 14 days after treatment: deaths tended to occur several days post-exposure, and 2/6 rabbits died several days after dermal exposure to 19.7 g/kg bw di(2-ethylhexyl) phthalate. The lethal effect of this compound appears to be cumulative, since the chronic LD_{50} value for i.p. administration to ICR mice five times weekly for 10 weeks was 1.36 g/kg bw, in comparison to a single-dose value of 37.8 g/kg bw (Lawrence *et al.*, 1975).

As could be predicted from the LD_{50} values, a high concentration of di(2-ethylhexyl) phthalate must be incorporated into the diet to produce acutely toxic effects. Shaffer *et al.* (1945) reported no deaths in male, albino rats given 30 000 mg/kg [ppm] (~ 1.9 g/kg bw per day) in the diet for 90 days, although body weight gain was reduced with this level, as well as with 15 000 mg/kg; no effects were observed with 7500 mg/kg. The only pathological lesion was testicular degeneration (see below). Similar findings were reported for Fischer 344 rats fed up to 25 000 mg/kg [ppm] for 90 days (National Toxicology Program, 1982) and in a 120-day feeding study in rats using 2000-20 000 mg/kg (Gray *et al.*, 1977). In the latter study, however, packed-cell volume (haematocrit) was reduced and liver size increased with the 10 000 mg/kg level; there was a reduction in renal concentrating and diluting ability in females receiving the 20 000 mg/kg level. Despite these functional effects, the microscopic appearances of the livers and kidneys in all animals were normal.

Di(2-ethylhexyl) phthalate produced increases in kidney weights, with renal cysts in mice fed diets containing 2500 or 25 000 mg/kg [ppm] (Omori, 1976). In $B6C3F_1$ mice, body weight gain was decreased with levels of 6300 or 12 500 mg/kg in the diet for 90 days, but no pathological effects were observed (National Toxicology Program, 1982).

Oral administration of 2 g/kg bw per day to rats for 21 days caused liver enlargement, hepatic mitochondrial swelling and a selective loss of succinic dehydrogenase activity in the periportal zone of the liver (Lake *et al.*, 1975; Mangham *et al.*, 1981). Daily oral administrations of di(2-ethylhexyl) phthalate to rats for six days or one month increased hepatic cytochrome P-450 concentration and the activities of some 'hepatic phase II drug metabolizing enzymes' (epoxide hydratase, UDP glucuronosyl transferase). The treatment had no effect on or reduced the activities of some 'hepatic phase I drug-metabolizing enzymes' (ethoxy coumarin deethylase, benzo[*a*]pyrene hydroxylase, aniline hydroxylase) (Aitio and Parkki, 1978; Zuccato *et al.*, 1980). Hexobarbital and pentobarbital sleeping times in mice were prolonged by pretreatment with di(2-ethylhexyl) phthalate (Rubin and Jaeger, 1973; Lawrence *et al.*, 1975), but aminopyrine serum half-life was unaffected (Zuccato *et al.*, 1980).

Oral treatment of Fischer 344 rats with di(2-ethylhexyl) phthalate increased the concentration of peroxisomes and the activities of peroxisomal enzymes in the liver, and reduced the concentrations of cholesterol and triglycerides in serum (Reddy *et al.*, 1976). Hepatic peroxisomal and mitochondrial fatty acyl-CoA oxidizing activities in rats and mice were also increased by such treatment (Osumi and Hashimoto, 1978,1979). These effects on the liver are similar to those of clofibrate and other hypolipidaemic drugs (Reddy, 1981).

Administration of a concentration of 4000 mg/kg [ppm] in the diet did not alter survival of male or female Sherman rats exposed throughout most of their life (Carpenter *et al.*, 1953). Male and female Wistar rats were similarly unaffected by two years' exposure to 5000 mg/kg in the diet, except that body weight gain was reduced (Harris *et al.*, 1956). Thirty percent mortality was reported in rats fed 3500 mg/kg in the diet for one year (10% in controls), but the cause of death was not determined (Nikoronow *et al.*, 1973).

Weekly i.v. infusions of blood from di(2-ethylhexyl) phthalate plasticized polyvinyl chloride blood bags (cumulative dose, 20.5-108 mg di(2-ethylhexyl) phthalate) into rhesus monkeys produced focal parenchymal liver necrosis and imflammatory cell infiltration, as well as a subtle change in the kinetics of exogenously-administered sodium sulphobromophthalein (BSP). Controls received blood stored in polyethylene containers (Jacobson *et al.*, 1977). [The number of animals used was small, however, and the Working Group did not consider the results to provide definitive evidence of the toxicity of di(2-ethylhexyl) phthalate.]

Human blood stored under standard blood bank conditions (polyvinyl-chloride packs) for 21 days contained 0.15 mM [59 μg/ml] of the plasticizer. Concentrations of 0.10 mM [39 μg/ml] inhibited the growth of cultured human diploid fibroblasts (Jacobson *et al.*, 1974).

The concentration of di(2-ethylhexyl) phthalate that inhibited growth of nine-day cultures of (human) WI-38 cells by 50%, as measured by protein synthesis, was reported to be 70 μM [2.7 μg/ml]. The toxic effect on the cultured cells, as measured by changes in protein concentration, was more prominent in replicating than in nonreplicating cells (Jones *et al.*, 1975).

Addition of 4 μg/ml di(2-ethylhexyl) phthalate *in vitro* caused dysfunction of cultured chicken embryo cells within 30 min of exposure, and cell death within 24 hours (Rubin and Jaeger, 1973).

Effects on reproduction and prenatal toxicity

Several studies in rats have demonstrated testicular damage following administration of di(2-ethylhexyl) phthalate (Shaffer *et al.*, 1945; Gray *et al.*, 1977; National Toxicology Program, 1982), the degree of which appears to be directly proportional to both the size of the dose and the length of exposure: Administration of 20 000 mg/kg [ppm] in the diet produced seminiferous tubular degeneration and testicular atrophy within seven days (Oishi and Hiraga, 1980a); 12 500 mg/kg produced similar effects within 90 days; and 6000 mg/kg produced the same effects by the end of two years of exposure (National Toxicology Program, 1982).

Reduced fertility has also been observed in ICR male mice given a single i.p. injection of 12.8, 19.2 or 25.6 ml/kg bw (Dillingham and Autian, 1973). However, mice fed a diet containing 20 000 mg/kg [ppm] did not show testicular atrophy (Oishi and Hiraga, 1980b).

Embryolethal and teratogenic effects of di(2-ethylhexyl) phthalate have been demonstrated in mice with single and repeated oral administration. Daily doses of 4000 or 10 000 mg/kg of diet [ppm] to ICR-JCL mice throughout pregnancy caused complete resorption; 1000 or 2000 mg/kg caused embryolethality and teratogenic effects of the central nervous system; while no adverse effect was observed with 500 mg/kg (Shiota *et al.*, 1980). Single doses of 0.05 g/kg bw given on day 7 of gestation to random-bred mice produced decreased foetal weight; embryolethality and malformed foetuses occurred with 0.1 g/kg bw (Nakamura *et al.*, 1979).

Teratogenic effects were also observed in CBA mice following a single oral administration of doses representing 1/3-1/12 of the acute LD_{50} dose (29.6 g/kg bw) on day 6 to 10 of gestation. Excess foetal deaths were observed when higher doses were given on day 7, but not when given on day 9 or 10. A significant number of external and skeletal malformations were found in the group given 7.5 g/kg bw on day 8 (Yagi *et al.*, 1976).

A metabolite of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, was more active than the parent compound in producing embryolethal and teratogenic effects in mice, suggesting that di(2-ethylhexyl) phthalate may act by conversion to this active metabolite (Yagi *et al.*, 1980).

Di(2-ethylhexyl) phthalate and its metabolites cross the placenta in rats (Singh *et al.*, 1975). Embryolethal and teratogenic effects in Sprague-Dawley rats were first demonstrated by Singh *et al.* (1972), with i.p. injections of 5 or 10 g/kg bw on days 5, 10 and 15 of gestation. Some or all of the following effects that were not seen in controls were observed: resorptions, gross abnormalities, foetal death or decreased foetal size.

I.p. administration to small numbers of Sprague-Dawley rats of 2 g/kg bw on days 3, 5 and 9 of gestation adversely affected both implantation and parturition with haemmorrhages and (on days 6 or 9) retention of foetuses at term (Peters and Cook, 1973). Sherman rats fed diets containing up to 4 g/kg di(2-ethylhexyl) phthalate (equivalent to 200 mg/kg bw per day) for two generations showed no adverse effects on reproduction, except for some decrease in body weight and increased liver and kidney weight in animals of both generations; 60 mg/kg bw per day was the 'no effect' level (Carpenter *et al.*, 1953). Reduced foetal and placental weights but no other adverse effects were seen in Wistar rats given up to 1700 mg/kg bw per day by gavage throughout pregnancy (Nikonorow *et al.*, 1973).

No significant effect on dams was observed when doses of up to 1% di(2-ethylhexyl) phthalate were administered in food to JCL/ICR mice; but there was significant embryolethality and teratogenicity with a dose of 0.1% DEHP. At higher doses, both maternal and embryonal toxicity were observed (Hamano *et al.*, 1977).

Plasma extracts of two samples of polyvinyl chloride containing di(2-ethylhexyl) phthalate as plasticizer, were injected intravenously on days 6-15 of gestation into a group of 25 Sprague-Dawley rats, giving average daily doses of about 1.5 and 5.0 mg/kg bw of the plasticizer. No adverse effect was observed on pregnancy, implants, resorptions, malformations or foetal weights (Lewandowski *et al.*, 1980).

Absorption, distribution, metabolism and excretion

Di(2-ethylhexyl) phthalate is well absorbed from the gut of rats, since more than 90% of the activity of a dose of 2000 mg/kg [ppm] of the ^{14}C-compound ingested from the diet is excreted in the urine (Williams and Blanchfield, 1974). Little, if any, is absorbed orally as the intact diester compound, since both intestinal cells and the gut contents from several species are efficient at degrading this compound to mono(2-ethylhexyl) phthalate and 2-ethylhexanol (Carter *et al.*, 1974; Rowland, 1974; Lake *et al.*, 1977; Rowland *et al.*, 1977).

Radioactivity is uniformly distributed thoughout the body and rapidly excreted following oral administration of ^{14}C-labelled di(2-ethylhexyl) phthalate to several species of experimental animals. Twenty-four hours after oral treatment of rats with 500 or 800 mg/kg [ppm] ^{14}C-DEHP, radioactivity was present primarily in the excretory organs (liver, kidney), the gut and body fat (Williams and Blanchfield, 1974; Tanaka *et al.*, 1975).

There is little or no tissue accumulation of radioactivity in rats following repeated dietary treatment with ^{14}C-di(2-ethylhexyl) phthalate. Highest concentrations were found in liver (110-165 mg/kg) and fat (60-80 mg/kg) after subchronic exposure of rats to 5000

mg/kg [ppm] in the diet. Estimated tissue half-lives for this compound (and its metabolites) in rats are 3-5 days for fat and 1-2 days for other tissues (Daniel and Bratt, 1974).

Rats have been reported to metabolize di(2-ethylhexyl) phthalate to 5-keto-2-ethylhexyl phthalate, 5-carboxyl-2-ethylpentyl phthalate, 5-hydroxy-2-ethylhexyl phthalate and 2-carboxymethylbutyl phthalate after initial hydrolysis to mono(2-ethylhexyl) phthalate (Albro *et al.*, 1973; Daniel and Bratt, 1974). Only a small percentage (<5%) of the administered dose was hydrolysed completely to phthalic acid, and no synthetic metabolism has been detected (Albro *et al.*, 1973; Daniel and Bratt, 1974; Williams and Blanchfield, 1974; Tanaka *et al.*, 1975). The major routes of elimination are urinary and biliary excretion.

African green monkeys (Albro *et al.*, 1981) and ferrets (Lake *et al.*, 1976), in contrast to rats, excrete di(2-ethylhexyl) phthalate metabolites in urine as glucuronide derivatives of mono(2-ethylhexyl) phthalate. Glucuronidation appears to occur at the free carbonyl group, while the 2-ethylhexyl substituent is oxidized to an alcohol.

Maintenance of rats on diets containing 5000 to 40 000 mg/kg [ppm] of the compound for four weeks lowered the concentrations of serum glucose and cholesterol and elevated those of serum-free fatty acid and ketone bodies (Sakurai *et al.*, 1978; Yanagita *et al.*, 1978). Livers were enlarged, deficient in glycogen and triglycerides and contained excessive amounts of phospholipids. This response is indicative of a transformation from glycolysis to lipolysis as an energy source. Sakurai *et al.* (1978) concluded that di(2-ethylhexyl) phthalate inhibited gluconeogenesis by preventing the conversion of 3-phosphoglycerate to fructose 1,6-diphosphate. Since this effect, like the inhibition of serum dehydrogenase activity, occurred in both target and non-target tissues, it may not be related to any direct toxic effect of the compound. However, the switch from hepatic glycolysis to lipolysis occurred at dietary concentrations in the range of those utilized in a chronic study [see section 3.1 (*g*)] (National Toxicology Program, 1982).

Mutagenicity and other related short-term tests

Di(2-ethylhexyl) phthalate did not induce reverse mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100, with or without metabolic activation (Simmon *et al.*, 1977).

It did not induce chromosomal aberrations in Chinese hamster cells (Abe and Sasaki, 1977; Ishidate and Odashima, 1977), human leucocytes (Stenchever *et al.*, 1976; Tsuchiya and Hattori, 1976) or human foetal lung cells (Stenchever *et al.*, 1976). It induced a non-dose-dependent, but significant (greater than two-fold background) increase in sister chromatid exchanges in Chinese hamster cells (Abe and Sasaki, 1977).

A single i.p. dose of 12.7-25.5 g/kg bw di(2-ethylhexyl) phthalate induced a small but significant dominant lethal effect in male mice (Dillingham and Autian, 1973). In another study, 12.5 or 15.0 g/kg bw of the parent comound or 175 or 350 mg/kg bw of monoethylhexyl phthalate administered once orally to ICR mice showed no dominant lethal activity (Hamano *et al.*, 1979).

(b) Humans

Toxic effects

Dermally-applied di(2-ethylhexyl) phthalate was judged to be moderately irritating, but only slightly or non-sensitizing, to human skin (Shaffer *et al.*, 1945; Mallette and von Haam, 1952). A single human subject who swallowed 10 g of the compound experienced gastritis and purgation, while a subject who swallowed 5 g did not (Shaffer *et al.*, 1945).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, metabolism and excretion

Intravenously administered di(2-ethylhexyl) phthalate is cleared rapidly from the serum; the majority is excreted in the urine within 24 hours post-transfusion (Rubin and Schiffer, 1976; Peck *et al.*, 1978). It was reported in an abstract that humans excrete this compound in urine primarily as derivatives of mono(2-ethylhexyl) phthalate glucuronide (Peck *et al.*, 1978).

Small amounts of di(2-ethylhexyl) phthalate (or its metabolites) have been isolated from human biopsies or autopsied materials; the presence of the plasticizer could not always be correlated with a history of blood transfusions (Jaeger and Rubin, 1972; Rubin and Nair, 1973; Mes *et al.*, 1974; Mes and Campbell, 1976; Overturf *et al.*, 1979).

Levels of 0.06 $\pm$ 0.02 mg/kg fresh weight have been reported in the placentas of 10 normal births of women living in Birmingham (Poole and Wibberley, 1977).

Mutagenicity and other chromosomal effects

Occupational exposure to atmospheric levels of 0.0006-0.01 ppm [0.001-0.016 mg/m^3] di(2-ethylhexyl) phthalate for 10-34 years did not increase the frequency of chromosomal aberrations in blood leucocytes (Thiess and Fleig, 1979).

3.3 Case reports and epidemiological studies of carcinogenicity in humans

In a small prospective cohort study, eight deaths were observed among 221 workers exposed to di(2-ethylhexyl) phthalate for periods of 3 months to 24 years. One carcinoma of the pancreas and 1 bladder papilloma were reported (Thiess *et al.*, 1978).

4. Summary of Data Reported and Evaluaton

4.1 Experimental data

Di(2-ethylhexyl) phthalate was tested in mice and rats by oral administration: It significantly increased the incidence of benign and malignant liver-cell tumours in animals of both species, and a dose-response relationship was observed.

Di(2-ethylhexyl) phthalate can cause testicular damage in rats. There is evidence that this compound and its metabolite, mono(2-ethylhexyl) phthalate, are teratogenic and embryolethal to rodents.

Di(2-ethylhexyl) phthalate was not mutagenic to *Salmonella typhimurium*. However, it caused dominant lethal mutations in mice after systemic but not oral administration.

4.2 Human data

Occupational exposure to di(2-ethylhexyl) phthalate probably occurs during its manufacture, its use as a plasticizer, its use in dielectric fluids for electrical capacitors and in the further processing or use of plasticized products containing it. Its reported widespread occurrence in ambient air, in drinking, river and ocean waters, in industrial effluents, in foods and in blood stored in plasticized bags indicates environmental exposure and exposure of the general human population.

No data were available to assess the mutagenicity or teratogenicity of this compound to man.

No adequate epidemiological study was available to the Working Group.

4.3 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of di(2-ethylhexyl) phthalate in mice and rats.

No adequate epidemiological study was available.

[1]This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DIRECT BLACK 38

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 1937-37-7

Chem. Abstr. Name: 2,7-Naphthalenedisulfonic acid, 4-amino-3-{[4′-((2,4-diaminophenyl)azo)(1,1′-biphenyl)-4-yl]azo}-5-hydroxy-6-(phenylazo)-, disodium salt

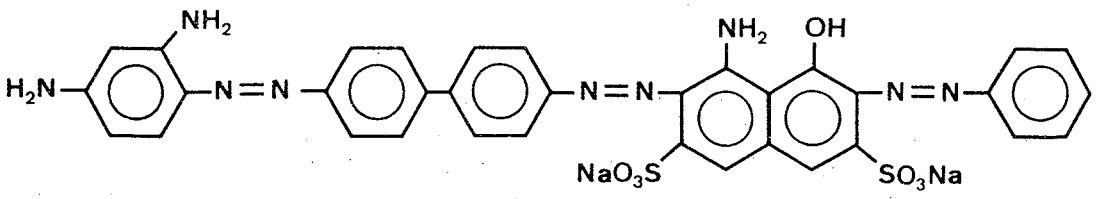
IUPAC Systematic Name: Disodium 4-amino-3-{[4′-((2,4-diaminophenyl)azo)-4-biphenyl]azo}-5-hydroxy-6-(phenylazo)-2,7-, naphthalene disulfonate

Synonyms: C.I. 30235; C.I. Direct Black 38, disodium salt

Trade names: Ahco Direct Black GX; Airedale Black ED; Aizen Direct Deep Black EH; Aizen Direct Deep Black GH; Aizen Direct Deep Black RH; Amanil Black GL; Amanil Black WD; Apomine Black GX; Atlantic Black BD; Atlantic Black C; Atlantic Black E; Atlantic Black EA; Atlantic Black GAC; Atlantic Black GG; Atlantic Black GXCW; Atlantic Black GXOO; Atlantic Black SD; Atul Direct Black E; Azine Deep Black EW; Azocard Black EW; Azomine Black EWO; Belamine Black GX; Bencidal Black E; Benzanil Black E; Benzo Deep Black E; Benzoform Black BCN-CF; Benzo Leather Black E; Black 2EMBL; Black 4EMBL; Brasilamina Black GN; Brilliant Chrome Leather Black H; C.I. 30235; C.I. Direct Black 38; Calcomine Black; Calcomine Black EXL; Carbide Black E; Chloramine Black C; Chloramine Black EC; Chloramine Black ERT; Chloramine Black EX; Chloramine Black EXR; Chloramine Black XO; Chloramine Carbon Black S; Chloramine Carbon Black SJ; Chloramine Carbon Black SN; Chlorazol Black E; Chlorazol Black EA; Chlorazol Black E (Biological Stain); Chlorazol Black EN; Chlorazol Burl Black E; Chlorazol Leather Black ENP; Chlorazol Silk Black G; Chrome Leather Black E; Chrome Leather Black EC; Chrome Leather Black EM; Chrome Leather Black G; Chrome Leather Brilliant Black ER; Coir Deep Black C; Columbia Black EP; Diacotton Deep Black; Diacotton Deep Black RX; Diamine Deep Black EC; Diamine Direct Black E; Diaphtamine Black V; Diazine Black E; Diazine Direct Black E; Diazine Direct Black G; Diazol Black 2V; Diphenyl Deep

Black G; Direct Black 3; Direct Black A; Direct Black BRN; Direct Black CX; Direct Black CXR; Direct Black E; Direct Black EW; Direct Black EX; Direct Black FR; Direct Black GAC; Direct Black GW; Direct Black GX; Direct Black GXR; Direct Black JET; Direct Black Meta; Direct Black Methyl; Direct Black N; Direct Black RX; Direct Black SD; Direct Black WS; Direct Black Z; Direct Deep Black E; Direct Deep Black EAC; Direct Deep Black EA-CF; Direct Deep Black E Extra; Direct Deep Black EW; Direct Deep Black EX; Enianil Black CN; Erie Black B; Erie Black BF; Erie Black GAC; Erie Black GXOO; Erie Black JET; Erie Black NUG; Erie Black RXOO; Erie Brilliant Black S; Erie Direct Black G Extra; Erie Fibre Black VP; Fenamin Black E; Fibre Black VF; Fixanol Black E; Formaline Black C; Formic Black C; Formic Black CW; Formic Black EA; Formic Black MTG; Formic Black TG; Hispamin Black EF; Interchem Direct Black Z; Kayaku Direct Deep Black EX; Kayaku Direct Deep Black GX; Kayaku Direct Deep Black S; Kayaku Direct Leather Black EX; Kayaku Direct Special Black AAX; Lurazol Black BA; Meta Black; Mitsui Direct Black EX; Mitsui Direct Black GX; Nippon Deep Black; Nippon Deep Black GX; Paper Black BA; Paper Black T; Paper Deep Black C; Paramine Black B; Paramine Black E; Peeramine Black E; Peeramine Black GXOO; Phenamine Black BCN-CF; Phenamine Black CL; Phenamine Black E; Phenamine Black E 200; Pheno Black EP; Pheno Black SGN; Pontamine Black E; Pontamine Black EBN; Sandopel Black EX; Seristan Black B; Telon Fast Black E; Tetrazo Deep Black G; Tetrodirect Black E; Tetrodirect Black EFD; Union Black EM; Vondacel Black N

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{34}H_{25}N_9Na_2O_7S_2$ Mol. wt: 783.7

1.3 Chemical and physical properties

(*a*) *Description*: Grey-black powder (Richter, 1951)

(*b*) *Solubility*: Soluble in water; moderately soluble in ethanol and ethylene glycol monoethyl ether; insoluble in other organic solvents (The Society of Dyers and Colourists, 1971a)

(*c*) *Conversion factor*: ppm = 0.0312 x mg/m^3

1.4 Technical products and impurities

The benzidine content of six US-produced samples of Direct Black 38 ranged from 2-20 mg/kg and that of seven samples imported from Egypt, India, The Netherlands and Poland ranged from 2-1254 mg/kg (Boeniger, 1980). In another study, the benzidine content of a Direct Black 38 sample was found to be <0.1 mg/kg, but 150 mg/kg 4-aminobiphenyl [see IARC, 1972] and 9200 mg/kg 2,4-diaminoazobenzene (the hydrochloride of which is chrysoidine [see IARC, 1975]) were present (Nony and Bowman, 1980).

The manufacture and testing of Direct Black 38 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Direct Black 38 was first synthesized in 1898 (The Society of Dyers and Colourists, 1971a). It is prepared commercially by: (1) coupling diazotized benzidine with 1 mol H-acid (8-amino-1-naphthol-3,6-disulphonic acid) under acid conditions, (2) reacting the resulting product with 1 mol diazotized aniline under alkaline conditions, (3) coupling this intermediate with *meta*-phenylenediamine, and (4) neutralization with sodium hydroxide.

Direct Black 38 was first produced in commercial quantities in the US in 1914 (US Tariff Commission, 1922). In 1976, US production of Direct Black 38 by five companies amounted to 1.71 million kg (US International Trade Commission, 1977), down sharply from the 3.1 million kg produced in 1973 (US International Trade Commission, 1975). It is presently produced commercially by only one US company, whose production in 1978 amounted to 374 thousand kg (National Institute for Occupational Safety and Health, 1980). It was the benzidine-based dye made in the largest quantity in the US in that year.

US imports of Direct Black 38 through the principal customs districts in 1980 amounted to nearly 95 thousand kg (US International Trade Commission, 1981), down from the 149 thousand kg imported in 1979 (US International Trade Commission, 1980).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or from eastern Europe.

In 1980, Japan imported an estimated 250 thousand kg of Direct Black 28, principally from South Korea but also from Taiwan.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, India, Mexico, the People's Republic of China, Poland, Romania and the USSR, but whether Direct Black 38 is one of the dyes produced is not known.

(*b*) *Use*

Direct Black 38 can be used to: (1) dye cellulose, wool, silk, bast and hog's hair; (2) print cellulose, wool and silk; (3) dye leather, plastics, vegetable-ivory buttons and wood flour used as a resin filler; (4) stain wool, silk, acetate, nylon, wood and biological materials; and (5) produce aqueous inks (The Society of Dyers and Colourists, 1971b). It has reportedly been used in hair dyes (National Cancer Institute, 1978) [see IARC, 1982].

Two US companies developed a non-benzidine-dye substitute for Direct Black 38 for use on cellulosic fibres (Auerbach Associates, Inc., 1978); however, in 1980 (Boeniger, 1980), Direct Black 38 was being used commercially to dye textiles, leather and paper.

In Japan, 60% of the Direct Black used is for dyeing fibres, 20% for dyeing paper and 20% for dyeing leather.

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty clause of existing occupational safety laws to control worker exposure' (Anon., 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to Direct Black 38 have been established.

2.2 Occurrence

(*a*) *Natural occurrence*

Direct Black 38 has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Direct Black 38 has been detected in the workplace air of a paper-dyeing facility, at total airborne particulate levels of 1.6-5.1 mg/m^3 [0.05-0.16 ppm], and of a textile-dyeing operation, at unspecified levels (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 13 000 US workers are exposed to Direct Black 38.

Direct Black 38 has been reported to be among the dyes used for painting kimonos in Japan, and occupational exposure to such dyes has resulted from the painters' practice of moistening their brushes with their tongues (Yoshida and Miyakawa, 1972; US Environmental Protection Agency, 1979).

(*c*) *Other*

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Black 38 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Black 38 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate (Schlegelmilch and Khodadadian, 1973). Typical methods for the analysis of Direct Black 38 in various matrices are summarized in Table 1.

Table 1. Methods for the analysis of Direct Black 38

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect sample with glass-fibre filter (method detects all dye particles)	Gravimetric	not given	Boeniger *et al.* (1980)
	Extract filter with appropriate solvent; scan from 400-700 nm and compare with scans of bulk dye sample solutions for quasi-specific identification	S	not given	
Bulk or mixtures	Elute solution of dye in distilled water on Silica Gel G (4:1 phenol:water)	TLC	not given	Mashruwala and Mehta (1979)
	Analyse distilled-water dye solutions or extracts from dyed yarn hanks (in colourless dimethylformamide) to detect most direct dyes (probably not dye-specific)	S/R	not given	

a Abbreviations: S, spectrometry; TLC, thin-layer chromatography; S/R, spectrometry/reflectance

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: A group of 60 ICR mice [sex unspecified], 14 weeks old, received 3000 mg/l Direct Black 38 in their drinking-water for 55-60 weeks, at which time the 59 surviving animals were killed. Hepatocellular carcinomas were found in 46/59 mice, and mammary carcinomas in 20/59; 9 animals developed both types of tumours. A further 40 mice were given the same concentration of Direct Black 38 in drinking-water, and 2 mice were killed every two weeks starting from the 16th week of treatment. The first liver tumour occurred in a mouse killed 20 weeks after the start of treatment. No liver or mammary tumour was reported to occur in a group of 20 untreated controls (Asada *et al.*, 1981).

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Black 38 and 1.3% corn oil. (The compound was determined by high-performance liquid chromatography to be 87.1 ± 3.4% pure, with the following impurities: water, 7.13 ± 0.54%; NaCl, 7.9%; benzidine, <0.004%; and traces of at least eight other impurities. The infra-red spectrum was as expected.) Surviving rats were killed at 13 weeks. All male and female animals administered 3000 mg/kg Direct Black 38 died prior to termination of the experiment: male rats survived for less than 5 weeks and female rats less than 12 weeks. Of the 9 surviving males that received 1500 mg/kg, 4 had hepatocellular carcinomas and 5, neoplastic nodules. No male receiving another dose exhibited a tumour, although 7 of 10 male animals given 375 mg/kg, 9 of 10 males given 750 mg/kg, and 5 of 9 males given 1500 mg/kg had foci of cellular alteration or basophilic foci in the liver. Of the female animals, 5 of 10 given 1500 mg/kg exhibited neoplastic nodules in the liver at the termination of the experiment; and all females administered 750 or 1500 mg/kg had foci of cellular alteration in the liver. In the same bioassay, no increased incidence of tumours, compared with that in controls, was found in groups of 10 male and 10 female $B6C3F_1$ mice fed diets containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] of Direct Black 38 and killed 13 weeks later (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

A group of 12 Wistar rats were administered 100 mg/l commercial Direct Black 38 (Direct Deep Black EX; benzidine-free, as shown by high-performance liquid chromatography) in their drinking-water. When the 8 rats still alive at 60 weeks were killed, no tumour was observed. Of 15 rats administered 500 mg/l Direct Black 38, 13 survived until 60 weeks; and 2 papillomas and 3 carcinomas of the urinary bladder, 3 carcinomas of the liver and 2 adenocarcinomas of the colon were observed in 6 animals. In addition, 9 survivors developed hyperplasia of the bladder mucosa, and 8, hyperplasia of the liver. No tumour was observed in a control group of 9 rats (Okajima *et al.*, 1975).

A group of 20 male and 25 female rats were given 400 mg/l Direct Black 38 [source and characteristics unspecified] in their drinking-water (0.04%) for 14 months, at which time 4 males and 2 females were still alive. One of the females had 'breast cancer' [pathological designation not specified]; no other neoplasm was noted (Niitsu, 1973). [The Working Group noted the poor survival of the animals and the short duration of the experiment; in addition, the number of control animals was not specified.]

(*b*) *Other experimental systems*

Bladder implantation: Two groups of 50 female dd *mice* (20 g) received either a paraffin wax pellet (20 mg) containing 10% Direct Black 38 or a wax pellet alone implanted in the bladder. After 40 weeks, when the surviving animals were killed, one bladder carcinoma was observed among the 21 mice still alive. In the control group, one bladder carcinoma was observed in 36 surviving mice (Niitsu, 1973). [The Working Group noted the short duration of the experiment.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Black 38 and mice 750-12 500 mg/kg for 13 weeks resulted in a series of dose- and substance-

related changes, seen when all animals were killed at the end of treatment. In rats, portal fibrosis and multifocal necrosis of the liver were observed; lymphoid depletion in spleen, and thymus and myeloid depletion of the bone marrow were also seen. Other effects included haemosiderosis of the spleen, and interstitial haemorrhage and seminiferous tubular degeneration in the testis. Biliary hyperplasia was seen with doses of 750 mg/kg and above. In mice, diffuse hepatocellular degeneration, biliary hyperplasia and pigment deposition in the liver, haemosiderosis of the spleen and kidney, and pigment deposition in the thyroid were observed (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

Wistar rats were injected subcutaneously on days 7, 8 and 9 of pregnancy with 10 mg (about 40 mg/kg bw per day) Direct Black 38 (Chlorazol black E, biological stain quality). Three of the 16 dams died and 4 resorbed completely, but no malformations were observed in the 70 fetuses that survived to term (Wilson, 1955).

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Black 38. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Black 38 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Black 38 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies, it cannot be concluded that Direct Black 38 is completely converted to benzidine.]

Following oral administration of a single dose of 10 mg/kg bw Direct Black 38 to Syrian golden hamsters, 10.7 μg benzidine, 535 μg monoacetylbenzidine, 27.6 μg diacetylbenzidine [see IARC, 1978], 11.5 μg 4-aminobiphenyl [see IARC, 1972] and, as alkaline hydrolysable conjugates, 328.5 μg benzidine and 6.3 μg 4-aminobiphenyl, were identified in the urine by parallel electron capture gas chromatography and high-performance liquid chromatography. Peak excretion occurred between 0-8 and 8-16 hours. These results indicate that a total of 10% of the dye is metabolized to benzidine and its metabolic follow-up products (National Center for Toxicological Research, 1979; Nony *et al.*, 1980).

Direct Black 38 is rapidly metabolized to benzidine by 14 common bacterial species (Dieckhues, 1960). Preparations of rat and mouse intestine *in vitro* have also been shown to convert Direct Black 38 to benzidine (Niitsu, 1973; Yoshida and Miyakawa, 1973). After increasing microbial activity in rats by feeding a meat-based diet, the azo-reductase level was enhanced (Goldin *et al.*, 1978).

Mutagenicity and other short-term tests

Direct Black 38 was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested in the presence of a mouse liver metabolic activation system; no mutagenicity was observed in the absence of activation (Lazear *et al.*, 1979). Monoacetylbenzidine, a major metabolite of benzidine (see above), and urine from hamsters given Direct Black 38 (100 mg/kg bw) were mutagenic for *S. typhimurium* strain TA1538, but only when tested in the presence of metabolic activation (Lazear *et al.*, 1979; Nony *et al.*, 1980).

Urine from rats given 500 mg/kg bw Direct Black 38 was also mutagenic for *S. typhimurium* strains TA98 and TA100 in the presence of metabolic activation (Tanaka, 1980).

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

Genin (1977) analysed the urine of 22 workers who had potential long-term exposure to benzidine-based dyes during the manufacture of Direct Black 38 and other direct azo dyes. Traces to 300 ppb benzidine were detected in the urine of eight workers, and dianisidine [see IARC, 1974] in three.

Using immunological methods, Korosteleva *et al.* (1974, 1977) identified a benzidine-albumin complex in the serum of female textile-mill workers. The amount present depended on the extent and duration of exposure to direct dyestuffs in the work place; and the complex was found only in workers exposed to direct azo dyes and not in those exposed to non-direct dyes or in controls.

Environmental and urine samples were collected at six factories where workers were potentially exposed to benzidine-based dyes (two benzidine-based dye manufacturers, two textile-dyeing plants, a leather-tanning and dyeing plant and a mill where paper was dyed). Monoacetylbenzidine was detected in the urine of 2/8 workers at one of the dye-manufacturing plants at levels of 3 and 7 ppb. At the second factory, 4 workers exposed to average levels of 7.9, 5.2, 11.7 and 17.4 mg total particulate/m^3 had corresponding urinary concentrations of 52, 11, 10 and 112 ppb benzidine; 590, 248 and 22 ppb monoacetylbenzidine were detected in urine samples containing 112, 52 and 11 ppb benzidine. Traces of diacetylbenzidine, *ortho*-tolidine [see IARC, 1972] and *ortho*-dianisidine were also detected. Benzidine (0-39 ppb) and/or monoacetylbenzidine was detected in the urine of workers in one textile-dyeing factory where Direct Black 38 and Direct Blue 2 were being used. The total level of airborne particulates (measured gravimetrically) was 1-4 mg/m^3. Benzidine was not detected in the urine of workers from

the other facilities (Boeniger, 1980; Lowry *et al.*, 1980). Minute levels of impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Black 38 was available to the Working Group. In the following reports, workers were exposed to dyestuffs which may have included Direct Black 38 (and/or Direct Blue 6 and/or Direct Brown 95).

Numerous case studies [described in more detail in section 3.3 of the monograph on benzidine] describe the occurrence of bladder tumours among workers in dye manufacture. Such reports include those of Oppenheimer (1927), Muller (1933), DiMaio (1937), Barsotti and Vigliani (1949), Goldblatt (1949), Scott (1952), Aboulker and Smagghe (1953), Uebelin and Pletscher (1954), Vigliani and Barsotti (1961), Maltoni and Ghetti (1964), Goldwater *et al.* (1965) and Ferber *et al.* (1976). Epidemiological studies, including those of Case *et al.* (1954) and of Tsuchiya *et al.* (1975), have also indicated increased incidences and mortality from cancer of the bladder among workers in dye manufacture. Three historical reviews (Hueper, 1942, 1969; Haley, 1975) described the international spread of cancer of the bladder among dye workers concomitantly with the spread of the industry.

Several epidemiological studies of dye users suggest that there may be excess mortality from bladder cancer in people possibly exposed occupationally to benzidine-based dyes. Such occupations include shoe and leather workers, tailors, textile workers and hairdressers (Wynder *et al.*, 1963; Anthony and Thomas, 1970; Cole *et al.*, 1972; Anthony, 1974; Viadana *et al.*, 1976). A proportional mortality study of 1429 bleachers and dyers in the UK showed no excess deaths from cancer of the bladder (Newhouse, 1978). [That study was limited in that no certificates of deaths occurring in the first 20 years after start of exposure were available, and only approximately one-third of the workers included in the analysis had actually been exposed to dyes.]

A hospital-based case-control study of 200 male bladder cancer cases and 148 male controls of the same age range with urinary disorders in Kyoto, Japan, showed that 17 (8.5%) of the cases and 2 (1.4%) of the controls had worked in the silk-dyeing industry. The relative risk for employment in the silk-dyeing industry was 6.8 ($p = 0.002$). At least 7 of the 17 patients with bladder cancer who had worked in the dyeing industry were kimono painters, some of whom may have ingested dyes by holding brushes or spatulas in their mouths while working. Among the dyes in wide use in Japan in the 1970s were the benzidine-based compounds, Direct Red 28, Direct Red 17, Direct Green 1 and Direct Black 38 (Yoshida *et al.*, 1971; Yoshida and Miyakawa, 1972). [The Working Group noted that the cases and the controls were recruited from different populations, a procedure which might introduce bias. No data on potential confounding factors were provided.]

In a metabolic evaluation of 22 workers engaged in drying and grinding benzidine-based azo dyes (Direct Black 38 and Direct Blue 2) and dyes based on *ortho*-dianisidine

(Direct Blue 15 and Direct Blue 218) [see section 3.2 (*b*)], benzidine was found in the urine of 8 and dianisidine in that of 3. A retrospective search of plant records showed 5 cases of bladder cancer in dryers and grinders (Genin, 1977).

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Direct Black 38 was tested by oral administration in mice and rats and by bladder implantation in mice. In one study in mice, the compound produced hepatocellular carcinomas and mammary carcinomas following its administration in drinking-water. The other study in mice was inadequate for evaluation. Oral administration to rats of one commercial sample of Direct Black 38 resulted in hepatocellular carcinomas in males and neoplastic nodules in males and females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. Lower doses produced only liver-cell changes such as foci of cellular alteration. In another study in rats, sacrificed after 60 weeks' exposure to the dye in drinking-water, mucosal hyperplasia and carcinoma of the bladder and carcinomas of the liver and colon were seen.

One study has shown that Direct Black 38 and the urine of hamsters given this compound are mutagenic to *Salmonella typhimurium* with metabolic activation.

One limited study in rats has shown it to be embryolethal but not teratogenic.

4.2 Human data

Occupational exposure to Direct Black 38 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or chromosomal effects of this compound to man.

No study of exposure to Direct Black 38 alone was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

There is *sufficient evidence* that commercial Direct Black 38 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity in man of Direct Black 38 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DIRECT BLUE 6

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 2602-46-2

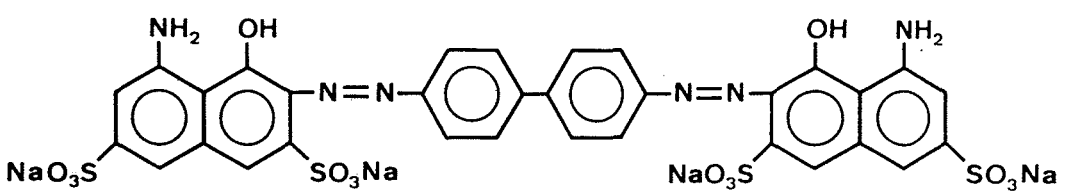
Chem. Abstr. Name: 2,7-Naphthalenedisulfonic acid, 3,3′-[(1,1′-biphenyl)-4,4′-diylbis(azo)]bis(5-amino-4-hydroxy)-, tetrasodium salt

IUPAC Systematic Name: Tetrasodium 3,3′-[4,4-biphenylenebis(azo)] bis(5-amino-4-hydroxy)-2,7-naphthalenedisulfonate

Synonyms: C.I. 22610; C.I. Direct Blue 6, tetrasodium salt; sodium diphenyl-4,4′-bis-azo-2"-8"-amino-1"-naphthol-3",6"-disulphonate

Trade Names: Airedale Blue 2BD; Aizen Direct Blue 2BH; Amanil Blue 2BX; Atlantic Blue 2B; Atul Direct Blue 2B; Azocard Blue 2B; Azomine Blue 2B; Belamine Blue 2B; Bencidal Blue 2B; Benzanil Blue 2B; Benzo Blue BBA-CF; Benzo Blue BBN-CF; Benzo Blue GS; Blue 2B; Blue 2B Salt; Brasilamina Blue 2B; Calcomine Blue 2B; Chloramine Blue 2B; Chlorazol Blue B; Chlorazol Blue BP; Chrome Leather Blue 2B; CI 22610; C.I. 22610; C.I. Direct Blue 6; C.I. Direct Blue 6, Tetrasodium Salt; Cresotine Blue 2B; Diacotton Blue BB; Diamine Blue 2B; Diamine Blue BB; Diaphtamine Blue BB; Diazine Blue 2B; Diazol Blue 2B; Diphenyl Blue 2B; Diphenyl Blue KF; Diphenyl Blue M2B; Direct Blue A; Direct Blue 2B; Direct Blue BB; Direct Blue GS; Direct Blue K; Direct Blue M2B; Direct Blue WBB; Enianil Blue 2BN; Fenamin Blue 2B; Fixanol Blue 2B; Hispamin Blue 2B; Indigo Blue 2B; Kayaku Direct; Kayaku Direct Blue BB; Mitsui Direct Blue 2BN; Naphtamine Blue 2B; Niagara Blue B; Niagara Blue 2B; Nippon Blue BB; Paramine Blue 2B; Phenamine Blue BB; Pheno Blue 2B; Pontamine Blue BB; Tertrodirect Blue 2B; Vondacel Blue 2B

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{32}H_{20}N_6Na_4O_{14}S_4$ Mol. wt: 936.8

1.3 Chemical and physical properties

(*a*) *Description*: Blue-violet solid (Richter, 1951)

(*b*) *Solubility*: Soluble in water; slightly soluble in ethanol and ethylene glycol monoethyl ether; insoluble in other organic solvents (The Society of Dyers and Colourists, 1971a)

(*c*) *Conversion factor*: ppm = 0.0261 x mg/m^3

1.4 Technical products and impurities

The benzidine content of two US-produced Direct Blue 6 samples were 4 and 12 mg/kg, that of a sample imported from Belgium was 6.6 mg/kg and that of a sample imported from India was 10 mg/kg (Boeniger, 1980). The manufacture and testing of Direct Blue 6 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Direct Blue 6 was first synthesized in 1890 (The Society of Dyers and Colourists, 1971a). It is prepared commercially by coupling diazotized benzidine with 2 mol H-acid (8-amino-1-naphthol-3,6-disulphonic acid) under alkaline conditions (Richter, 1951).

Direct Blue 6 was first produced in commercial quantities in the US in 1914 (US Tariff Commission, 1922). In both 1973 and 1976, US sales amounted to 148 thousand kg (US International Trade Commission, 1975; National Institute for Occupational Safety and Health, 1980). It is presently produced commercially by only one US company, whose production in 1978 totalled 28 thousand kg (National Institute for Occupational Safety and Health, 1980), making it the benzidine-based dye produced in the seventh largest volume in the US in that year.

US imports of Direct Blue 6 through the principal customs districts were last reported in 1978, when they totalled 2.0 thousand kg (US International Trade Commission, 1979).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or from eastern Europe.

Direct Blue 6 is not produced commercially in Japan; imports from South Korea and Taiwan, are estimated to have been less than 1 thousand kg per year in recent years.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, the People's Republic of China, India, Mexico, Poland, Romania and the USSR, but whether Direct Blue 6 is one of the dyes produced is not known.

(*b*) *Use*

Direct Blue 6 can be used to: (1) dye cellulose and silk; (2) stain silk, wool and nylon fibres; (3) print cellulose fabrics; (4) dye leather and paper; (5) stain biological materials; and (6) produce aqueous writing inks (The Society of Dyers and Colourists, 1971b; Boeniger, 1980). Direct Blue 6 has reportedly been used in hair dyes (National Cancer Institute, 1978) [see IARC, 1982].

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty portion of existing occupational safety laws to control worker exposure' to Direct Blue 6 (Anon. 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to Direct Blue 6 have been established.

2.2 Occurrence

(*a*) *Natural occurrence*

Direct Blue 6 has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Direct Blue 6 has been detected in the workplace air of a textile-dyeing operation, at total airborne particulate levels of 1.20-3.94 mg/m^3 (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 800 US workers are exposed to Direct Blue 6.

(*c*) *Other*

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Blue 6 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Blue 6 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate, and are separated from each other by chromatography on silica gel (Schlegelmilch and Khodadadian, 1973). However, several eluent systems were tried without success in an effort to move Direct Blue 6 during thin-layer chromatographic separation of dye mixtures (Mashruwala and Mehta, 1979).

Typical methods for the analysis of Direct Blue 6 in various matrices are summarized in Table 1.

Table 1. Methods for the analysis of Direct Blue 6

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect sample on glass-fibre filter (method detects all dye particles)	Gravimetric	not given	Boeniger *et al.* (1980)
	Extract filter with appropriate solvent; scan from 400-700 nm and compare with scans of bulk dye sample solutions for quasi-specific identification	S	not given	
Bulk or mixtures	Analyse distilled-water-dye solutions or extracts of dye-yarn hanks (in colourless dimethylformamide) to detect most direct dyes (probably not dye-specific)	S/R	not given	Mashruwala and Mehta (1979)

a Abbreviations: S, spectrometry; S/R, spectrometry and reflectance

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Blue 6 and 1.3% corn oil. (The compound was determined by high-performance liquid chromatography to be 59.9 $\pm$ 1.9% pure, with the following impurities: water, 9.18 $\pm$ 0.51%; NaCl, 20.8%; benzidine, $<$ 0.004%; and traces of at least eight other impurities.) Survivors were killed at 13 weeks. All male and female animals administered 3000 mg/kg Direct Blue 6 and 1 male administered 1500 mg/kg diet of the dye died prior to termination of the study; all males given the highest dose died before 5 weeks on the study, and all females at that dose were dead by 10 weeks on test. Liver-cell tumours were seen in 8 of 10 males given 1500 mg/kg; 2 were hepatocellular carcinomas and 6, neoplastic nodules. Of animals given 3000 mg/kg, 1 of 9 males and 7 out of 9 females were found to have liver-cell tumours at autopsy prior to the termination of the experiment; 4 of the tumours in females were

hepatocellular carcinomas and 3 were neoplastic nodules. No neoplastic lesion was seen in animals of either sex given lower doses. The first tumours appeared after 4 weeks of feeding in the males and after 5 weeks of feeding in the females. Almost all animals fed 750 or 1500 mg/kg exhibited foci of cellular alterations in the liver and some basophilic foci were seen in the livers of animals receiving 3000 mg/kg. In the same bioassay, no increased incidence of tumours, compared with that in controls, was found in groups of 10 male and 10 female B6C3F$_1$ mice fed diets containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] of Direct Blue 6 and killed 13 weeks later (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

Twenty female Wistar rats [age unspecified] were given 400 mg/l Direct Blue 6 [purity unspecified] in their drinking-water (0.04%) for 14 months. At 12 months, 12 animals were still alive, and 1 exhibited a 'glandular tumour' of the outer ear. No other neoplasm was found (Niitsu, 1973). [The Working Group noted the small number of animals and lack of a control group.]

(*b*) *Subcutaneous and/or intramuscular administration*

The results of a study by Fujita *et al.* (1957) in rats were inconclusive because of poor survival and lack of proper controls.

(*c*) *Other experimental systems*

Bladder implantation: A group of 50 female dd *mice* (20 g) received either a paraffin wax pellet (20 mg) containing 10% Direct Blue 6 or a wax pellet alone implanted in the bladder. After 40 weeks, when the surviving animals were killed, bladder carcinomas were found in 3 of 21 treated mice and in 1 of 36 controls still alive at that time (Niitsu, 1973). [The Working Group noted that the difference from controls was not significant ($p = 0.13$) by the Fisher exact test.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Blue 6 and mice 750-12 500 mg/kg for 13 weeks resulted in a series of dose-related changes seen when all animals were killed at the end of treatment. In rats, hepatocellular degeneration, cholangiofibrosis and portal fibrosis were observed in the liver. Lymphoid depletion of the spleen, thymus and lymph nodes, and myeloid depletion of the bone marrow were also seen. Other effects included oedema of the large intestine and interstitial haemorrhage of the testes. Biliary hyperplasia was seen in animals fed 750 mg/kg and more. In mice, pigment deposition in the liver and haemosiderosis of the kidneys were observed in the groups that received the highest dose, and haemosiderosis of the spleen in all groups that received 1 500 mg/kg or more; extramedullary haematopoiesis was also seen in females (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

In Wistar rats, s.c. administration of 150 mg/kg Direct Blue 6 (as Niagara Blue 2B) on day 8.5 of pregnancy was highly teratogenic (25% of fetuses were malformed) (Beck and Lloyd, 1966; Lloyd and Beck, 1966). I.p. administration of 140 or 200 mg/kg on day 8 of pregnancy to Wistar rats caused 29 or 15% maternal mortality and was teratogenic (2.5 or 11% of fetuses malformed). Hydrocephalus and eye defects were the commonest malformations observed (Beaudoin, 1968). It seems likely that the teratogenic action is an indirect one on the yolk sac epithelium (Beck and Lloyd, 1966; Jensh and Brent, 1972).

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Blue 6. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Blue 6 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Blue 6 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies it cannot be concluded that Direct Blue 6 is completely converted to benzidine.]

Mutagenicity and other short-term tests

No data were available to the Working Group.

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

Using immunological methods, Korosteleva *et al.* (1974, 1977) identified a benzidine-albumin complex in the serum of female textile-mill workers. The amount present depended on the extent and duration of exposure to direct dyestuffs in the work place; and the complex was found only in workers exposed to direct azo dyes and not in those exposed to non-direct dyes or in controls.

Environmental and urine samples were collected at six factories where workers were potentially exposed to benzidine-based dyes (two benzidine-based dye manufacturers, two textile-dyeing plants, a leather dyeing plant and a mill where paper was dyed). Monoacetylbenzidine was detected in the urine of 2/8 workers at one of the dye-manufacturing plants at levels of 3 and 7 ppb. At the second factory, 4 workers exposed to average levels of 7.9, 5.2, 11.7 and 17.4 mg total particulate/m^3 had corresponding urinary concentrations of 52, 11, 10 and 112 ppb benzidine; 590, 248 and 22 ppb monoacetylbenzidine were detected in urine samples containing 112, 52 and 11 ppb benzidine. Traces of diacetylbenzidine, *ortho*-tolidine [see IARC, 1972] and *ortho*-dianisidine [see IARC, 1974] were also detected. Benzidine (0-39 ppb) and/or monoacetyl-benzidine was detected in the urine of workers in one textile-dyeing factory where Direct Black 38 and Direct Blue 2 were being used. The total level of airborne particulates (measured gravimetrically) was 1-4 mg/m^3. Benzidine was not detected in the urine of workers from the other facilities (Boeniger, 1980; Lowry *et al.*, 1980). Minute levels of impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Blue 6 was available to the Working Group. Studies of exposures to benzidine-based dyes are summarized in the monograph on Direct Black 38.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Oral administration to rats of one commercial sample of Direct Blue 6 resulted in hepatocellular carcinomas and neoplastic nodules in males and females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. Lower doses produced only liver-cell changes such as foci of cellular alteration.

Direct Blue 6 is teratogenic in rats only when injected during the first half of pregnancy.

No data were available to assess the mutagenicity of this compound.

4.2 Human data

Occupational exposure to Direct Blue 6 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or chromosomal effects of this compound to man.

No study of exposure to Direct Blue 6 was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

There is *sufficient evidence* that commercial Direct Blue 6 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of Direct Blue 6 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

---

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

5. References

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DIRECT BROWN 95

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 16071-86-6

Chem. Abstr. Name: Cuprate (2-), (5-[(4′-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)phenyl)azo)(1,1′-biphenyl)-4-yl)azo]- 2-hydroxy benzoato(4-))-, disodium salt

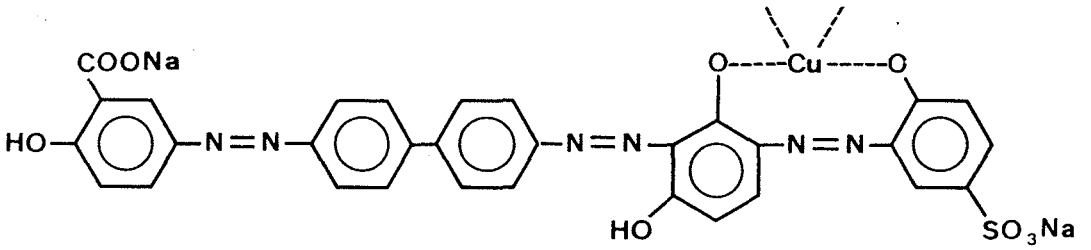
IUPAC Systematic Name: Disodium (5-[(4′-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)phenyl)azo)-4-biphenyl)azo]salicylato (4))cuprate(2-)

Synonyms: C.I. 30145; copper, dihydrogen(5-[(4′-((2,6-dihydroxy-3-((2-hydroxy-5-sulphophenyl)azo)phenyl)azo)-4-biphenylazo]salicyla to(2-))-, disodium salt

Trade Names: Aizen Primula Brown BRLH; Aizen Primula Brown PLH; Amanil Fast Brown BRL; Amanil Supra Brown LBL; Atlantic Fast Brown BRL; Atlantic Resin Fast Brown BRL; Belamine Fast Brown BRLL; Benzanil Supra Brown BRLL; Benzanil Supra Brown BRLN; Brown 4EMBL; C.I. 30145; C.I. Direct Brown; Calcodur Brown BRL; Chloramine Fast Brown BRL; Chloramine Fast Cutch Brown PL; Chlorantine Fast Brown BRLL; Chrome Leather Brown BRLL; Chrome Leather Brown BRSL; Cuprofix Brown GL; Derma Fast Brown W-GL; Dermafix Brown PL; Dialuminous Brown BRS; Diaphtamine Light Brown BRLL; Diazine Fast Brown RSL; Diazol Light Brown BRN; Dicorel Brown LMR; Diphenyl Fast Brown BRL; Direct Brown BRL; Direct Fast Brown BRL; Direct Fast Brown LMR; Direct Light Brown BRS; Direct Supra Light Brown ML; Durazol Brown BR; Durofast Brown BRL; Eliamina Light Brown BRL; Enianil Light Brown BRL; Fastolite Brown BRL; Fastusol Brown LBRSA; Fastusol Brown LBRSN; Fenaluz Brown BRL; Helion Brown BRSL; Hispaluz Brown BRL; KCA Light Fast Brown BR; Kayarus Supra Brown BRS; Paranol Fast Brown BRL; Peeramine Fast Brown BRL; Pontamine Fast Brown BRL; Pontamine Fast Brown NP; Pyrazol Fast Brown BRL; Pyrazoline Brown BRL; Saturn Brown LBR; Sirius Supra Brown BRL; Sirius Supra Brown BRS; Solantine Brown BRL; Solar Brown PL; Solex Brown R; Solius Light Brown BRLL; Solius Light Brown BRS; Sumilight Supra Brown BRS;

Suprazo Brown BRL; Suprexcel Brown BRL; Tertrodirect Fast Brown BR; Tetramine Fast Brown BRDN Extra; Tetramine Fast Brown BRP; Tetramine Fast Brown BRS; Triantine Brown BRS; Triantine Fast Brown OG; Triantine Fast Brown OR; Triantine Light Brown BRS; Triantine Light Brown OG

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{31}H_{18}CuN_6Na_2O_9S$ Mol. wt: 760.1

1.3 Chemical and physical properties

(*a*) *Solubility*: Soluble in water; slightly soluble in ethanol; insoluble in acetone (The Society of Dyers and Colourists, 1971a)

(*b*) *Conversion factor*: ppm = 0.0322 x mg/m^3

1.4 Technical products and impurities

The benzidine contents of two US-produced Direct Brown 95 samples were 19 and 270 mg/kg (Boeniger, 1980). The manufacture and testing of Direct Brown 95 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Direct Brown 95 was first synthesized in 1931. It is prepared commercially by: (1) coupling diazotized 2-amino-1-phenol-4-sulphonic acid with resorcinol; (2) coupling 1

mol of the resulting intermediate with 1 mol diazotized benzidine; (3) coupling the resulting intermediate with salicylic acid; and (4) forming a copper complex with a copper salt. In one product (Sirius Supra Brown BRLN), 20% of the salicylic acid is replaced by 2,3-cresotic acid (The Society of Dyers and Colourists, 1971a).

Direct Brown 95 was first produced in commercial quantities in the US in 1937 (US Tariff Commission, 1938). In 1976, US production of Direct Brown 95 by four companies totalled 270 thousand kg (US International Trade Commission, 1977), up somewhat from the 257 000 kg produced in 1973 (US International Trade Commission, 1975). It is presently produced commercially by one US company, whose production in 1978 amounted to 34.5 thousand kg (National Institute for Occupational Safety and Health, 1980), making it the benzidine-based dye produced in the fifth largest volume in the US in that year.

US imports of Direct Brown 95 through the principal customs districts in 1980 were 10.9 thousand kg (US International Trade Commission, 1981).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or eastern Europe.

Direct Brown 95 is not produced commercially in Japan. Imports in 1980 are estimated to have been 14 thousand kg, all from South Korea and Taiwan.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, the People's Republic of China, India, Mexico, Poland, Romania and the USSR, but whether Direct Brown 95 is one of the dyes produced is not known.

(b) Use

Direct Brown 95 can be used to: (1) dye cellulose and silk fibres; (2) stain wool, acetate and nylon fibres; (3) print cellulose and silk fabrics; (4) dye leather, paper and casein-formaldehyde plastics; and (5) produce its heavy metal salts which can be used as pigments (The Society of Dyers and Colourists, 1971b; Boeniger, 1980).

In Japan, 70% of the Direct Brown used is for dyeing leather and 30% for dyeing paper.

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty portion of existing occupational safety laws to control worker exposure to Direct Brown 95' (Anon. 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to this compound have been established.

2.2 Occurrence

(a) Natural occurrence

Direct Brown 95 has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Total airborne particulate levels of Direct Brown 95 detected in the workplace air of a textile-dyeing operation were 1.3-1.54 mg/m^3; those in a leather-dyeing operation, 1.12-14.72 mg/m^3; and those in a paper-dyeing facility, 0.17-3.30 mg/m^3 (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 700 US workers are exposed to Direct Brown 95.

(*c*) *Other*

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Brown 95 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Brown 95 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate, and separated from each other by chromatography on silica gel (Schlegelmilch and Khodadadian, 1973).

Direct dyes can be collected from air with a glass-fibre filter and analysed gravimetrically. (This method will detect all dye particles.) For more specific identification, the filter may be extracted with an appropriate solvent and scanned in a spectrometer from 400-700 nm for comparison with scans of bulk dye sample solutions (Boeniger *et al.*, 1980).

Direct Brown 95 may be detected as an impurity in reactive dyes by thin-layer chromatography. Achwal and Abhyankar (1979) studied several eluent systems for separating Direct Brown 95 from Reactive Brown 9 by this method; the most successful were 6:9:5 *n*-butyl acetate:pyridine:water and 4:2:1:3 *n*-propanol:isobutanol:ethyl acetate:water.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Brown 95 and 1.3% corn oil. [The compound was determined by high-performance liquid chromatography to be 72.2 $\pm$ 7.0% pure, with the following impurities: water, 4.99 $\pm$ 0.22%; NaCl, 14.9%; benzidine, $<$ 0.004%; and traces of at least eight other impurities.] Surviving rats were

killed at 13 weeks. All male and female animals administered 1500 or 3000 mg/kg Direct Brown 95 died prior to termination of the studies; male rats survived for less than 5 weeks, females given the high dose less than 6 weeks on the study, and females fed 1500 mg/kg about 12 weeks; 2 males receiving 750 mg/kg Direct Brown 95 also died prior to the end of the study. Among male rats, basophilic foci or foci of cellular alteration were seen in 2/9 animals given 3000 mg/kg, in 7/8 given 1500 mg/kg and in 8/10 given 750 mg/kg. In female animals, 4/8 given the 1500 mg/kg dose exhibited neoplastic nodules, and 1 of these showed a hepatocellular carcinoma; basophilic foci or foci of cellular alteration in the liver were seen in 3/8 females given 3000 mg/kg, 6/8 given 1500 mg/kg and 3/10 given 750 mg/kg. No other relevant findings in relation to neoplastic development were seen in these animals. In the same bioassay, groups of 10 male $B6C3F_1$ mice, 6-7 weeks of age, were fed a diet containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] Direct Brown 95 and 1.3% corn oil. Groups of 10 female $B6C3F_1$ mice, 6-7 weeks of age, were fed similar diets containing 350, 750, 1500, 3000 or 6000 mg/kg of the dye. Control diets contained corn oil in amounts equal to that in the diets of groups given the highest doses. The compound was administered for 13 weeks, when all animals were killed. The only suggestive neoplastic lesion observed was foci of basophilic cellular alteration in one male mouse administered 12 500 mg/kg Direct Brown 95 (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Brown 95 and mice 375-12 500 mg/kg for 13 weeks resulted in a series of dose- and substance-related changes seen when all animals were killed at the end of treatment. In rats, biliary hyperplasia and portal fibrosis were observed in the liver; lymphoid depletion in spleen and thymus, lymphoid necrosis in lymph nodes and myeloid depletion in the bone marrow were also seen. Other effects included subacute glomerulonephritis, interstitial haemorrhage and degeneration of germinal epithelium of the testes, and some extramedullary haematopoiesis in the liver. Biliary hyperplasia was seen in all animals given 375 mg/kg or more. In mice, pigment deposition in the liver and haemosiderosis of the spleen were observed (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Brown 95. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount

of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Brown 95 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Brown 95 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies, it cannot be concluded that Direct Brown 95 is completely converted to benzidine.]

Mutagenicity and other related short-term tests

No data were available to the Working Group.

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

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impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Brown 95 was available to the Working Group. Studies of exposures to benzidine-based dyes are summarized in the monograph on Direct Black 38.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Oral administration to rats of one commercial sample of Direct Brown 95 resulted in one hepatocellular carcinoma and several neoplastic nodules in females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. The study in mice was inadequate for evaluation.

No data were available to assess the mutagenicity or teratogenicity of Direct Brown 95.

4.2 Human data

Occupational exposure to Direct Brown 95 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or teratogenicity of Direct Brown 95 to man.

No study of exposure to Direct Brown 95 alone was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

The number of preneoplastic lesions in rats and the precocity of their onset indicate a carcinogenic effect similar to that of Direct Black 38. The present data, however, provide only *limited evidence* that commercial Direct Brown 95 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of Direct Brown 95 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

[1]This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

5. References

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2-NITROPROPANE

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 79-46-9

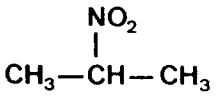
Chem. Abstr. Name: Propane, 2-nitro-

IUPAC Systematic Name: 2-Nitropropane

Synonyms[1]: Dimethylnitromethane; isonitropropane; nitroisopropane; 2-NP

Trade Names: NiPar S-20 Solvent; Ni Par S-30 Solvent

1.2 Structural and molecular formulae and molecular weight



$C_3H_7NO_2$ Mol. wt: 89.1

1.3 Chemical and physical properties of the pure substance

From Baker and Bollmeier (1981), unless otherwise specified

(*a*) *Description*: Colourless liquid

(*b*) *Boiling-point*: 120.25°C

(*c*) *Melting-point*: -93°C (Weast, 1979); freezing-point, -91.3°C

(*d*) *Density*: d_4^{20} 0.988

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[1] 2-Nitropropane exists in tautomeric equilibrium with its enolic or aci form, $[CH_3C(=NO_2H)CH_3]$, which is called 2-isonitropropane, aci-2-nitropropane or 2-propane-nitronic acid (Baker and Bollmeier, 1981).

(*e*) *Refractive index*: n_D^{20} 1.3944

(*f*) *Spectroscopy data*: λ_{max} 260 nm (in ethanol) (Weast, 1979); infra-red, nuclear magnetic resonance and mass spectra have been reported (NIH/EPA Chemical Information System, 1980).

(*g*) *Identity and purity test*: Secondary nitroparaffins such as 2-nitropropane react with nitrous acid to form alkali-insoluble nitroso derivatives (pseudonitroles) which are blue as monomeric liquids and white as dimeric crystals.

(*h*) *Solubility*: At 20°C, 1.7 wt % dissolves in water, and 0.5 wt % water dissolves in 2-nitropropane; soluble in chloroform (Weast, 1979)

(*i*) *Viscosity*: 0.770 cP at 20°C

(*j*) *Volatility*: Vapour pressure, 13 mm at 20°C

(*k*) *Stability*: Flash-point, 24°C (Windholz, 1976); lower inflammability limit, 2.6 vol % in air (Martin and Baker, 1967)

(*l*) *Reactivity*: May be chlorinated in the presence of alkali, condensed with carbonyl compounds to give nitro alcohols, or hydrogenated to isopropylamine

(*m*) *Conversion factor*: ppm = 0.274 x mg/m^3

1.4 Technical products and impurities

2-Nitropropane is available in the US as a commercial grade and as part of a mixture with 1-nitropropane. Specifications for the two grades are identical: 94% minimal nitropropane; 99% minimal total nitroparaffins; 0.1% maximal acidity (as acetic acid); and 0.1% maximal water (International Minerals and Chemical Corp., 1979).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

2-Nitropropane was first prepared by the reaction of isopropyl iodide with silver nitrite in the 1870s. It has since been prepared by the reaction of methyl zinc with 1,1-

bromonitroethane or of dimethyl zinc with 1,1-dibromo-1-nitroethane (Prager *et al.*, 1918). Nitration of propane in the liquid phase with nitric acid and nitration of the vapour phase with nitric acid or nitrogen dioxide have also been used to make 2-nitropropane (Martin and Baker, 1967). It can be made conveniently in the laboratory by the oxidation of isopropyl amine with *meta*-chloroperbenzoic acid in 1,2-dichloroethane (Baker and Bollmeier, 1981).

2-Nitropropane is made commercially in the US by the vapour phase reaction of nitric acid with excess propane at 370-450°C and 8-12 atm. The reaction product, which is further processed and fractionally distilled to recover nitroparaffins, is a mixture of nitromethane, nitroethane and 1- and 2-nitropropane; the exact composition depends on the temperture at which the reaction is carried out (Baker and Bollmeier, 1981). The following composition has been reported: nitromethane, 28%; nitroethane, 8%, 1-nitropropane, 18%; and 2-nitropropane, 46% (Anon., 1976).

2-Nitropropane is also made commercially in France, where propane is nitrated with nitrogen peroxide in the presence of oxygen at 150-330°C and 9-12 atm. The crude reaction product from this process has been reported to contain 20-25% nitromethane, 10-15% nitroethane, 10-20% 1-nitropropane and 60% 2-nitropropane (Anon., 1976).

2-Nitropropane was first produced in the US in a pilot plant in 1940 (Martin and Baker, 1967), and pilot-plant production was started in France in the early 1970s (Anon., 1976). Because there is only one US commercial producer, separate production and exports data for 2-nitropropane are not reported (see preamble section 8(*b*)(ii)). However, US production in 1977 was estimated to be 13.6 million kg per year, 8.2 million kg of which was either used internally by the manufacturer or exported (Finklea, 1977). US imports of all nitroparaffins in 1979 totalled 7.2 thousand kg (US Department of Commerce, 1980).

2-Nitropropane is produced by one company in France. It is not produced commercially in Japan, but it has been imported since 1960; imports (all from the USA) in 1979 amounted to 217 thousand kg and those in 1980 to 139 thousand kg.

(*b*) *Use*

2-Nitropropane is believed to be used principally as a solvent and as a chemical intermediate. Its major uses, in decreasing order of importance, in 1976 were: as a solvent in inks, paints, varnishes, polymers and synthetic materials; as an intermediate in the manufacture of 2-nitro-2-methyl-1-propanol; and as an intermediate in the manufacture of 2-amino-2-methyl-1-propanol (Quibel *et al.*, 1976).

In 1977, 5.4 million kg of the 2-nitropropane made by the single US producer (equivalent to 40% of the annual US production) were sold in the USA (Finklea, 1977). Nearly all is used as a solvent for products such as coatings, inks and adhesives, principally at low concentrations in blends with other solvents in order to impart some desired property to the product, e.g., greater solvency, improved drying time, or low resistivity in electrostatic coatings. 2-Nitropropane is also used as a processing solvent to separate closely related materials in natural products or in reaction mixtures and as a medium for chemical reactions (Baker and Bollmeier, 1981). The amount used in these applications may have decreased dramatically in recent years because of concern about its possible carcinogenic activity and the attendant proposed regulation of its use in certain applications.

2-Nitropropane is reacted with formaldehyde to produce 2-nitro-2-methyl-1-propanol. Although this compound has been reported to be useful as a bactericide and fungicide in cutting oils and synthetic resins, as a polymerization inhibitor for styrene/methyl methacrylate and as a disinfectant (Anon., 1976), its principal uses are believed to be as an intermediate in the manufacture of tyre-cord adhesive components and in the production of 2-amino-2-methyl-1-propanol.

2-Amino-2-methyl-1-propanol, produced by hydrogenation of the nitro alcohol, is reportedly useful for a variety of applications, the most important of which are believed to be as an emulsifier, as a pigment dispersant and in the manufacture of oxazolines (Anon., 1976). One of these oxazolines, 2-(8-heptadecenyl)-4-hydroxymethyl-4-methyl-2-oxazoline, is a cationic surface active agent which is used as a pigment dispersant and as a corrosion inhibitor. Another commercially significant derivative of the amino alcohol is 2-dimethylamino-2-methyl-1-propanol, which is also used as an emulsifier.

Although 2-nitropropane has reportedly been used or studied for possible use in explosives based on ammonium nitrate, in propellants for rocket motors, and in engine fuels, e.g., for model engines and racing cars (Baker and Bollmeier, 1981), no evidence was found that it is presently being used for these purposes.

One company in the Federal Republic of Germany converts 2-nitropropane imported from the USA into 2-nitro-2-methyl-1-propanol, 2-amino-2-methyl-1-propanol and 2-dimethylamino-2-methyl-1-propanol. Another company in France makes the 2-nitro- and 2-amino- compounds and also makes oxazolines.

In Japan, 2-nitropropane is used principally as a solvent in paint and ink formulations.

Nine countries have been reported to limit occupational exposure to 2-nitropropane by regulation or recommended guideline. These standards are listed in Table 1. In the Federal Republic of Germany, 2-nitropropane has been included in a list of substances which so far have been proved to be carcinogenic only in animal experiments under conditions comparable to the possible exposure of men in the work process. In Sweden, 2-nitropropane is included in a list of carcinogenic substances for which limit values are specified (International Labour Office, 1980). The American Conference of Governmental Industrial Hygienists (1981) has designated it as an industrial substance suspected of having carcinogenic potential for man.

The US Food and Drug Administration (1978) has proposed to delete 2-nitropropane from a list of ingredients approved for use as components of adhesives intended to come into contact with food.

The US Environmental Protection Agency (1980a) has identified 2-nitropropane as a toxic waste and requires that persons who discard it comply with the regulations of a Federal hazardous waste managment programme.

The US Department of Treasury (1981) permits the denaturing of alcohol with (among other denaturants) mixed isomers of nitropropane. The resulting denatured alcohol is authorized for use as a solvent in a variety of industrial products.

As part of the US Department of Transportation (1981) Hazardous Materials Regulations, shipments of 2-nitropropane are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

Table 1. National occupational exposure limits for 2-nitropropane[a]

Country	Year	Concentration mg/m³	Concentration ppm	Interpretation[b]	Status
Australia	1978	90	25	TWA	Guideline
Belgium	1978	90	25	TWA	Regulation
German Democratic Republic	1979	50		TWA	Regulation
		100		Maximum (45 mn)	
The Netherlands	1978	90	25	TWA	Guideline
Romania	1975	50		TWA	Regulation
		75		Maximum	
Sweden	1978	36	10	TWA	Guideline
USA					
OSHA	1980	90	25	TWA	Regulation
ACGIH	1981	90	25	Ceiling	Guideline
USSR	1977	30		Maximum	Regulation
Yugoslavia	1971	90	25	Ceiling	Regulation

a From International Labour Office (1980); US Occupational Safety and Health Administration (OSHA) (1980); American Conference of Governmental Industrial Hygienists (ACGIH) (1981)
b TWA - time-weighted average

2.2 Occurrence

(*a*) *Natural occurrence*

2-Nitropropane is not known to occur as such in Nature.

(*b*) *Occupational exposure*

The number of US workers potentially exposed to 2-nitropropane has been estimated variously to be 100 000 (Finklea, 1977), a maximum of 15 000 (US Environmental Protection Agency, 1980b) or 185 000 (National Institute for Occupational Safety and Health, 1980). Industries in which occupational exposure may occur include industrial construction and maintenance, printing, highway maintenance and food packaging (Finklea, 1977).

2-Nitropropane was detected in the air of a rubber tyre manufacturing plant at a concentration of <0.2 mg/m³ [0.05 ppm] (National Institute for Occupational Safety and Health, 1978a). Nitroparaffin workers exposed to 2-nitropropane in a chemical plant had a daily exposure of about 1 ppm [3.65 mg/m³] (National Institute for Occupational Safety and Health, 1977).

2.3 Analysis

Methods for analysing nitroparaffins, including 2-nitropropane, have been reviewed (Martin and Baker, 1967; Baker and Bollmeier, 1981).

2-Nitropropane has been identified qualitatively in a bulk sample of nitroparaffin by gas chromatography-mass spectrometry (National Institute for Occupational Safety and Health, 1977). For its analysis in air, it is absorbed in charcoal, desorbed with a solvent and measured by gas chromatography (National Institute for Occupational Safety and Health, 1978b).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Inhalation studies*

Rat: Groups of 50 male rats of a Sprague-Dawley-derived strain were used in these experiments. One group of animals, weighing approximately 100 g, was exposed to 98 $\pm$ 11 mg/m^3 [27 $\pm$ 3 ppm] 2-nitropropane (94.5% pure by weight, 3.1% of the 1-isomer, 2% nitroethane and 0.4% 2-nitro-2-methylpropane) vapour; and another group of 50 weanlings was exposed to 754 $\pm$ 55 mg/m^3 [207 $\pm$ 15 ppm]. A further group of animals was exposed to 1456 mg/m^3 [400 ppm] nitropropane; however, since this level resulted in excessive mortality, experiments at that dose were terminated. Equal numbers of controls were employed for each test group. Exposure was for seven hours/day, five days/week, for periods of two days, 10 days, one month, three months or six months to atmospheres generated under controlled, standardized conditions. Ten animals from the exposed groups and their respective controls were sacrificed at each of these times. All animals except those exposed to 1456 mg/m^3 survived to the end of the study, and no significant change in body weight from that of control animals was seen in either group. Three months after exposure, 4/9 rats exposed to 754 mg/m^3 exhibited 'basophilic foci of hyperplastic small hepatocytes'. At six months, all of 10 rats exposed to 754 mg/m^3 2-nitropropane exhibited multiple hepatocellular carcinomas. No significant neoplastic lesion was noted in animals exposed to the low dose (Lewis *et al.*, 1979)[1].

Groups of 125 male and 125 female Sprague-Dawley rats were exposed under controlled, standardized conditions to 0 or 91 mg/m^3 [25 ppm] 2-nitropropane (96.65% pure, with 3.63% of the 1-isomer, 0.2% nitroethane and 0.51% 2-nitromethylpropane) vapour for seven hours per day on five days per week. Ten male and ten female animals from exposed and control groups were killed after one, three, six and 12 months, and all remaining animals were sacrificed 22 months after beginning exposure. No increase in the incidence of malignancies was observed in the livers or other organs of treated animals as compared with controls; however, focal areas of hepatocellular nodules were noted in 3/250 control animals and in 13/249 exposed animals [$p < 0.01$] (Griffin *et al.*, 1980, 1981). [No definite indication of the time at which these lesions occurred was given.]

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[1] The Working Group was aware of a study in progress, which included similar levels of exposure to 2-nitropropane (National Institute for Occupational Safety and Health, 1980).

Rabbit: Fifteen male New Zealand white rabbits, each weighing apprximately 2 kg, were exposed for six months to levels of 98 ± 11 mg/m³ [27 ± 3 ppm] or 754 ± 55 mg/m³ [207 ± 15 ppm] nitropropane in chambers and conditions as described for rats by Lewis *et al.* (1979), above. Five rabbits each were sacrificed after one, three and six months of exposure; all other animals survived the length of the experiment. No neoplastic lesion was found in any of the rabbits examined (Lewis *et al.*, 1979). [The Working Group noted the short duration and small numbers of animals used in this experiment.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The lethal oral dose of 2-nitropropane in rabbits was reported to be 0.50-0.75 g/kg bw. After oral administration (dose unspecified) to rabbits, the following symptoms were observed: After a latent period of 20-40 minutes, progressive weakness and collapse, unsteadiness and uncoordination, ending in complete ataxia and a decrease followed by an increase in ventilation rate. Severe liver damage of a necrotizing type was the predominant change in animals that died after inhaling the compound. Skin application daily for five days did not result in local irritation, nor in signs of systemic disease (Machle *et al.*, 1940).

The LC_{50} (single six-hour inhalation exposure) in male Sprague-Dawley rats was reported to be 400 ppm [1460 mg/m³]; female rats were less sensitive (Lewis *et al.*, 1979).

High doses of 2-nitropropane administered by inhalation or intraperitoneally to rats produced methaemoglobinaemia and increased tissue levels of free nitrite (Dequidt *et al.*, 1972).

Systematic inhalation studies with 4.5-hour exposures revealed widely differing interspecies susceptibilities. The maximum tolerated (non-lethal) concentrations and the lowest lethal concentrations, respectively, were: cats, 328/714 ppm [1197/2606 mg/m³]; rats, 714/1513 ppm [2606/5522 mg/m³]; rabbits 1401/2381 ppm [5133/8690 mg/m³]; guinea-pigs, 2381/4622 ppm [8690/16 869 mg/m³]. Rats, rabbits and guinea-pigs survived 130 seven-hour inhalations of 328 ppm [1197 mg/m³] in a chronic experiment. Liver damage, pulmonary oedema and haemorrhage, selective disintegration of brain neurones and general vascular endothelial damage in all tissues were observed. Cats developed dose-dependent methaemoglobinaemia and Heinz-body formation; these changes were observed to a lesser degree in rabbits, and were not seen in rats or guinea-pigs (Treon and Dutra, 1952).

A 24-week inhalation study was performed with Sprague-Dawley rats and New Zealand white rabbits given 27 or 207 ppm [99 or 755 mg/m³] 2-nitropropane for seven hours/day on five days/week. No significant abnormality was detected in rabbits with either concentration. With 755 mg/m³, rats demonstrated mild pulmonary oedema and haemorrhagic foci in the lungs. There was considerable liver damage, as evidenced by marked elevation of serum glutamic-pyruvic transaminase levels, an increase in liver weight, pale colour and necrotic foci, and hypertrophic areas with distorted architecture of the acini, which were considered to be preneoplastic lesions. No change was seen in rats given

the lower level (Lewis *et al.*, 1979). These results were confirmed in a six-month study in which male rats were found to be more susceptible to the hepatotoxic effects than females (Griffin *et al.*, 1978).

In a 22-month inhalation experiment with Sprague-Dawley rats exposed to 25 ppm [91 mg/m^3] 2-nitropropane for seven hours/day on five days/week, all parameters of blood chemistry and morphology remained normal; there was no methaemoglobin formation and no indication of an increase in nitrite levels. Body and organ weights were normal. A slight increase in focal vacuolization of the cytoplasm of hepatocytes was observed in males but not in females. In addition, focal areas of hepatocellular nodules in excess over that in controls were detected (Griffin *et al.*, 1981).

Effects on reproduction and prenatal toxicity

No adequate data were available to the Working Group.

Absorption, distribution, excretion and metabolism

2-Nitropropane has been shown to be metabolized to nitrous acid and acetone in rats (Dequidt *et al.*, 1972; Ullrich *et al.*, 1978) and in a variety of other mammals (Treon and Dutra, 1952). The denitrifying enzymes found in rat liver microsomes all have the properties of monooxygenases, and it has been speculated that *N*-nitroso compounds or other reactive intermediates may be formed during the denitrification reaction (Ullrich *et al.*, 1978).

The denitrifying enzymes were also found in *Neurospora crassa*, in pea seedlings (Little, 1957) and in *Hansenula mrakii* (Kido *et al.*, 1975). The active enzyme of *H. mrakii* (IFO 0895) has been isolated and characterized as a FAD- and Fe^{+3}-containing dioxygenase (Kido *et al.*, 1976).

Mutagenicity and other short-term tests

2-Nitropropane induced reverse mutations in *Salmonella typhimurium* strains TA1537, TA92, TA98 and TA100 in the presence or absence of an exogenous metabolic activation system (Hite and Skeggs, 1979; Speck *et al.*, 1982); activity was enhanced in the presence of activation. The compound did not induce micronuclei in the polychromatic erythrocytes of Charles River (CD-1) mice when given at the highest tolerated doses (0.1-0.3 g/kg bw per day orally for two days) (Hite and Skeggs, 1979).

(*b*) *Humans*

Toxic effects

In one study of eight workers, no ill effect was observed in two exposed intermittently for various periods of time to air concentrations of 10-30 ppm [36-108 mg/m^3] 2-nitropropane; but definite symptoms occurred in six workers exposed to 20-45 ppm [73-164 mg/m^3]: anorexia and nausea, eventually followed, after prolonged exposure, to vomiting and diarrhoea, with complete recovery overnight. Others reported occipital headache, the intensity of which increased during a work day's exposure. All symptoms were more severe in damp weather conditions (Skinner, 1947).

Four fatal and one nonfatal case of acute intoxication were reported in workers

handling 2-nitropropane or 2-nitropropane-containing industrial products. All occurred after exposure in confined spaces with poor or absent ventilation, after exposures of six to 16 hours in broken shifts. The first signs were nausea, vomiting, diarrhoea, headache, dyspnoea, ataxia, chest pain and abdominal pain. In the course of several hours to a few days, toxic hepatitis and gastrointestinal bleeding developed, leading to a typical hepato-renal syndrome, with severe metabolic acidosis. Death occurred between six and 10 days after exposure. Post-mortem findings were ascites and pulmonary oedema, but there was no overt liver enlargement. Histological investigation indicated centrolobular necrosis, proliferation of bile ducts, fatty degeneration of parenchymal cells in the liver, and severe degenerative changes in the tubular epithelia of the kidney. In other organs, the typical signs secondary to hepatic and renal insufficiency were seen (Hine *et al.*, 1978).

Two more cases of acute intoxication by 2-nitropropane have been described, one with a fatal outcome, again characterized by acute necrotizing hepatitis (Gaultier *et al.*, 1964).

Toxic hepatitis was reported in construction workers using epoxy resin preparations which contained 2-nitropropane as well as the hepatotoxic agent 4,4′-diaminophenyl methane. The degree to which each agent was involved in the hepatotoxicity reported could not be clearly differentiated (Williams *et al.*, 1974).

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No case report of cancer in humans exposed to 2-nitropropane was available to the Working Group.

The Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health (National Institute for Occupational Safety and Health, 1980) summarized a retrospective epidemiological study of mortality in 1481 workers employed since 1955 in the manufacture of 2-nitropropane in the US. [The original report, which was prepared by the chemical manufacturers, is unpublished and could therefore not be evaluated by the Working Group.] No excess mortality from cancer at all sites combined was evident among male workers. Seven deaths from sarcomatous cancer were observed: four of the sarcomas were classified histologically as lymphatic cancer; only one case of lymphatic cancer was expected on the basis of US mortality rates. [The summary does not comment on the exposure classification of the seven sarcoma cases.] No excess mortality from hepatic cancer was observed among male workers. [This study had only a 35% probability of detecting a five-fold excess of mortality from liver cancer, the site indicated from studies in experimental animals. Also, the period of follow-up since first exposure was generally short. Only a small number of workers had been exposed for more than 15 years. Inadequate data on exposure of the workers were provided.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

2-Nitropropane was tested in two experiments in rats by inhalation exposure. Hepatocellular carcinomas were produced in one experiment, and an increased incidence of hepatocellular nodules in the other. An inhalation study in rabbits was considered to be inadequate for evaluation.

2-Nitropropane is mutagenic to *Salmonella typhimurium*, with or without activation, but was negative in the micronucleus test in mice.

No adequate data were available to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to 2-nitropropane occurs during its manufacture, its widespread use as a solvent and its conversion to chemical derivatives. Its use as a solvent is probably the greatest source of human and environmental exposure.

There have been several reports of liver toxicity, sometimes with fatal outcome, in workers occupationally exposed to 2-nitropropane. No data were available to assess the mutagenicity or teratogenicity of this compound to man.

The one epidemiological study of workers exposed to 2-nitropropane was available to the Working Group only in the form of an abstract in which the data reported were inadequate for evaluation.

4.3 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of 2-nitropropane in rats.

No adequate epidemiological data were available to the Working Group.

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[1] This evaluation should be read in conjuction with section 7(*b*)(*c*) of the preamble.

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FORMALDEHYDE[1]

1. Chemical and Physical Data

1.1 Synonyms and trade names

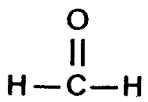
Chem. Abstr. Services Reg. No.: 50-00-0

Chem. Abstr. Name and IUPAC Systematic Name: Formaldehyde

Synonyms[2]: Formaldehyd; formaldehyde gas; formaldehyde solution; formalin; formalin 40; formalin 100%; formic aldehyde; methaldehyde; methanal; methyl aldehyde; methylene glycol; methylene oxide; oxomethane; oxymethylene; paraform; paraformaldehyde; polyoxymethylene glycols; α-polyoxymethylene; β-polyoxymethylene; tetraoxymethylene; α-trioxane; trioxane; α-trioxymethylene

Trade Names[3]: BFV; Fannoform; Formalith; Formol; Fyde; Ivalon; Lysoform; Morbicid; Superlysoform

1.2 Structural and molecular formulae and molecular weight



CH_2O Mol. wt: 30.0

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[1] This compound was first considered by a Working Group which met on 10-17 February 1981 (for list of members, see front of volume). Because certain papers were not available, however, publication of the monograph was postponed; the data were reviewed again by the present Working Group.

[2] Including common names for polymeric forms of formaldehyde from which monomeric formaldehyde can be generated

[3] Not including the large number of formulated products containing formaldehyde along with many other ingredients

1.3 Chemical and physical properties of the pure substance

From Gerberich *et al.* (1980), unless otherwise specified

(*a*) *Description*: Colourless gas with a pungent, suffocating odour

(*b*) *Boiling-point*: -19°C

(*c*) *Melting-point*: -118°C

(*d*) *Density*: d_4^{20} 0.8153; d 1.067 (air = 1.000) (Windholz, 1976)

(*e*) *Spectroscopy data*: λ_{max}^{vapour} 155.5 nm, 175 nm (Weast, 1979). Infra-red, nuclear magnetic resonance (Sadtler Research Laboratories, Inc., undated) and mass spectral data have been published (Mass Spectrometry Data Centre, 1974).

(*f*) *Solubility*: Miscible with water, acetone, benzene, diethyl ether, chloroform and ethanol (Weast, 1979)

(*g*) *Volatility*: Vapour pressure is 400 mm at -33°C (Weast, 1979).

(*h*) *Stability*: Ignition temperature, 430°C; uncatalysed decomposition is very slow below 300°C; relatively stable to polymerization at 80-100°C but slowly polymerizes at lower temperatures

(*i*) *Reactivity*: Very reactive; undergoes self-condensation, particularly under alkaline conditions; condenses with numerous compounds to produce methylol ($-CH_2OH$) or methylene ($=CH_2$) derivatives; can react with hydrogen chloride to form bis(chloromethyl)ether, which is a human carcinogen [see IARC 1974a, 1979a].

(*j*) *Conversion factor*: ppm = 0.82 x mg/m^3

1.4 Technical products and impurities

Anhydrous gaseous formaldehyde is not available commercially. Most formaldehyde is sold in the form of aqueous solutions containing 30-56% formaldehyde with 0.5-15% methanol (Gerberich *et al.*, 1980) as an inhibitor of polymerization. Formaldehyde is also available in the US as its cyclic trimer, trioxane, and as its linear low-molecular-weight homopolymer, paraformaldehyde.

The following specifications have been reported for uninhibited (low methanol) and methanol-inhibited grades of 37% aqueous solutions: a maximum of 1.8% methanol (uninhibited) or 5-8% methanol (inhibited); a maximum of 0.03% acidity (as formic acid); a maximum of 60 mg/kg ash (uninhibited) or 40 mg/kg ash (inhibited); a maximal turbidity of 10 mg/kg; and a maximum of 1 mg/kg iron. Polymerization may also be inhibited by the addition of up to 100 mg/kg of stabilizers such as cellulose ethers or isophthalobisguanamine (Gerberich *et al.*, 1980).

A pharmaceutical solution of formaldehyde is available in the US, as a USP grade containing a minimum of 37.0% formaldehyde, with methanol added to prevent polymeri-

zation (US Pharmacopeial Convention, Inc., 1980), and in the United Kingdom as a solution containing 34-38 weight % formaldehyde, with methanol as a stabilizing agent (British Pharmacopoeia Commission, 1973).

Paraformaldehyde is available in the US as a powdered or flaked product containing the equivalent of 91-93% formaldehyde, a maximum of 9% water; a maximum of 0.03% acidity (as formic acid); and with a melting-range of 110-150°C. A formulation available in western Europe contains 98% formaldehyde.

No information was available on specifications for trioxane.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Formaldehyde was discovered by Butlerov in 1859, and it has been manufactured commercially since the early 1900s. It is produced by the oxidation of methanol with air in the presence of a silver or an iron oxide-molybdenum oxide catalyst. Earlier processes no longer in use commercially include catalytic oxidation of propane and butanes (which produced a complex mixture of products from which formaldehyde was isolated) and oxidation of dimethyl ether (Gerberich *et al.*, 1980).

Total US production of formaldehyde (which is reported in terms of a 37% aqueous solution called 'formalin', even though a variety of forms are actually produced) by 16 companies in 1979 amounted to 2710 million kg (US International Trade Commission, 1980); 2900 million kg were produced by 16 companies in 1978 (US International Trade Commission, 1979). US imports of formaldehyde are negligible; exports of 'formaldehyde, including solutions' in 1979 were 8.7 million kg (US Department of Commerce, 1980).

In 1978, formaldehyde production by 11 companies in Mexico totalled 45 million kg; and total production by four companies in Canada is estimated to have been 238 million kg. Canadian imports were about 15 million kg in 1978.

Total production of formaldehyde in western Europe by 59 companies in 14 countries reached an estimated 3643 million kg in 1978. Imports were negligible; exports were approximately 21 million kg.

Total production of formaldehyde (also reported as 37% aqueous solutions) by 24 companies in Japan in 1979 is estimated to have been 1210 million kg, compared with about 1142 million kg in 1978. Imports and exports were negligible. Formaldehyde is also produced in Central America, South America, eastern Europe, Asia (in addition to Japan), Australia and Africa. Eastern Europe, the Asian countries and South America are believed to have the largest production capacity.

(b) Use

The US use pattern for formaldehyde in 1978 is estimated to have been as follows: 60% for plastics and resin manufacture; 22% for production of intermediates; and 18% for miscellaneous uses.

The plastics and resins which are based on formaldehyde are as follows (listed in order of decreasing US consumption of formaldehyde in 1978): urea-formaldehyde resins, phenolic resins, polyacetal resins and melamine resins. Most of the formaldehyde used for the production of intermediates was in the manufacture of acetylenic chemicals; smaller quantities went into the production of pentaerythritol, hexamethylenetetramine and urea-formaldehyde concentrates. The largest miscellaneous uses of formaldehyde are production of diphenylmethanediamine (4,4′-methylene dianiline) [see IARC, 1974b] and 4,4′-methylenediphenyl diisocyanate [see IARC, 1979b], chelating agents and trimethylolpropane.

Urea-formaldehyde resins, which accounted for over 25% of the formaldehyde used in the US in 1978, are used primarily as adhesives in the manufacture of particleboard, medium-density fibreboard and hardwood plywood [see IARC, 1981]. Other important applications include compounds for moulding, paper treating and coating, textile treating, surface coating and foams for insulation.

Phenolic resins, which accounted for 20-25% of US consumption of formaldehyde in 1978, are used principally as adhesives in water-resistant plywood and as binders in fibreglass insulation. They are also involved in the production of moulding compounds and foundry resins and in a variety of other smaller applications (e.g., laminates, fibrous and granulated wood, abrasives and ion exchange).

Polyacetal resins, produced from formaldehyde or its trimer, trioxane, are used as unreinforced thermoplastic resins in a variety of applications where they can replace metals in mechanical working parts (e.g., in automobiles, lorries, consumer articles, plumbing, industrial machinery and appliances). Melamine resins are thermosetting resins principally used in surface coatings and moulding compounds but also in laminates and in paper and textile treating and coating.

Production of acetylenic chemicals from formaldehyde, which represented 5-10% of US consumption in 1978, is based principally on the reaction of two molecules of formaldehyde with acetylene to produce 2-butyne-1,4-diol, which is then hydrogenated to 1,4-butanediol. This diol is the basis of tetrahydrofuran (a widely used resin solvent, reaction medium and intermediate in the manufacture of Spandex^{R} fibres and polyurethane elastomers), polybutylene terephthalate resins, polyurethanes [see IARC, 1979b], and α-butyrolactone, an intermediate in the production of a family of 2-pyrrolidone derivatives, including *N*-vinylpyrrolidone and its polymer [see IARC, 1979c].

Pentaerythritol, made by the alkaline condensation of formaldehyde with acetaldehyde, is an important polyol used principally in the manufacture of alkyd resins. It is also involved in the production of synthetic lubricants, rosin and tall-oil esters, and of pentaerythritol tetranitrate which is used as an explosive and as a vasodilator. Hexamethylenetetramine, the condensation product of formaldehyde and ammonia, is used principally in the manufacture of curing agents for phenolic thermosetting resins. Urea-formaldehyde concentrates are used mainly to produce slow-release nitrogen fertilizers, commonly called 'ureaforms'.

Separate data on US production of diphenylmethanediamine are not published, but it is believed that nearly all of it is used in the manufacture of 4,4′-methylenediphenyl diisocyanate and polymethylene polyphenyl isocyanate. US production of the latter was 221 million kg in 1979 (US International Trade Commission, 1980).

Among the chelating agents produced from formaldehyde, trisodium nitriloacetate is believed to be the most important. The principal use for trimethylolpropane is as an intermediate in the manufacture of polyols (both polyester and polyether types), used to make polyurethane resins. Other important miscellaneous uses of formaldehyde include textile-treating applications and the production of pyridine chemicals and nitroparaffin derivatives.

One recent source (Suta, 1980) reported the following additional minor use of formaldehyde:

Agriculture (e.g., seed treatment, soil disinfectant)
Analysis (as a reagent)
Concrete and plaster (to make them impermeable to water and grease)
Cosmetics (e.g., foot antiperspirants) (it is also used as a preservative in such products, see section *(c),* below)
Deodorants (for odour control in rooms)
Disinfectants and fumigants (to destroy bacteria in a variety of structures)
Dyes (as a chemical intermediate for dyes and processing aids)
Embalming (as a preservative and hardener of tissues)
Histopathology (Slater, 1981)
Hydrocarbon products (e.g., as a biocide in drilling fluids, as a stabilizer in petrol)
Leather (tanning agent) [see IARC, 1981]
Medicine (e.g., treatment of athlete's foot)[1]. Until recently, formaldehyde was used to sterilize *Echinococcus* cysts prior to their surgical removal (Hunter *et al.,* 1976).
Metal industries (e.g., as a corrosion inhibitor)
Paper (as a chemical intermediate for wet-strength and other paper-treating resins) [see IARC, 1981]
Photography (e.g., for film hardening)
Rubber (e.g., as a biocide in latex rubber, and as a chemical intermediate for the manufacture of rubber-processing chemicals) [see IARC, 1982]
Solvents (as a chemical intermediate)
Starch (for modification of properties)
Surface-active agents (as a chemical intermediate)
Textiles (e.g., to modify the properties of fibres)
Vasectomies (Davis, 1981)
Wood (as an ingredient in wood preservatives) [see IARC, 1981]

The consumption pattern for formaldehyde in western Europe in 1978 is estimated to have been as follows: 40% for urea-formaldehyde resins; 18% for phenolic resins; 5.5% for acetylene chemicals; 5% each for melamine resins, pentaerythritol and hexamethylenetetramine; 4% for polyacetal resins; and 17.5% for other uses. In some individual countries, 90% or more may be used solely in the manufacture of urea-formaldehyde resins.

In Japan, the consumption pattern in 1978 is estimated to have been 36% for urea-formaldehyde resins; 11% for melamine resins; 9% each for pentaerythritol and polyacetal resins; 7% for phenolic resins; 6% for hexamethylenetetramine; and 22% for other uses.

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[1] According to Windholz (1976), formaldehyde has been used in human medicine as a disinfectant and in veterinary medicine as an antiseptic and fumigant in the treatment of tympany, diarrhoea, mastitis, pneumonia and internal bleeding. According to another source, formaldehyde is used in one country in cough sweets, footcare products, skin disinfectants, mouthwashes, spermicide creams and as a disinfectant for root canals. In association with iodine it is used as a coccidiostat in chickens.

Total world demand for formaldehyde (37% aqueous solutions) is estimated to have been 8862 million kg in 1978.

(c) Examples of legislation concerning formaldehyde

Permissible levels of formaldehyde in the working environment have been established in at least 18 countries by regulation or recommended guidelines. These standards are listed in Table 1.

Table 1. National occupational exposure limits for formaldehyde[a]

Country	Year	Concentration mg/m^3	ppm	Interpretation[b]	Status
Australia	1978	3	2	Ceiling	Guideline
Belgium	1978	3	2	Ceiling	Regulation
Bulgaria	1971	1	—	Maximum	Regulation
Czechoslovakia	1976	2	—	TWA	Regulation
		5	—	Ceiling (10 min)	
Finland	1975	3	2	Ceiling	Regulation
German Democratic Republic	1979	2	—	Maximum (30 min)	Regulation
		2	—	TWA	
Federal Republic of Germany	1979	1.2	1	TWA[c]	Guideline
Hungary	1974	1	—	TWA[d]	Regulation
Italy	1978	1.2	1	TWA	Guideline
Japan	1978	2.5	2	Ceiling	Guideline
The Netherlands	1978	3	2	Ceiling	Guideline
Poland	1976	2	—	Ceiling	Regulation
Romania	1975	4	—	Maximum	Regulation
Sweden	1978	3	2	Maximum (15 min)	Guideline
Switzerland	1978	1.2	1	TWA	Regulation
USA					
OSHA	1980	3.7	3	TWA	Regulation
		6.2	5	Ceiling	Regulation
		12.3	10	Ceiling (30 min)	Regulation
ACGIH	1981	3	2	Ceiling	Guideline
NIOSH	1976	1.2	1	Ceiling (30 min)	Guideline
USSR	1977	0.5	—	Maximum	Regulation
Yugoslavia	1971	1	0.8	Ceiling	Regulation

a From International Labour Office (1980), National Institute for Occupational Safety and Health (NIOSH) (1976), the American Conference of Governmental Industrial Hygienists (ACGIH) (1981) and the US Occupational Safety and Health Administration (OSHA) (1980).

b TWA - time-weighted average

c Skin irritant

d May be exceeded 5 times per shift as long as average does not exceed value

Table 2. Recommended and promulgated ceiling limits for exposure to formaldehyde[a]

Exposure	Country	Exposure limit mg/m³	ppm	Status
Outdoor, ambient air	USA	0.123	0.1	Recommended[b]
	Federal Republic of Germany	0.031	0.025	Promulgated
	USSR	0.010	0.008	Promulgated
Indoor air	Denmark	0.148	0.12	Recommended
	The Netherlands	0.123	0.1	Promulgated
	Sweden	0.123-0.492	0.1-0.4	Recommended
	Federal Republic of Germany	0.123	0.1	Recommended

a From National Research Council (1980)
b American industrial Hygiene Association

Table 2 provides a summary of recommended and promulgated ceiling limits for exposure to formaldehyde in outdoor and indoor air.

Notification must be given to the Environmental Protection Agency (EPA) (1979) of any discharges into waterways of 454 kg or more of formaldehyde. The EPA has also identified formaldehyde as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. The following wastes are stated to contain formaldehyde among the hazardous constituents present in them: 'distillation bottoms and distillation side cuts from the production of acetaldehyde from ethylene'; 'wastewater from the washing and stripping of phorate (an organophosphate insecticide) production'; and 'wastewater treatment sludge from the production of phorate' (US Environmental Protection Agency, 1980).

Formaldehyde is registered by the EPA for use alone as a volatile fumigant in agriculture in the form of the 37% aqueous solution, but only in nonfood applications, including vegetable crops, field crops, ornamental crops, seed treatments and others. Paraformaldehyde is registered for use alone or in combinations with several other chemicals in the control of mould, mildew, etc, in agricultural seed treatments; miscellaneous agricultural practices; household vegetable crops, ornamental crops and nonagricultural uses; and commercially. It can be used only in nonfood applications, except in the control of taphole microbiological growths on sugar maple trees, where a tolerance of 2.0 ppm [mg/kg] in the final syrup has been established (US Environmental Protection Agency, 1970).

Formaldehyde is approved for use in the US as a preservative in various aspects of food production. Most of these relate to its presence in a number of food packaging products, but it is also approved for inclusion in defoaming agents containing dimethylpolysiloxane, which are used in food processing; the level of formaldehyde is limited to a maximum of 1.0% of the dimethylpolysiloxane content. Formaldehyde is also approved as an additive in the manufacture of animal feeds based on animal fats and oilseed meals in order to improve the handling characteristics of the feed. The dried feed may contain a maximum of 1% formaldehyde (US Food and Drug Administration, 1980).

Shipments of formaldehyde solutions are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with the designation of formaldehyde as a hazardous material (US Department of Transportation, 1980).

The Commission of the European Communities (1980) requires that bulk solutions of 5-30% formaldehyde be labelled as irritating to the eyes and skin and that concentrated solutions (>30%) be labelled toxic by inhalation, in contact with skin or if swallowed. Formaldehyde is permitted for use in a limited number of cosmetics in the European Communities, with the following authorized concentrations in the finished cosmetic product: 5% in nail hardeners; 0.2% as a preservative (not permitted in aerosol dispensers or in mouth hygiene products) and 0.1% in mouth hygiene products (Commission of the European Communities, 1976). It can be used in grana padano cheese provided that when the final product is marketed, the level of formaldehyde, free and/or combined, not exceed 0.5 mg/kg [ppm] (Commission of the European Communities, 1978).

The US Consumer Product Safety Commission (1981) proposed a ban on the manufacture and sales of urea-formaldehyde foam insulation because it can release

Table 3. Potential occupational exposures to formaldehyde[a]

Anatomists	Glass etchers
Agricultural workers	Glue and adhesive makers
Bakers	Hexamethylenetetramine makers
Beauticians	Hide preservers[b]
Biologists	Histology technicians [assumed to include necropsy and autopsy technicians]
Bookbinders	Ink makers
Botanists	Lacquerers and lacquer makers
Crease-resistant textile finishers	Medical personnel [assumed to include pathologists]
Deodorant makers	Mirror workers
Disinfectant makers	Oil-well workers
Disinfectors	Paper makers[b]
Dress-goods shop personnel	[Particleboard makers[b]]
Dressmakers	Pentaerythritol makers
Drugmakers	Photographic film makers
Dyemakers	Plastic workers
Electrical insulation makers	Resin makers
Embalmers	Rubber makers[c]
Embalming-fluid makers	Soil sterilizers and greenhouse workers
Ethylene glycol makers	Surgeons
Fertilizer makers	Tannery workers[b]
Fireproofers	Taxidermists
Formaldehyde resin makers	Textile mordanters and printers
Formaldehyde employees	Textile waterproofers
Foundry employees	Varnish workers[b]
Fumigators	Wood preservers[b]
Fungicide workers	
Furniture dippers and sprayers[b]	
Fur processors[b]	

a From National Institute for Occupational Safety and Health (1976)
b See IARC (1981)
c See IARC (1982)

significant amounts of formaldehyde. No final rule has been promulgated. The Government of Canada has adopted a temporary ban, effective as of December 1980, on urea-formaldehyde foam insulation sales because of the risks of injury associated with the product.

2.2 Occurrence

(a) Occupational exposure

In 1976, the number of employees engaged directly in the production of formaldehyde in the US was estimated to be 8000. The occupations listed in Table 3 were identified as those in which there was potential exposure to formaldehyde (National Institute for Occupational Safety and Health, 1976). A more recent estimate of the number of US workers occupationally exposed to formaldehyde, including those in industries which use formaldehyde and its derivatives, ranged from 1.4-1.75 million (Booz, Allen and Hamilton, Inc., 1979).

Concentrations of formaldehyde reported in various occupational environments are summarized in Table 4.

Workers in the following occupations were said to have experienced skin irritation resulting from dermal exposure to formaldehyde: seamstress, hairdresser, glue worker, pathologist, nurse, foundry employee, resin manufacturing employee, wood laminating worker and fabric worker (National Institute for Occupational Safety and Health, 1976).

Airborne formaldehyde concentrations in seven US funeral homes have been reported to be in the range of 0.1-0.4 ppm [0.12-0.49 mg/m^3] during embalming of nonautopsied

Table 4. Concentrations of formaldehyde in occupational environments[a]

Occupational environment	Concentration range mg/m^3	ppm	Year
Fabric cutting and sewing	1.23-13.53	1-11	1955
Dress shop	0.16-0.55	0.13-0.45	1959
Resin manufacture and paper production	19.68-36.9	16-30	1961
Paper conditioning	1.11-1.97	0.9-1.6	1961
Textile garment production	1.11-3.32	0.9-2.7	1966
Clothing store	1.11-4.06	0.9-3.3	1966
Textile production	0.0-3.3	0.0-2.7	1968
Wood processing	38.38 max	31.2 max	1968
Laminating plants	0.05-13.41	0.04-10.9	1971
Textile processing	< 6.15	< 5.0	1971
Sheepskin dyeing	5-78	4.07-63.41	1971
Funeral home embalming	0.11-6.47	0.09-5.26	1975
Rubber processing	0.49-0.98	0.4-0.8	1975

a From National Institute for Occupational Safety and Health (1976); National Research Council (1980)

Table 5. Airborne formaldehyde concentrations in occupational and nonoccupational environments. (Air samples were taken during 10 min to 2 h and analysed by the chromotropic acid method.)[a]

Environment	Number (years of measurements)	Formaldehyde concentration (cm^3/m^3)			Source
		Arithmetic mean	Range	Number of measurements	
Textile plants	2 (1977-1979)	0.2	0.1-0.5	16	Finishing and dyeing substances
Shoe factories	1 (1977)	1.9	0.9-2.7	4	Formalin spraying
Particleboard plants	3 (1977-1979)	1.15	0.1-4.9	220	Urea and melamine resins
Plywood plants	6 (1977-1979)	0.35	0.1-1.2	91	Phenolic and urea resins
Wooden furniture manufacturing plants	19 (1977-1979)	1.35	0.1-5.4	134	Adhesives, lacquers, paints
Adhesive plants	1 (1977)	1.75	0.8-3.5	17	Urea-formaldehyde resin
Foundries	10 (1972-1975)	2.7		43	Furan resin
	3 (1977-1979)	0.6	0.05-2.0	8	
Welding and machine shops	3 (1977-1980)	0.5	0.05-1.2	9	Plastic tape, paints, corrosion prevention
Workshops manufacturing electrical machinery: soldering, lacquering, treatment of plastic	10 (1977-1979)	< 0.1		47	Solder
		0.35	0.2-0.5	8	Lacquer, melamine-formaldehyde plastic
Construction sites	7 (1974-1975)	2.8	0.5-7.0	10	Lacquer
Hospitals, clinics	7 (1977-1979)	0.7	0.05-3.5	25	Formaldehyde disinfectant
Offices, schools	4 (1977-1980)	0.24	0.05-0.77	12	Insulation foam, adhesive, lacquer

a From Niemela and Vainio (1981)
b 1 cm^3/m^3 = 1.25 mg/m^3

bodies and up to 2.1 ppm [2.58 mg/m^3] during embalming of autopsied bodies (Anon., 1980a). In a study of formaldehyde exposure in an embalming laboratory, levels of up to 4.8 mg/m^3 were found when the exhaust ventilation system was not functioning (Anon., 1980b).

Studies carried out in Finland during 1972-1980 provided data on airborne formaldehyde concentrations in various occupational environments (Table 5).

(*b*) *Air*

Formaldehyde occurs in air as a product of the natural photooxidation of atmospheric hydrocarbons emitted from sources such as automobile exhaust (Kitchens *et al.*, 1976). Automobile exhaust itself has also been reported to contain formaldehyde at concentrations of 29-43 ppm [35.7-52.9 mg/m^3] (Altshuller *et al.*, 1961). This source has been reported to account for much of the formaldehyde present in the atmosphere (National Research Council, 1980).

Ambient air concentrations of formaldehyde, measured in Los Angeles, California, during the autumn of 1961 and 1966, were 0.005-0.16 ppm [0.006-0.197 mg/m^3] (Kitchens *et al.*, 1976) in 1961 and a daily average of 0.05-0.12 ppm [0.06-0.148 mg/m^3] in 1966 (Patterson *et al.*, 1976). Concentrations of formaldehyde in the Los Angeles area ranged from 0.002-0.136 ppm [0.003-0.167 mg/m^3] in 1969 (Kitchens *et al.*, 1976). More recent air measurements taken during 1979 in Los Angeles indicated levels of less than 15 ppb [18.5 μg/m^3] formaldehyde (Versar, Inc., 1980). Measurements taken in four cities in New Jersey showed median daily concentrations in the range of 3.8-6.6 ppb [4.67-8.12 μg/m^3] (Cleveland *et al.*, 1977).

A study in Switzerland showed concentrations of 9.3-10 ppb [11.4-12.3 μg/m^3] formaldehyde in street air (Wanner *et al.*, 1977). Maritime air in the northern part of the Federal Republic of Germany has been reported to contain formaldehyde at levels of 0.1-6.5 ppb [0.12-8 μg/m^3] (Platt *et al.*, 1979).

In addition to automobile exhaust, combustion processes in power plants, manufacturing facilities, incinerators and petroleum refineries have been reported to be sources of formaldehyde emissions (Table 6).

Another source of airborne formaldehyde is absorber vents of formaldehyde production processes in which metal oxide catalysts are used. Measurements of the composition of the absorber vent stream indicated levels of 0-0.1% of 37% formaldehyde solution when the silver catalyst process was used and 0.8-0.9% with the metal oxide catalyst process. Formaldehyde may also be emitted during the bleeding of storage tanks, in the use of formaldehyde as a fumigant or soil disinfectant, and during embalming and leather-tanning processes (Kitchens *et al.*, 1976).

Formaldehyde may occur in indoor air as an emission from urea-formaldehyde foam insulation or from particleboard containing adhesives based on urea-formaldehyde resins. Measurements taken in Danish houses where particleboard was used in walls, floors and ceilings showed air concentrations of 0.07-1.84 ppm [0.08-2.24 mg/m^3] (Andersen *et al.*, 1975). Formaldehyde concentrations of 0.03-2.5 ppm [0.04-3.07 mg/m^3] reported in mobile homes are believed to be associated with use of particleboard (National Research Council, 1980). A study involving 186 measurements of airborne concentrations of formaldehyde in 65 Finnish dwellings during 1976-1980 reported an arithmetic mean of

Table 6. Estimated formaldehyde concentrations in emissions from various sources[a]

Emission source	Formaldehyde level
Natural gas combustion	
Home appliances and industrial equipment	2400-58 800 μg/m³
Power plants	15 000 μg/m³
Industrial plants	30 000 μg/m³
Fuel-oil combustion	0.0-1.2 kg/barrel oil
Coal combustion	
Bituminous	< 0.005-1.0 g/kg coal
Anthracite	0.5 g/kg coal
Power plant, industrial and commercial combustion	2.5 mg/kg coal
Incinerators	
Municipal	0.3-0.4 g/kg refuse
Small domestic	0.03-6.4 g/kg refuse
Backyard	11.6 g/kg (max) refuse
Oil refineries	
Catalytic cracking units	4.27 kg/barrel oil
Thermofor units	2.7 kg/barrel oil
Mobile sources	
Automobiles	0.2-1.6 g/l fuel
Diesel engines	0.6-1.3 g/l fuel
Aircraft	approx. 0.3-0.5 g/l fuel

a From Kitchens *et al.* (1976)

0.29 cm^3/m^3 [0.36 mg/m^3] and a range of 0.01-0.93 cm^3/m^3 [0.012-1.162 mg/m^3]. The source of the formaldehyde was stated to be particleboard, insulation foam and the glue of wall panels (Niemela and Vainio, 1981).

In two US studies in which formaldehyde concentrations were attributed to emissions from urea-formaldehyde foam insulation, indoor air was found to contain 0.8-0.95 ppm [0.98-1.17 mg/m^3] (Gunby, 1980) and 0.01-31.7 ppm [0.01-39 mg/m^3] (National Research Council, 1980). Another US study of 20 houses, including 17 trailers, reported airborne formaldehyde concentrations of 0.02-4.15 ppm [0.02-5.10 mg/m^3] (National Research Council, 1980).

In 1980 it was reported that urea-formaldehyde foam insulation had been used in more than 500 000 US houses in the preceding five years (Gunby, 1980). Suta (1980) used available information on the levels of formaldehyde in trailers and houses insulated with urea-formaldehyde to develop estimates of human exposure to residential atmospheric formaldehyde. The resulting figures (the calculation of which required several assumptions when data were lacking) are summarized in Table 7.

(*c*) *Water and sediments*

Formaldehyde has been found in municipal and industrial aqueous effluents, including those resulting from chemical and oil and coal processing (Shackelford and Keith, 1976;

Table 7. Estimated human exposures to residential atmospheric formaldehyde[a]

Measure of exposure	Mobile homes	Foam-insulated conventional houses	Nonfoam-insulated conventional houses
People exposed (thousands)	9844	1001	98 354
Exposure (10^6 person-h/year)	56 577	5773	566 938
Total exposures [10^6 ppm (mg/m^3)/person-h per year]	14 930[b] (18 364)	2021 2486	5700-57 000[c] 7011-70 110
Average inhalation exposure[d] (mg/year per person)	1200	1560	45-450

a From Suta (1980)
b Based on an estimation procedure that allows concentration to change with house age
c Range of exposures if an average concentration of 0.01-0.10 ppm [0.012-0.123 mg/m^3] is assumed
d Recalculated values

Hushon *et al.*, 1980). Effluents from the production of urea-, melamine- and phenol-formaldehyde resins also contain formaldehyde: 4% of the total effluent from urea and melamine resin production and 0.1% of the phenolic resin effluent was measured as formaldehyde (Kitchens *et al.*, 1976).

Other effluents that contain formaldehyde include those of manufacturers and users of phenol- and urea-formaldehyde resin glues, such as plywood producers (usually located near lumber mills), which generate waste water from the washing of gluing equipment (Kitchens *et al.*, 1976).

Formaldehyde has been reported in rainwater and in atmospheric aerosol in western Europe (Table 8). A study undertaken in 1969 reported concentrations of 0.31-1.38 mg/l in rainwater (Kitchens *et al.*, 1976).

Formaldehyde is transported in waterways, but it usually undergoes rapid biodegradation. In 1966, stagnant lake water was found to contain detectable concentrations of formaldehyde only in the hypolimnion (Kitchens *et al.*, 1976).

(d) Soil and plants

Formaldehyde is degraded by certain bacteria in soil, and therefore bioaccumulation does not occur. Although completely polymerized urea-formaldehyde resins persist in the environment, partially polymerized, low-molecular-weight condensation products degrade gradually, releasing formaldehyde vapour which can be broken down by soil microflora (Kitchens *et al.*, 1976; Hsiao and Villaume, 1978).

Kidney bean and barley plants can absorb gaseous formaldehyde through their leaves. Maize leaves can form formaldehyde *via* photosynthesis (Kitchens *et al.*, 1976; Szarvas and Pozsar, 1979).

Table 8. Formaldehyde concentrations in rainwater and aerosol[a]

Location (year)	Rainwater concentration (μg/g)	Aerosol concentration (ng/m³)
Mainz (1974-1977)	0.174 ± 0.085	—
Deuselbach (1974-1976)	0.141 ± 0.048	40.9 ± 26.0
Ireland (1975, 1977)	0.142 ± 0.059	5.36 ± 2.4
Ireland[b] (1977)	0.111 ± 0.059	—

a From Klippel and Warneck (1978)
b Very clean air

(*e*) *Food, beverages and animal feed*

Formaldehyde may be present in foods either naturally or as a result of contamination (Kitchens *et al.*, 1976). Determination of the formaldehyde concentration in fruits and vegetables by two different methods produced the following results: tomato, 5.7 and 7.3 μg/g; apple, 17.3 and 22.3 μg/g; cabbage, 4.7 and 5.3 μg/g; spinach, 3.3 and 7.3 μg/g; green onion, 13.3 and 26.3 μg/g; carrot, 6.7 and 10.0 μg/g; and white radish 3.7 and 4.4 μg/g (Tsuchiya *et al.*, 1975).

Hexamethylenetetramine, which is used as a food additive, has been reported to decompose gradually to formaldehyde under acidic conditions or in the presence of proteins (Hutschenreuter, 1956; WHO, 1974).

Formaldehyde can be eluted from formaldehyde-resin (not further identified) plastic dishware with water and acetic acid in an amount directly proportional to the temperature of the water (Table 9).

(*f*) *Tobacco and tobacco smoke*

Concentrations of formaldehyde in cigarette smoke, determined by high-performance liquid chromatography and by colorimetry, have been reported as 45.2-73.1 and 37.5-44.5 μg/cigarette, respectively (Mansfield *et al.*, 1977).

Table 9. Formaldehyde eluted from plastic dishware by water and by 4% acetic acid[a]

Temperature (°C)	Average concentration of formaldehyde (μg/ml)	
	Water	4% acetic acid
20	—	0.5
30	—	1.6
40	0.51	6.9
50	1.7	9.4
60	5.3	16.0
70	7.1	37.4
80	9.9	—
90	15.1	216.5
Boiling	18.2	—

a From Tsuchiya *et al.* (1975)

(g) Other

Free formaldehyde is emitted from formaldehyde resins used in durable-press cotton when they are heat-cured and stored (Cashen, 1979). In the US, concentrations in 112 fabric samples ranged from 1-3517 ppm [mg/kg]; 18 samples had a free formaldehyde content greater than 750 ppm (Schorr *et al.*, 1974).

Formaldehyde occurs in fingernail hardeners, and several cases have been reported of human exposure to formaldehyde through this medium (National Institute for Occupational Safety and Health, 1976). It also occurs in 'wet-strength' facial tissues and paper towels treated with urea-formaldehyde resins (Fisher *et al.*, 1962).

When hexamine hippurate is used in the treatment of urinary infections, 13% is converted to formaldehyde. Hexamine, hexamine hippurate and hexamine mandelate have been used as antimicrobial agents (Wade, 1977).

Formaldehyde is formed endogenously in mammals as a consequence of oxidative metabolism of many xenobiotics (Hutson, 1970) [see also section 3.2 (*a*)].

2.3 Analysis

Typical methods for the analysis of formaldehyde in various matrices are summarized in Table 10. Analytical methods based on colorimetry, spectrophotometry, gas chromatography, microwave spectrometry, laser fluorescence, polarography, atomic absorption spectroscopy and ion-exchange chromatography have been reviewed (Walker, 1964; Kitchens *et al.*, 1976; Kamens and Jeffries, 1978; National Research Council, 1980).

Table 10. Methods for the analysis of formaldehyde

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Commercial solution	Add to neutralized sodium sulphite solution	SST[b]	not given	Walker (1964)
Air	Draw air into acidic solutions to give colour reaction	S[b]	0.002-0.005 ppm (0.002-0.006 mg/m^3)	Kamens and Jeffries (1978)
	Draw air through adsorbent; desorb with high-purity helium as carrier gas	GC/MS	0.3 ppb (0.37 ng/m^3)	Yokouchi *et al.* (1979)
	Directly monitor ultra-violet absorption	DOA[b]	0.1 ppb (0.123 ng/m^3)	Platt *et al.* (1979)
	Draw air through absorbent; extract with acetonitrile	HPLC/UV	not given	Beasley *et al.* (1980)
Effluent waters	Neutralize and filter; add to magnesium metal; add hydrochloric, sulphuric and chromotropic acids; mix; heat; dilute with water For preconcentration, extract water with diethyl ether	S[b]	0.05 µg/ml; 0.05 ng/ml (with pre-concentration)	Jordan (1980)
Fabric samples, clothing, chipboard and plastics	Extract with chromotropic and sulphuric acid	S[b]	not given	Hsiao and Villaume (1978)
Tobacco smoke	Draw sample into 2,4-dinitrophenylhydrazine solution; extract with chloroform; wash with hydrochloric acid and water; dry; dissolve in dichloromethane	HPLC/UV	not given	Mansfield *et al.* (1977)

a Abbreviations: SST, sodium sulphite titration; S, spectrophotometry; GC/MS, gas chromatography/mass spectroscopy; DOA, differential optical absorption; HPLC/UV, high-performance liquid chromatography/ultra-violet detection

b Not specific for formaldehyde

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Inhalation and/or intratracheal administration*

Mouse: Groups of 42-60 C3H mice [sex and age not specified] were exposed to formaldehyde (USP grade) vapour at a concentration of 0, 0.05, 0.1 or 0.20 mg/l [0, 50, 100 or 200 mg/m^3] for one hour per day, three times weekly for 35 weeks. Thirty-six of the mice exposed to the lower concentration were subsequently exposed to 150 mg/m^3 [120 ppm] for 29 weeks. In mice exposed to the higher concentration, exposure was discontinued after the eleventh day of exposure because of severe intoxication. Animals were killed at 35 or 70 weeks. There was no evidence of induction of pulmonary tumours in any dose group. The nasal epithelium was not examined, either grossly or microscopically. Basal-cell hyperplasia, squamous metaplasia and atypical metaplasia were seen in the trachea and bronchi of most of the exposed mice, but not in untreated controls. An additional 26 C3H mice were exposed to 100 mg/m^3 formaldehyde vapour for 35 weeks and then to a coal-tar aerosol for 35 weeks. The formaldehyde did not modify the pulmonary carcinogenesis of coal-tar (Horton *et al.*, 1963). [The Working Group noted the short duration of the experiment.]

Groups of 119-120 male and 120-121 female B6C3F$_1$ mice, 6 weeks of age were exposed to 0, 2.0, 5.6 or 14.3 ppm [0, 2.5, 6.9, 17.6 mg/m^3] formaldehyde (>97.5% pure) vapour by whole-body exposure for six hours per day on five days per week, for up to 24 months, followed by a six-month observation period. Ten males and 10 females from each group were killed at six and 12 months, 0-20 of each sex at 18 months, 17-41 at 24 months and 0-16 at 27 months. Between 0 and 24 months, the numbers of animals that died were 78 male and 30 female controls, 77 and 34 exposed to 2 ppm formaldehyde vapour, 81 and 19 exposed to 5.6 ppm and 82 and 34 exposed to 14.3 ppm. Histopathological examinations were made of all major tissues from each organ system (approximately 40/animal) from control and high-exposure mice, multiple sections of the nasal cavity and all gross lesions from all low and mid-exposure mice, and the same tissues from all animals that died before sacrifice. Squamous-cell carcinomas occurred in the nasal cavities of 2 male mice at the high dose level, but not in females. [The incidence was not statistically significant by life-table analysis nor by the Fisher exact test.] The incidences of a variety of non-neoplastic lesions were significantly increased in mice exposed to formaldehyde [see section 3.2 (*a*)] (Kerns *et al.*, 1982).

Rat: Groups of 119-120 male and 120 female Fischer 344 rats, 7 weeks of age, were exposed to 0, 2, 5.6 or 14.3 ppm [0, 2.5, 6.9, 17.6 mg/m^3] formaldehyde (>97.5% pure) vapour by whole-body exposure for six hours per day on five days per week for up to 24 months, followed by a six-month observation period. Ten males and 10 females from each group were killed at 6 and 12 months, 19-20 of each sex at 18 months, 13-54 at 24 months, 0-10 at 27 months and 0-6 at 30 months. Between 0 and 24 months, the numbers of animals that died were 6 males and 13 females in the control group, 10 and 16 exposed to 2 ppm, 19 of each sex exposed to 5.6 ppm and 57 and 67 exposed to 14.3 ppm. Histopathological evaluations were made of all major tissues from each organ system

Table 11. Neoplastic lesions in the nasal cavity of Fischer 344 rats exposed to formaldehyde vapour

	Exposure (ppm)							
	0		2.0		5.6		14.3	
Sex	M	F	M	F	M	F	M	F
No. of nasal cavities examined	118	114	118	118	119	116	117	115
Squamous-cell carcinoma	0	0	0	0	1	1	51[a]	52[a]
Nasal carcinoma	0	0	0	0	0	0	1[b]	1
Undifferentiated carcinoma or sarcoma	0	0	0	0	0	0	2[b]	0
Carcinosarcoma	0	0	0	0	0	0	1	0
Osteochondroma	1	0	0	0	0	0	0	0
Polypoid adenoma	1	0	4	4	6	0	4	1

a Statistically significant in life-table test for positive trend with dose ($p < 0.0167$) and life-table comparison of control group *versus* 14.3-ppm group

b One animal also had a squamous-cell carcinoma.

(approximately 40/animal) from control and high-exposure rats, multiple sections of the nasal cavity and all gross lesions from all low and mid-exposure rats, and the same tissues from all animals that died before sacrifice. The neoplastic lesions found in the nasal cavity are summarized in Table 11. Life-table analysis of these data revealed significant increases ($p < 0.0167$) in the incidences of squamous-cell carcinomas in both male and female rats exposed to 14.3 ppm formaldehyde vapour; no other neoplasm was increased significantly. The incidences of a variety of non-neoplastic lesions were significantly increased in rats exposed to formaldehyde [see section 3.2 (*a*)] (Swenberg *et al.*, 1980; Kerns *et al.*, 1982).

Groups of 100 male Sprague-Dawley rats were exposed from 9 weeks of age to (1) 14.3 ppm [17.44 mg/m^3] formaldehyde [purity unspecified] and 10 ppm [16.2 mg/m^3] hydrogen chloride gas before dilution in the exposure chamber to maximize formation of bis(chloromethyl)ether; (2) 14.1 ppm [17.2 mg/m^3] formaldehyde and 9.5 ppm [15.48 mg/m^3] hydrogen chloride not mixed before introduction into the exposure chamber; (3) 14.2 ppm [17.32 mg/m^3] formaldehyde vapour alone; (4) hydrogen chloride gas alone [10.2 ppm]; or (5) air (sham-exposed controls). After a total of 382 exposures over a period of 588 days (19.4 months), 10 histologically confirmed, grossly visible nasal squamous-cell carcinomas were observed in the rats exposed to formaldehyde alone; none were seen in the controls or in the rats exposed to hydrogen chloride alone ($p = 0.001$ by the Fisher exact test). [No grossly visible spontaneous nasal cancer had been seen in 1920 control rats in this laboratory over a period of 14 years.] Combined exposure to formaldehyde and hydrogen chloride did not produce a statistically significant increase in the incidence of histologically confirmed, grossly visible nasal squamous-cell carcinomas over that obtained with formaldehyde alone: there were 12 in the group exposed to formaldehyde and hydrogen chloride mixed at high concentration (374 exposures) and 6 nasal cancers (5 squamous-cell and 1 adenocarcinoma) in the group exposed to formaldehyde and hydrogen chloride not mixed before introduction into the exposure chamber (378

exposures). There was 10-20% mortality in the exposed groups when the first cancers were observed (Albert *et al.*, 1982).

In another experiment in the same laboratory, 99 male Sprague-Dawley rats were exposed from 8 weeks of age to 14.7 ppm [17.9 mg/m^3] formaldehyde vapour and 10.6 ppm [17.3 mg/m^3] hydrogen chloride gas mixed at high concentrations, for six hours per day on five days per week. The average level of bis(chloromethyl)ether was 1 ppb [5.13 ng/m^3]. Groups of 50 rats were sham-exposed in air or were untreated colony controls. Of the treated rats, 28 (28%) developed tumours of the nasal cavity, 25 of which were squamous-cell carcinomas and 3 of which were papillomas. No nasal cancers were seen in the concurrent control rats. There was about 50% mortality in the exposed group when the first carcinoma was observed at 223 days. About two-thirds of the exposed rats showed squamous metaplasia of the nasal mucosa, which was not seen in controls (Albert *et al.*, 1982).

Hamster: In a study reported as an abstract, male Syrian golden hamsters were given 10 weekly s.c. injections of 0.5 mg *N*-nitrosodiethylamine concurrently with weekly 5-hour exposures to 30 ppm [36.7 mg/m^3] formaldehyde for life. The number of tumours per tumour-bearing animal was increased over that with *N*-nitrosodiethylamine alone (Dalbey and Nettesheim, 1981). [The Working Group noted the inadequate reporting of this study.]

(*b*) *Subcutaneous and/or intramuscular administration*

Rat: Ten rats were injected subcutaneously once weekly for 15 months with 1 ml of 0.4-0.5% formaldehyde. Sarcomas were found in 4 rats: 2 in the skin at the injection site, 1 in the liver and 1 in the peritoneal cavity. No controls were noted (Watanabe *et al.*, 1954). [The Working Group noted the absence of controls and the small number of animals used.]

(*c*) *Other experimental systems*

Oral tanks: Six *rabbits*, 2-3 kg, were fitted by a muzzle-like holder with oral mucosa tanks containing a 3% formaldehyde solution for 90 min, five times a week for 10 months. Four rabbits received tanks without formaldehyde solution, and 10 rabbits were untreated. All rabbits were sacrificed at 11 months. Leucoplakia was grossly visible in 2/6 rabbits exposed to formaldehyde; and in these lesions dyskeratosis and intraepithelial carcinoma of the exposed mucosa were confirmed histologically (Muller *et al.*, 1978). [The Working Group noted the short duration, the small number of animals used and inadequate reporting of this experiment.]

(*d*) *Studies of precursors*

Since under acid conditions, or in the presence of proteins, hexamethylenetetramine decomposes gradually to yield ammonia and formaldehyde (Hutschenreuter, 1956), the two studies reported below are relevant for the evaluation of the carcinogenicity of formaldehyde.

Oral administration: Sixteen groups of 29-99 male or 27-100 female CTM, SWR or C3Hf strain *mice* received 0, 0.5, 1 or 5% hexamethylenetetramine in their drinking-water over 60 weeks. The two groups receiving 5% were treated for 30 weeks only. After the end of treatment, animals were observed up to 110-130 weeks of age. The groups given 5% hexamethylenetetramine showed slightly reduced growth rate and survival; the SWR mice given 1% also showed slight growth retardation. There was no significant difference in tumour incidence between treated and control groups (Della Porta *et al.*, 1968).

Table 12. LD_{50} values for formaldehyde in various species

Species	Route	LD_{50} (mg/kg bw)	Reference
Rat	oral	800	Smyth *et al.* (1941)
	s.c.	420	Skog (1950)
	i.v.	87	Langecker (1954)
Mouse	s.c.	300	Skog (1950)
Rabbit	dermal	270	Lewis and Tatken (1980)
Guinea-pig	oral	260	Smyth *et al.* (1941)

Four groups of 48 male or 48 female Wistar *rats* received 0 or 1% hexamethylenetetramine in their drinking-water for 104 weeks. All animals were still alive at 60 weeks of age. Tumour incidence was essentially similar in control and treated animals (Della Porta *et al.*, 1968).

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The toxicity of formaldehyde has been reviewed (Berry, 1975; Einhorn, 1975; National Research Council, 1980, 1981). LD_{50} values for various species are given in Table 12.

Acute toxic effects of formaldehyde have been studied in rats, mice, rabbits, guinea-pigs, cats, dogs and monkeys. Acute exposure to low (<1 ppm) or moderate (10-50 ppm) concentrations of formaldehyde vapour resulted in increased airway resistance, decreased sensitivity of the nasopalatine nerve, irritation of eyes and of the respiratory system, and changes in the hypothalamus (Amdur, 1960; Fassett, 1963; Davis *et al.*, 1967; Kulle and Cooper, 1975; Palkovits and Mitro, 1968; Kane and Alarie, 1977). Exposure to high doses (>100 ppm) of formaldehyde vapour caused salivation, acute dyspnoea, vomiting, cramps and death of the test animals (Skog, 1950; Horton *et al.*, 1963; Bitron and Aharonson, 1978).

Exposure of $B6C3F_1$ mice and Fischer 344 rats to 2.0, 5.6 or 14.3 ppm [2.5, 6.9 or 17.6 mg/m^3] formaldehyde vapour for up to 24 months [see section 3.1 (*a*)] resulted in chronic toxicity. Survival of mice did not appear to be related to the concentration of formaldehyde to which they were exposed; however, exposure to 14.3 ppm vapour resulted in lowered body weight. Several lesions were seen in the nasal cavities of mice exposed to concentrations of 5.6 and 14.3 ppm, whereas no effect of exposure to 2.0 ppm was evident. The lesions seen in animals at 14.3 ppm included dysplasia and squamous metaplasia of respiratory epithelium, purulent or seropurulent rhinitis and atrophy of the olfactory epithelium. Three months after exposure was discontinued (27 months), the nasal lesions were seen to have regressed. In the rats, several lesions occurred in the nasal cavities with concentrations as low as 2 ppm; these increased in extent and severity

with increasing concentrations. The lesions included dysplasia and squamous metaplasia of respiratory epithelium, goblet-cell hyperplasia and purulent or seropurulent rhinitis. Rats exposed to 14.3 ppm also exhibited goblet-cell metaplasia of the olfactory epithelium, respiratory epithelial hyperplasia, squamous epithelial hyperplasia, squamous atypia and papillary hyperplasia; dysplasia and squamous metaplasia of the tracheal epithelium were also detected. The incidence of squamous metaplasia in rats exposed to 2.0 or 5.6 ppm regressed within three months after the end of exposure (Kerns *et al.*, 1982).

Acute cell degeneration, necrosis and inflammation were evident in the nasal cavities of rats exposed to 15 ppm [18.3 mg/m^3] formaldehyde vapour for six hours per day for one to nine days. Early squamous metaplasia was detected over the naso- and maxilloturbinates, median septum and lateral wall after as little as five days. Examination of turbinates from rats exposed for five days and allowed to recover for 48 hours showed considerable regeneration. In contrast, only mild serous rhinitis was evident in regions of olfactory epithelium; mild degenerative and inflammatory changes were also evident in the nasopharynx. Mice were less severely affected than rats in their acute response to formaldehyde toxicity. In comparing these data with results from a six-month interim sacrifice (Kerns *et al.*, 1982), it was evident that adaptive changes had occurred. The extent and severity of formaldehyde-induced toxicity diminished with time (Swenberg *et al.*, 1982).

Rats and mice were implanted with pumps containing ^{3}H-thymidine and then exposed to formaldehyde vapour for six hours/day for three days. Tissue autoradiography showed increased replication of respiratory epithelial cells in rats exposed to 6 or 15 ppm [7.3 or 18.3 mg/m^3] and in mice exposed to 15 ppm. No increase was detected in rats exposed to 0.5 or 2 ppm [0.6 or 2.4 mg/m^3] or in mice exposed to 0.5, 2 or 6 ppm [0.6, 2.4 or 7.3 mg/m^3] (Swenberg *et al.*, 1982).

Local necrosis was observed in rabbits following intrapulmonary administration of aqueous solutions of formaldehyde (Garschin and Schabad, 1936; Garschin, 1937). Exposure by inhalation to formaldehyde for up to 90 days produced interstitial inflammation in the lungs of dogs, rats, monkeys, rabbits and guinea-pigs (Coon *et al.*, 1970). Chronic exposure led to hyperplasia and metaplasia of the trachea and major bronchi in mice (Horton *et al.*, 1963).

Groups of six male cynomolgus monkeys, 20 male and 20 female Fischer 344 rats and 10 male and 10 female Syrian golden hamsters were exposed to 0, 0.2, 1.0 or 3.0 ppm [0, 0.24, 1.2 or 3.7 mg/m^3] formaldehyde vapour (98.8% pure) for 22 hours per day on seven days per week for 26 weeks. Squamous metaplasia of the nasal turbinates was evident in 6/6 monkeys exposed to 3 ppm and in 1/6 exposed to 1 ppm. Squamous metaplasia and basal-cell hyperplasia of the respiratory epithelium of the nasal cavity were significantly increased in rats exposed to 3 ppm. The same group exhibited marked depressions in body weight gain. No exposure-related effects were demonstrated in hamsters (Rusch *et al.*, 1982).

Dermal contact with aqueous formaldehyde caused irritation of the skin; when contact was repeated, sensitization was noted in rats and guinea-pigs (El-Sayad, 1972; Ostapovich, 1975). Hair depigmentation was observed in black mice at the sites of s.c. injection of 100 μg formaldehyde (Boyland and Sargent, 1951).

It was reported in an abstract that mice treated with formaldehyde on the skin developed severe liver damage (Searle, 1968).

Effects on reproduction and prenatal toxicity

This topic has been reviewed (Staples, 1982). CD-1 mice were given up to 185 mg/kg bw formaldehyde by gavage on days 6-15 of gestation. The highest dose was definitely toxic to the dams, but no embryotoxicity or teratogenicity was seen with any dose (Marks *et al.*, 1980).

Absorption, distribution and excretion

Formaldehyde is absorbed by inhalation and ingestion in dogs (Malorny *et al.*, 1965; Egle, 1972) and, to a lesser extent, by skin absorption in guinea-pigs (Usdin and Arnold, 1979). Whole-body autoradiography of mice sacrificed 5 min after an i.v. injection of ^{14}C-formaldehyde showed localization of radioactivity primarily in the liver and, to a lesser degree, in the kidneys. Following a survival time of 30 min or more, radioactivity appeared in tissues with high cell turnover (blood-forming organs, lymphoid system, gastrointestinal mucosa) and in tissues with a high rate of protein synthesis (exocrine pancreas, salivary glands) (Johansson and Tjalve, 1978).

Detailed studies on the distribution of ^{14}C-formaldehyde in the rat nasal cavity have demonstrated that it is absorbed primarily in the upper respiratory system. Following a six-hour exposure by inhalation, the amount of ^{14}C-formaldehyde absorbed appeared to be directly proportional to the airborne concentration. Since the amount absorbed did not appear to vary following pre-exposure, these findings, which were based on single exposures, may also be relevant to the chronic toxicity of formaldehyde. Radioactivity was extensively distributed to other tissues, with highest concentrations in the oesophagus, and then in the kidney, liver, intestine and lung, indicating that absorbed ^{14}C-formaldehyde or its metabolites were rapidly removed by the mucosal blood supply. Studies of distribution and pharmocokinetics indicated that inhaled formaldehyde is extensively metabolized and incorporated (Heck *et al.*, 1982).

In order to localize absorption within the nasal cavity, naive (not previously exposed) or pretreated rats and mice were exposed to 15 ppm [18.3 mg/m^3] ^{14}C-formaldehyde for six hours and prepared for whole-body autoradiography. Formaldehyde-associated ^{14}C was heavily deposited in the anterior nasal cavity of rats and mice. The amount of radioactivity correlated well with the distribution of lesions in similarly exposed animals. However, the radioactivity may represent covalently bound material rather than reactive formaldehyde. No differences were apparent between naive rats and mice in the distribution of formaldehyde. Rats that had been exposed to 15 ppm of non-radioactive formaldehyde for nine days prior to exposure to ^{14}C-formaldehyde had less radioactivity than naive rats (Swenberg *et al.*, 1982); this decrease in radioactivity parallels a greater decrease in minute volume in mice than in rats exposed to formaldehyde (Barrow *et al.*, 1982). When the minute volumes for rats and mice exposed to 15 ppm formaldehyde were used to calculate the amount of formaldehyde inspired and this amount was normalized to the surface area of the nasal cavity (Gross *et al.*, 1982), the 'dose' of formaldehyde available for absorption was 0.156 and 0.076 μg/min per cm^2 in rats and mice, respectively (Barrow *et al.*, 1982). Thus, the mouse nasal mucosa is likely to be exposed(to half the amount of formaldehyde that the rat nasal mucosa is. This 'dose' correlated well with tumour response [see section 3.1 (*a*)] (Kerns *et al.*, 1982).

Metabolism and covalent binding

Formaldehyde, which is also formed endogenously as a consequence of xenobiotic metabolism (Hutson, 1970), is converted to carbon dioxide and formate. It is rapidly cleared from the blood in monkeys (McMartin *et al.*, 1979), rats, guinea-pigs, rabbits and cats (Rietbrock, 1969). Conversion to formate has been demonstrated in sheep liver; rat brain, kidney and muscle; rabbit brain; and bovine brain and adrenals (Goodman and Tephly, 1971); and by horse liver homogenates *in vitro* (Kendal and Ramanathan, 1952).

The metabolism of inhaled ^{14}C-formaldehyde appears to be similar to that after other routes of administration (Heck *et al.*, 1982). After s.c. (du Vigneaud *et al.*, 1950) or i.p. (Neely, 1964) injection of ^{14}C-formaldehyde to rats, 81% and 82% of the label, respectively, was recovered as respired $^{14}CO_2$.

As formaldehyde is converted to formate, which is partially incorporated *via* normal metabolic pathways into the one-carbon pool of the body or further oxidized to carbon dioxide, studies using ^{14}C-formaldehyde showed the presence of ^{14}C-labelled cellular macromolecules, both *in vivo* and *in vitro* (Williams, 1959; Kitchens *et al.*, 1976; Pruett *et al.*, 1980). Formaldehyde also reacts with proteins (French and Edsall, 1945) and nucleic acids (Haselkorn and Doty, 1961; Lewin, 1966; Collins and Guild, 1968; Feldman, 1973; Chaw *et al.*, 1980); and it has been reported to induce DNA protein crosslinks (Thomas, 1976). A recent study on human lung cells *in vitro* indicated that ^{14}C from ^{14}C-formaldehyde is incorporated preferentially into RNA rather than DNA or nuclear protein (Pruett *et al.*, 1980).

Formaldehyde is a normal metabolite in mammalian systems and, in small quantities, is rapidly metabolized. The major route of biotransformation appears to be oxidation to formic acid followed by further oxidation to carbon dioxide and water. Administration of radiolabelled formaldehyde to rats by the oral or i.p. route resulted in the appearance of 40% and 82%, respectively, of the label in respiratory carbon dioxide. The remaining isotope in the study by i.p. injection was found in urine as methionine, serine, and an adduct formed from cysteine and formaldehyde (National Research Council, 1980).

Numerous enzymes capable of catalysing the reaction of formaldehyde to formic acid have been identified in liver preparations and erythrocytes. Formaldehyde is a compound that reacts rapidly with amino acids, histones and proteins to form both reversible methylol adducts and stable methylene bridges (National Research Council, 1980).

Effects on intermediary metabolism

Exposure of cultured monkey kidney cells to 1-16 mM formaldehyde for 15 min resulted in the formation of short RNA chains; concentrations $\geqslant$2 mM, produced complete inhibition of thymidine incorporation and cell growth. Almost complete reversal of these effects was seen within 24 hours after the removal of formaldehyde; such recovery was not accompanied by unscheduled DNA synthesis (Nocentini *et al.*, 1980).

Mutagenicity and other short-term tests

The mutagenicity of formaldehyde has been reviewed (Auerbach *et al.*, 1977; Boreiko *et al.*, 1981).

Formaldehyde was mutagenic to an RNA-containing virus (Zasukhina and Marinina, 1967). It was also mutagenic for *Escherichia coli* (Demerec *et al.*, 1951; Englesberg, 1952; Iyer and Szybalski, 1958; Szybalski, 1958; Panfilova *et al.*, 1966; Voronina *et al.*, 1968; Voronina, 1971), *Pseudomonas fluorescens* (Englesberg, 1952) and *Staphylococcus aureus* (Clark, 1953). Both positive and negative results have been reported in *Salmonella typhimurium* (Sasaki and Endo, 1978; Brusick *et al.*, 1980; Boreiko *et al.*, 1981; Ashby and Lefevre, 1982).

E. coli strains deficient in various DNA repair enzymes are more sensitive to the lethal action of formaldehyde than are DNA repair-proficient strains (Rosenkranz, 1972; Nishioka, 1973; Bilimoria, 1975; Poverenny *et al.*, 1975; Mitsevich *et al.*, 1979; Rosenkranz and Leifer, 1980). This phenomenon appears to be due to DNA-protein cross-linkages which are removed by DNA-repair enzymes (Poverenny *et al.*, 1975; Wilkins and Macleod, 1976).

Formaldehyde induced mutations in *Saccharomyces cerevisiae* (Chanet and von Borstel, 1979), in *Neurospora crassa* (Jensen *et al.*, 1951) and in *Chaetomium aureum* (Ghora, 1974). It also induced mitotic recombination in *S. cerevisiae* (Chanet *et al.*, 1975). *S. cerevisiae* strains deficient in DNA repair were more sensitive to the lethal and mutagenic actions of formaldehyde than DNA repair-proficient strains (Chanet *et al.*, 1976; Chanet and von Borstel, 1979). These genetic effects may involve the formation of DNA-protein crosslinks that are recognized by DNA-repair enzymes (Magana-Schwencke and Ekert, 1978; Magana-Schwencke *et al.*, 1978; Magana-Schwencke and Moustacchi, 1980).

Formaldehyde was not mutagenic for growing Chinese hamster ovary (CHO) cells (Hsie *et al.*, 1978). It induced sister chromatid exchanges in cultured CHO cells and human lymphocytes (Obe and Beek, 1979), unscheduled DNA synthesis in HeLa cells (Martin *et al.*, 1978), preferential killing of xeroderma pigmentosum cells (Coppey and Nocentini, 1979), and has been shown to cause DNA-protein crosslinks in mouse L1210 cells (Ross and Shipley, 1980; Ross *et al.*, 1981) and Chinese hamster V79 cells (Swenberg *et al.*, 1982). The DNA-protein crosslinks were repaired within 24 hours after removal of the compound in both cell types.

The effects of formaldehyde have been evaluated in the C3H/10T1/2 Cl 8 cell transformation system. Treatment of cells with 0.1-2.5 μg/ml of formaldehyde alone for 24 hours killed 5-88% of the cells but did not result in significant rates of transformation. When formaldehyde treatment was followed by continuous incubation with 0.1 μg/l of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), however, significantly enhanced transformation frequencies were observed (Ragan and Boreiko, 1981; Boreiko and Ragan, 1982). In a published discussion of one of these papers (Boreiko *et al.*, 1981), Dr Andrew Sivak reported that he had found that 2-20 μg/ml of formaldehyde alone induced dose-dependent transformation of mouse Balb/c 3T3 cells.

Formaldehyde caused X-chromosome breakages in the spermatocytes of the grasshopper (*Tristria pulvinata*) (Manna and Parida, 1967); it did not produce mutations in the silkworm (*Bombyx mori*) (Tazima, 1980).

The genetic effect of formaldehyde in *Drosophila melanogaster* is dependent upon the mode of administration. Addition to food resulted in a strong mutagenic effect, which was restricted to early larval spermatocytes (Rapoport, 1946; Kaplan, 1948; Slizynska, 1957, 1963; Nafei and Auerbach, 1964; Auerbach, 1967; Auerbach *et al.*, 1977). The effects observed included chromosomal deletions, induced repeats and dominant lethal muta-

tions (Auerbach, 1967; Auerbach *et al.*, 1977; Sram, 1970). Exposure to formaldehyde vapour was ineffective (Auerbach, 1949), while injection of aqueous formaldehyde resulted in mutations mainly at the mature sperm stage (Auerbach, 1952).

No dominant lethal mutations were induced in Swiss (ICR/Ha) mice injected intraperitoneally with 16-40 mg/kg bw formaldehyde (Epstein *et al.*, 1972). However, Fontignie-Houbrechts (1981) reported an increase in early fetal deaths and preimplantation losses in strain Q mice after males were given i.p. injections of 50 mg/kg bw formaldehyde; no chromosomal aberrations were observed in meiotic preparations of spermatocytes from the treated males.

Formaldehyde induced genetic effects in barley but not in onion root tips (Auerbach *et al.*, 1977).

(b) Humans

Toxic effects

The acute effects of formaldehyde have been well documented (National Institute for Occupational Safety and Health, 1976; Loomis, 1979; National Research Council, 1980).

Symptoms associated with exposure to formaldehyde include irritation of eyes, nose and throat leading to lachrymation, sneezing, shortness of breath, sleeplessness, tight chest, nausea and excess phlegm (Schuck *et al.*, 1966; Rader, 1974; Kerfoot and Mooney, 1975; Schoenberg and Mitchell, 1975; Breysse, 1977; Sardinas *et al.*, 1979; Garry *et al.*, 1980). An outbreak of haemolytic anaemia, attributed to accidental exposure to formaldehyde, occurred among patients on haemodialysis (Orringer and Mattern, 1976).

A 41-year-old woman died 28 hours after ingesting 120 ml of a formaldehyde solution (37% w/v formaldehyde, 12.5% v/v methanol, containing no formic acid) (Eells *et al.*, 1981).

Sixteen healthy young subjects were exposed to 0.25, 0.42, 0.83 or 1.6 ppm [0.3, 0.5, 1 or 2 mg/m^3] formaldehyde for five hours per day on four days. Physiological parameters, subjective discomfort and mathematical performance were measured during the control period and after 1-3 and 3-5 hours of exposure: no significant changes were observed in pulmonary function, nor was there any difference in performance of mathematical tests. The nasal mucus flow rate was decreased with all concentrations of formaldehyde except 0.83 ppm; the effect was observed only in the upper third of the nose. Subjects reported 'slight discomfort' (averaging 9, 5, 11 and 18, respectively, on a scale of 0 to 100); specifically, they complained of conjunctival irritation and dryness of the nose and throat (National Research Council, 1980).

In another study, 33 subjects (24 men and 9 women) were exposed to 0.03-3.2 ppm [0.037-3.9 mg/m^3] formaldehyde for a total of 35 min, and 48 others (35 men and 13 women) were exposed five times to 0.03-4 ppm [0.037-5 mg/m^3] for 1.5 min (Weber-Tschopp *et al.*, 1977). Several responses were measured, including eye, nose and throat irritation, odour, 'desire to leave the room' and eye-blinking rate. An approximately linear relationship was found for the average responses over the range of concentrations. There was no difference in the average response to exposure to 0.03 ppm formaldehyde and to control air; and significant changes began to appear only with 1.2 ppm [1.5 mg/m^3]. The thresholds for the specific responses ranged from 1.2 to 2.1 ppm [1.5-2.5 mg/m^3]. There

was some suggestion of adaptation to the irritating effects of formaldehyde: at the same concentrations, responses to 1.5-min exposures were generally greater than the responses during 35-min exposures.

Formaldehyde has been shown to be a potent experimental allergen in humans. Skin sensitization was produced in about 8% of male subjects given repeated occlusive applications of 5 or 10% formalin (1.8 or 3.7% formaldehyde) for 3.5 weeks and then challenged with a 1% application two weeks later (Marzulli and Maibach, 1973). Approximately 4% of 1200 dermatology patients exhibited positive skin reactions to 2% formalin (0.8% formaldehyde) under an occlusive patch (Rudner *et al.*, 1973). Experiments suggest that most sensitized subjects can tolerate exposure to 30 ppm aqueous formaldehyde applied to the axilla (Jordan *et al.*, 1979). Sensitized subjects who tolerate formaldehyde-containing products may react to occluded-patch tests of lower concentrations; Marzulli and Maibach (1973) reported that 1/5 sensitized subjects reacted to a challenge concentration as low as 0.01% formalin (0.004% formaldehyde).

Although formaldehyde is a potent experimental allergen in man and animals, many daily exposures to formaldehyde (in shampoos, clothing, etc) may involve quantities below the threshold for induction of sensitization and contact times less than that required to produce a response (National Research Council, 1980).

Contact urticaria also occurs with exposure to formaldehyde; however, no epidemiologic data are available, nor has the mechanism been identified (National Research Council, 1980).

With 1-3 ppm [1.23-3.69 mg/m^3] formaldehyde in air, most people experience irritation of the eyes, nose and throat (Schuck *et al.*, 1966; Weber-Tschopp *et al.*, 1977). With increasing concentrations, the irritation increases rapidly; many people cannot tolerate long exposure to 4-5 ppm [4.92-6.15 mg/m^3]. With 10-20 ppm [12.3-24.6 mg/m^3], the symptoms are more severe, and difficulty in breathing is encountered. Exposures to more than 50 ppm [61.5 mg/m^3] may cause serious injury to the respiratory tract (Fassett, 1963), such as pulmonary oedema and pneumonitis.

The fatal oral dose of formaldehyde has been reported to range from 'a few drops' to 7.5 oz (222 ml) of solutions containing various concentrations of formaldehyde. Pathological examination of fatal cases has revealed congestion, oedema, tissue erosion and haemorrhage (Kline, 1925). A person who swallowed 2-3 oz (59-89 ml) of a 'commercial' solution collapsed and died within 20 min (Levison, 1904).

Chronic effects associated with exposure to formaldehyde include respiratory impairment (Yefremov, 1970; Kratochvil, 1971; Kerfoot and Mooney, 1975; Schoenberg and Mitchell, 1975; Hendrick and Lane, 1977; Ishenko and Pushkina, 1978) and dermatitis (Harris, 1953; Engel and Calnan, 1966; Sneddon, 1968; Helander, 1977).

Effects on reproduction and prenatal toxicity

Effects in women attributed to exposure to formaldehyde include menstrual disorders and secondary sterility (Shumilina, 1975).

Absorption, distribution, excretion and metabolism

People exposed to 0.78 mg/m^3 [0.64 ppm] formaldehyde gas for three hours demonstrated

a rapid rise in formate levels in blood and urine (Einbrodt *et al.*, 1976). Rapid conversion of formaldehyde to formate occurred in many tissues, including human erythrocytes (Malorny *et al.*, 1965) and liver (Uotila and Koivusalo, 1974).

In the case of fatal poisoning described above (Eells *et al.*, 1981), formic acid accumulated in the blood 30 min after ingestion of formaldehyde at a level of about 7 mM.

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans[1]

The Working Group was aware of many studies of cancer incidence or mortality in workers in industries in which formaldehyde is used (see e.g., IARC, 1981). Since workers in such industries are also exposed to many other chemicals, and since the studies did not make specific mention of exposure to formaldehyde, most have not been reviewed here. However, medical personnel, in particular pathologists and certain laboratory technicians, are most likely to be exposed to formaldehyde.

In a letter to the Editor of *The Lancet*, Jensen (1980) reported that according to data recorded in the Danish Cancer Registry during the period 1943-1976, three cases of cancer of the nasal cavities, sinuses or nasopharynx were notified in Danish doctors. None had ever worked in a pathology department or as an anatomist.

In a mortality study of pathologists and medical laboratory technicians in the UK, a total of 2709 pathologists alive and active between 1 January 1955 and 21 December 1973 and a total of 12 944 medical laboratory technicians registered between August 1963 and December 1973 were followed up to the end of 1973 and their mortality compared with that of the population of England and Wales or Scotland. The standardized mortality ratios (SMRs) for all causes of death were 60 for pathologists and 67 for medical laboratory technicians. Statistically significant excesses of observed *versus* expected deaths were found for suicide (SMR 250 for pathologists and 243 for medical laboratory technicians). In male pathologists a statistically significant increase in lymphatic and haematopoietic neoplasms was observed (8 observed, 3.3 expected). Similar findings were not seen in laboratory technicians (Harrington and Shannon, 1975).

The Registrar General's decennial supplement for England and Wales on occupational mortality for the period 1970-1972 (Registrar General for England and Wales, 1978) reported a SMR of 81 for qualified medical practitioners, a group which includes pathologists; a deficit of lung cancer deaths was noted. In a prospective study of 34 400 British doctors, no unusual pattern of respiratory cancers was noted among nonsmokers (Doll and Peto, 1976); and examination of mortality rates in 11 occupational groups among 20 540 male doctors revealed a deficit of cancers of the lung, mouth or oesophagus in doctors working in scientific research, pathology or biochemistry (Doll and Peto, 1977).

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[1] The Working Group was aware of several epidemiological studies in progress on the relationship between formaldehyde and cancer (Gunby, 1980; Muir and Wagner, 1981).

[The Working Group noted that in all of these studies, actual exposure to formaldehyde is unknown.]

Walrath and Fraumeni (1982) carried out a proportional mortality analysis of 1106 (1101 males and 5 females) deceased morticians who had been licensed to practice embalming in New York State between 1902 and 1979. Formaldehyde has been the main preservative used in commercial embalming fluids, and its presence in embalming fluids is required by US state laws. The number of observed deaths due to each cause was compared with the expected number calculated by the application of age-, sex- and time-specific proportions of deaths in the US general population in five-year age and time periods to the deaths in the study group. The proportional mortality ratio (PMR) for all cancers in white male embalmers was 108 (not significantly greater than 100). There was, however, a significant proportional excess of deaths from skin cancer (sites not specified) (PMR = 253) based on 8 deaths from skin cancer, 4 of which were melanomas. This elevation in PMR was greater among those licensed for more than 35 years than among those licensed for less than 35 years (PMRs of 354 and 196), among those licensed after age 30 than those licensed before age 30 (PMRs of 424 and 151) and among those licensed for embalming only than among those licensed for both embalming and funeral directing (PMRs of 337 and 178). The PMRs for kidney cancer [types not specified] and brain cancer were also significantly elevated among white males licensed only for embalming (256 and 245, respectively, based, in each case, on 6 deaths). There was no proportionate excess of deaths from respiratory cancer (PMR = 102) and no death from cancer of the nose or nasal sinuses. The authors noted that embalming fluid contains a variety of chemicals apart from formaldehyde. Smoking habits were not documented.

Wong (1982) reported follow-up of 2026 white male employees of a chemical factory in the US where formaldehyde was manufactured between the early 1940s (when the plant began operation) and 1977. The workers were exposed to formaldehyde, other oxygenated hydrocarbons, benzene, asbestos, and inorganic and organic pigments. A total of 1975 workers (97.5%) were traced to 31 December 1977. Death certificates were obtained for 136 of 146 who had died. A total of 32 514 person-years of follow-up was accumulated. Expected numbers of deaths by cause were estimated by use of age-specific mortality rates in US males in five-year periods between 1925 and 1975. Overall mortality in the workers was significantly lower than expected (ratio of observed to expected deaths, 0.74); but for all cancers the number of deaths observed equalled that expected (O/E = 1.01). Excess numbers of deaths over those expected occurred from cancers at the following sites that were the cause of more than 1 death: prostate (4 observed, 1.31 expected), brain (3 observed, 1.61 expected), Hodgkin's disease (2 observed, 0.83 expected). The number of deaths from respiratory cancer equalled that expected (12 observed, 12.36 expected), and there were fewer digestive system cancers (5) than expected (9.46). There were no deaths from cancers of the nose or nasal sinuses. None of these differences was statistically significant. When analysis was confined to deaths that occurred more than 20 years after first employment in the factory (in 5948 person-years of follow-up accumulated after this latency), there was a statistically significant excess of deaths from prostatic cancer (4 observed, 0.93 expected). Two of these deaths, however, occurred in men who had worked for less than 5 years in the plant; and for this, and for other cancer sites, there was no evidence of increasing risk with increasing duration of employment. [The second Working Group noted that the power of this study to detect the presence of increasing risk with increasing duration of employment was slight.] A proportional mortality analysis showed a significant proportional excess of deaths from all cancers (PMR = 148) and from cancer of the prostate (PMR = 367). Smoking habits were not documented. [The first Working Group noted that this report

provides no information on the levels of exposure to formaldehyde or to the other chemicals present in the working environment. Only a small proportion of the person-years covered were accumulated more than 20 years after first employment in the factory.]

Marsh (1982) carried out a proportional mortality study on a cohort of 2490 male employees in a large chemical plant where formaldehyde was both produced and used as a raw material in the production of resins, hexamethylenetetramine and resorcinol. The cohort was constituted of workers employed for a minimum of one year between 1 January 1949 and 31 December 1966 and followed up from 1950 to 1976. Complete work histories, available for the 592 deaths included in the analysis, revealed that 136 (23%) had worked for at least one month in areas of the plant where exposure to formaldehyde, as well as to other chemicals, could have occurred. Only 36 had worked for the majority of their employment in areas where significant exposure to formaldehyde *vapour* could have occurred routinely. PMRs by race were calculated for the groups exposed to formaldehyde and for all the others. Expected numbers of deaths were calculated by applying the cause-specific proportional mortality of US white and non-white males to the total number of white and non-white deaths in the study group, after adjusting for age and time period. The overall cancer mortality was lower among the group exposed to formaldehyde than in the others; and no excess mortality by cancer site observed in the former reached statistical significance, while in the latter group there was a significant excess of neoplasms of the genito-urinary tract (PMR = 192, 22 observed deaths) among whites and for all cancers (PMR = 251, 5 deaths) among non-whites. When analysed by age at death, the youngest age group among those exposed to formaldehyde showed excess mortality from cancer of the digestive tract (PMR = 413, 2 observed deaths). No death from cancers of the nose or nasal sinuses was observed among the workers. A proportional cancer mortality analysis was also performed, using US age-, time- and site-specific proportional cancer mortality data to compute the expected numbers of deaths. The results showed a slight increase in the proportional cancer mortality ratios (PCMRs) compared with the PMRs for cancer. However, the mortality excesses were still not statistically significant. An elevated PMR for cancer of the digestive system (PMR = 320, based on 5 observed deaths; $p < 0.01$) was reported for white males exposed for a total of less than 5 years to formaldehyde and with more than 20 years from onset of exposure. The author pointed out that exposure to other chemicals, such as phenol, cellulose acetate, polyvinyl butyral, aminoplastics, polystyrene and vinyl chloride products, could not be excluded for workers handling formaldehyde. Smoking habits were not documented.

[The second Working Group further reviewed the three papers and came to a number of additional conclusions on quantitative aspects:

[None of the studies reported measurements of environmental exposure.

[The duration of employment in the cohorts was generally short: in the study by Marsh, the mean duration of exposure to formaldehyde was 4.5 years; in the study by Wong, the estimated mean length of employment was 11.4 years, and 48% of the cohort had been exposed for <10 years. Only indirect information on duration of exposure was given by Walrath and Fraumeni.

[All three studies had limited power to detect increases in mortality from nasal cancer: the probabilities of detecting a three-fold excess of mortality from nasal cancer in the studies by Walrath and Fraumeni, by Marsh and by Wong were 12%, 7% and 8%, respectively. The studies generally had good statistical power to detect increased

mortality from lung cancer: probabilities of detecting a doubling of lung cancer mortality were 99%, 71% and 88% in the three studies, respectively. The power of the studies to detect a three-fold increase in mortality from brain cancer was 95% for that of Walrath and Fraumeni, uncalculable for that of Marsh, and 58% for that of Wong.

[Two of the three studies were proportional mortality analyses, only: as such they may provide biased estimates of risk.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Formaldehyde was tested for carcinogenicity by inhalation exposure in two strains of rats and one strain of mice. A significant incidence of squamous-cell carcinomas of the nasal cavity was induced in both strains of rats but not in mice. Another study in mice and one in hamsters by inhalation exposure, one in rats by subcutaneous administration and one in rabbits by exposure in oral tanks were considered inadequate for evaluation. Levels of formaldehyde that cause nasal tumours also cause acute degeneration, necrosis, inflamation and increased cell replication in the nasal mucosa of rats and mice following inhalation exposure.

DNA damage has been observed in bacteria, yeast and mammalian cells exposed to formaldehyde. The chemical is mutagenic to bacteria, yeasts and *Drosophila melanogaster*, but not to the silkworm nor to mammalian cells in culture. Chromosomal aberrations were seen in formaldehyde-treated mammalian cells, plants, and the spermatocytes of grasshoppers and fruit flies. In one study, it induced morphological transformation in mouse 10T1/2 cells when treatment was followed by exposure to 12-*O*-tetradecanoyl phorbol-13-acetate.

One positive and one negative study on the induction of dominant lethal mutations by formaldehyde in mice have been reported.

Data were insufficient to evaluate adequately the teratogenicity/embryotoxicity of this compound.

4.2 Human data

Formaldehyde has been produced commercially since the early 1900s. Its widespread use in a variety of applications (world demand in 1978 is estimated to have been 8862 million kg) is known to result in appreciable exposure of workers and of sections of the

general population. Notable recent observations are that formaldehyde is present in the indoor air of buildings and mobile homes containing particleboard made with formaldehyde-based adhesives and in houses insulated with urea-formaldehyde foams.

Allergic sensitization which can result in dermatitis or acute or chronic respiratory distress is associated with exposure to formaldehyde.

Three mortality studies of workers manufacturing formaldehyde and other chemicals or using formaldehyde have given inconclusive results. A study of embalmers who used embalming fluid containing formaldehyde showed a proportional excess of deaths from skin cancer, which increased with both duration of employment in embalming and intensity of exposure (as judged by whether a man was involved only in embalming or also directed funerals). Men involved only in embalming also had increased proportional mortality from cancers of the brain and kidney. A second study showed a significant excess of deaths from prostatic cancer in the period after 20 years from first employment in a factory. A third study showed a proportional excess of deaths from digestive-tract cancer in two subgroups of men, but not overall. The total number of deaths or person-years observed after a suitable latent period in all three studies was small and would be insufficient to show an increased risk of an uncommon cancer.

4.3 Evaluation[1]

There is *sufficient evidence* that formaldehyde gas is carcinogenic to rats.

The epidemiological studies provide inadequate evidence to assess the carcinogenicity of formaldehyde in man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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ANNEX: SOME ASPECTS OF QUANTITATIVE CANCER RISK ESTIMATION

Introduction

Quantitative risk estimation is the process by which the risk of disease or death in a population exposed to a toxic agent is related quantitatively to the intensity and duration of exposure. Quantitative risk estimation is based upon the assessment of dose-response relationships in exposed populations. It is to be distinguished from risk-benefit analysis and from other forms of analysis, such as standard setting, threshold limits, 'acceptable' or 'safe' levels, etc, which weigh social, political and economic factors (varying from country to country) against the risk of disease or death.

Quantitative estimation of the risk of human cancer, particularly in man, is a difficult task. Many issues complicate the process. Among them are: the long induction-latent period, typically of many years' duration between beginning of exposure and appearance of disease; the possible two-stage process of carcinogenicity; the paucity of epidemiological data on most chemical compounds; the mixed nature of most chemical exposures; and the modification of carcinogenic effects not only by other chemical exposures but also by a variety of 'life-style' factors.

Neither the principles nor the techniques for the quantitative estimation of cancer risk to man are yet well developed. Further, most previous studies of the epidemiology of human cancer are such that the data necessary for quantitative risk estimation are available only infrequently; detailed quantitative data on exposure or dose in particular are lacking.

The Working Group was of the opinion that quantitative estimation of risk will be important in future studies of the epidemiology of human cancer, and the Group felt that development of the theory, principles and methods for quantitative risk estimation should be encouraged. Specifically, the Working Group recommended to IARC that a special monograph be prepared on quantitative cancer risk estimation. As a minimum, such a monograph should address the following subjects:

1. The carcinogenic process and its relevance to risk estimation
1.1 Exposure evaluations, including concentration and duration of exposure in relation to a dose concept
1.2 Initiation, promotion; latency-induction
1.3 Environmental effect modifiers and host factors (e.g., heterogeneity of populations and of their exposures)

2. Cancer frequency and dose-response or exposure-effect estimations[1]
2.1 Absolute measures of effect as a function of time
2.2 Relative measures of effect as a function of time

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[1] Whereas 'dose-response' in toxicology often refers to the number of subjects affected at a given dose, epidemiological terminology tends traditionally to use 'effect' rather than 'response'. Moreover, the 'dose' has usually to be approximated by some sort of ascertainment of 'exposure'; hence, 'exposure-effect', 'measures of effect', 'effect-modification', etc.

3. Mathematical models and extrapolation
3.1 Over time, e.g., lifetime, post-exposure
3.2 Over dose, e.g., low doses

In the present early state of development of the field and in the absence of a systematic treatise on risk assessment, any attempt at quantitative cancer risk estimation will be imperfect and will contain areas of disagreement and uncertainty. The Working Group was, however, of the opinion that an attempt to derive quantitative estimations of cancer risk for certain compounds is important and justifiable, because the resulting estimates will: (a) complement the qualitative evaluations presented elsewhere in these monographs, and (b) stimulate interest in further refinement of the technique of quantitative cancer risk estimation.

Concepts of exposure and exposure-effect

Quantitative cancer risk estimations that are based on epidemiological data require that assumptions be developed on: (1) the nature (shape) of the exposure-effect relationships and (2) the duration of exposure. With regard to the issue of the exposure-effect relationship, which is the better understood of these two issues, a common approach is to construct a risk model that can be used to evaluate the effects of various hypothetical levels of exposure. Epidemiologists have frequently assumed a linear model for dose-response. This model has some scientific plausibility (Crump *et al.*, 1976; Acheson and Gardner, 1980; Committee on the Biological Effects of Ionizing Radiation, 1980). It is, however, recognized to be a rather simplified model which may vary with chemical, species and treatment schedule and which may ideally require modification, e.g., with regard to mechanisms influencing susceptibility, such as variations in metabolic activation of carcinogens, in saturation of systems for detoxification, or in dose-related alterations in the rates and fidelity of DNA repair (Anderson *et al.*, 1980). As a result of such variation, a given total exposure, accumulated at different concentration and different duration, may give rise to widely varying effects. There is at least the theoretical possibility that supplementary biological information can be obtained to answer these issues for a given problem. In-vivo and in-vitro experimentation might help in these instances.

With regard to assumptions concerning dose, it was recognized that data on past exposure are almost always incomplete. However, one may sometimes be confident that the historical dose rate is known within an order of magnitude. It is of first importance in dealing with risk calculations to insure that they reflect the degree of uncertainty in the estimates of dose rate. This is often most simply done by citing upper and lower bounds of such estimates.

Duration of exposure and also fractionation of dose are less well understood problems in modelling exposure-effect relationships. Two issues are particularly important: first of all, the probability of effect may be related as a power (3 to 6) to duration of exposure. This is expressed, for example, by the multistage model and has been well illustrated by Doll's and Peto's (Doll and Peto, 1978) analysis of lung cancer in British physicians who smoke. A second issue concerns the cessation of exposure and subsequent follow-up. If, on the one hand, the exposure acts as an early-stage initiator, then the risks remain somewhat the same after cessation. If, on the other hand, the chemical is a promoter,

then the risk decreases after elimination of the exposure. Whittemore (1978), followed by Day and Brown (1980), have discussed this issue in some detail. The typical occupational study involves continued follow-up of workers after they have left the workplace. The resulting risk ratios may thereby misrepresent the true risks for a continued exposure.

To avoid these problems, estimates of risk based on continuous exposure would seem preferable. One might thereby propose as a measure of risk, for a given exposure level, an estimate of the risk associated with continuous exposure throughout a working lifetime, or, alternatively, continuous exposure for 25 years. The latter would have the advantage of being more often directly observable; the former might be of greater relevance and more readily comparable with animal data.

External effect modifiers and host factors

Exposures of human populations are rarely, if ever, to single substances, but to a variety of agents. Given that control of confounding is achieved, exposures other than the one under study may still influence or modify the disease outcome and, therefore, any derived exposure-effect relationship. In theory, the nature, direction and size of such effect modifications should be identified and quantified, a task which may, however, prove difficult because of lack of pertinent information. In such a case, exposure-effect relationships can only be confidently generalized to environments and populations close to those from which they were obtained.

Similarly, host factors like age, nutritional status, inherited differences in enzyme activities etc, i.e., internal modifiers of effect, could influence the final outcome of a particular exposure. Sometimes, therefore, seemingly conflicting results might be obtained in qualitative risk estimates derived from various study populations, as merely reflecting the incomplete character of the necessarily somewhat simplified models that have to be used practically. However, this does not rule out the feasibility and the usefulness of quantified risk estimations, as long as their limitations are recognized.

Relative risk *versus* risk difference

The most frequently used measure of the carcinogenic effect of an exposure is the relative risk, often expressed as a ratio of the observed to expected number of cases. Empirical evidence, theoretical reasons and arguments for simplicity support this choice when cancer is the end-point (Doll and Peto, 1981), although this may not necessarily be the case for other biological end-points, for which a risk difference, for example, may turn out to be a preferable representation.

The Working Group thus attempted to construct hazard functions of risk of cancer over time following exposure (ideally continuous).

The epidemiological analyses of cigarette smoking and lung cancer and various radiation studies possibly provide the best data for risk modelling. However, for the chemicals benzidine and benzene there are some epidemiological data available that would permit an attempt at risk quantification, although very different in terms of both quality and quantity; one must be careful not to provide risk estimates beyond what the data permit.

The remainder of this section of the annex presents examples of quantitative cancer risk estimations, which were undertaken for benzidine and for benzene by the Working Group. It should be noted that these risk estimations are based entirely on human epidemiological data and do not incorporate the results of animal studies.

Benzidine production

Of the epidemiological studies available to the Working Group, only that of Zavon *et al.* (1973) provides sufficient information on levels of exposure to benzidine to serve as a basis for quantitative risk assessment. This study allows very crude estimates of the carcinogenic potency of benzidine to be made.

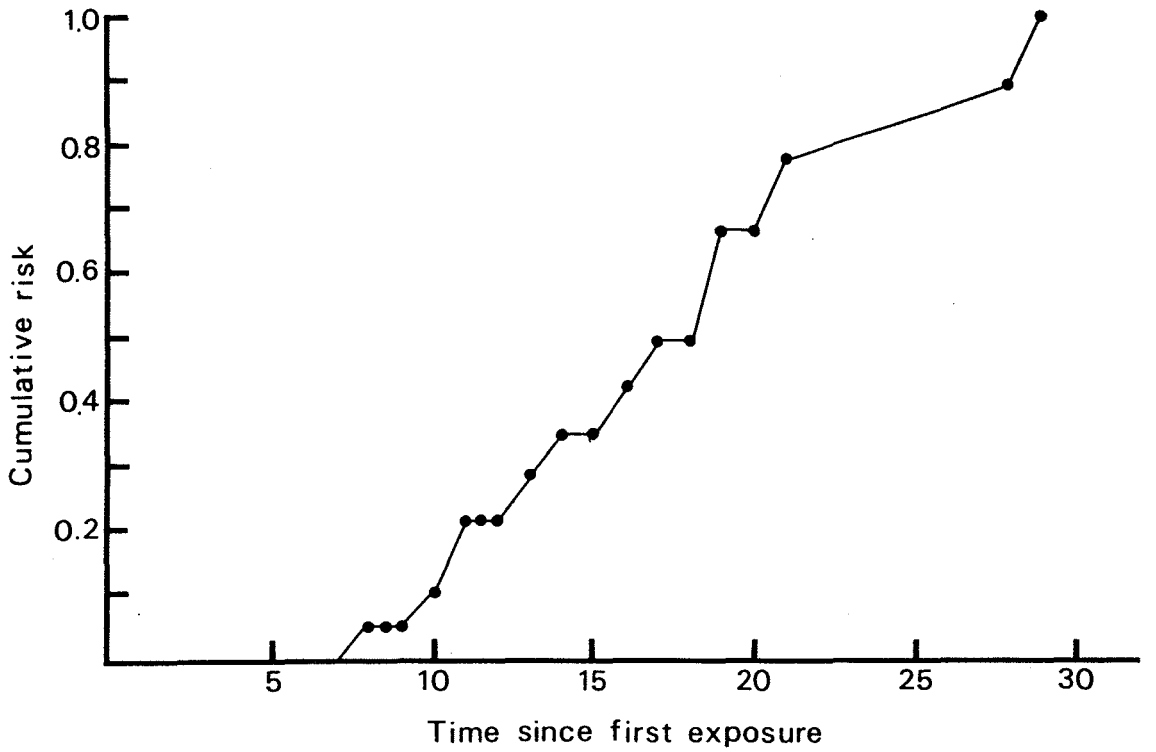
In that study, 13 of 25 exposed men developed bladder tumours. Atmospheric samples of benzidine at different work locations in the plant showed levels ranging from less than 0.005 mg/m^3 up to 0.4 mg/m^3, except at a work area where benzidine was shovelled into drums, where a concentration of 17.6 mg/m^3 was measured. Since only one worker is likely to have been in that area at a given time, it is reasonable to assume that a bladder tumour incidence of about 50% would be observed for exposure to between 0.005 mg/m^3 (assuming no job rotation and removal of the highly exposed worker) and $(0.4^{24} \times 17.6)^{1/25}$ = 0.5 mg/m^3 (assuming job rotation).

Tumours occurred, on average, after exposure of 13.5 years' duration; mean duration of exposure for the entire cohort was 11.5 years. A working lifetime exposure of 45 years would probably increase an already high tumour rate. Because of the small size of the cohort and uncertainty about the measurements of exposure, it does not seem reasonable to attempt to predict with much confidence effects at lower levels of exposure.

In Figure 1, the cumulative risk of bladder tumour for the cohort evaluated by Zavon *et al.* is given as a function of duration of exposure for those workers continuously exposed. A latency of 8 years from first exposure is observed, with cumulative incidence rates of 25% and 75% after 15 and 20 years' exposure, respectively. The curve, particularly that part reflecting 15 years or more of exposure, is based on small numbers. The final cumulative risk of 100% is based on only 3 cases - the last 3 individuals - all of whom developed bladder cancer. An alternative way of expressing the risk is in terms of the average annual incidence, which is approximately 5% in this group 5 years or more after exposure began. However, averaging incidence in this manner is based on the assumption that risk varies linearly with duration of exposure.

If this risk estimate were extrapolated to lower levels of exposure by reducing exposures by a factor of 100, and assuming a proportionally reduced risk, then a cumulative incidence of bladder tumour of 0.25% would be anticipated after 15 years' exposure (e.g., 25%/100). After a working lifetime of 45 years at the reduced exposure, an incidence of 3/4% (i.e., 1/4% x 45/15) would be expected if linearity in duration of exposure were the appropriate model. However, if the cumulative incidence were related to duration of exposure as t^5 (as might be inferred from population incidence data), then the projected lifetime incidence would be 60% instead of 3/4%. These differences in the risk estimates illustrate the central importance in risk estimation of the choice of model. This issue could be resolved for benzidine only if additional epidemiological data were available on people exposed to lower levels, or if information on the biological mechanisms whereby benzidine induces bladder tumours could be obtained.

Fig. 1 Cumulative risk of developing a bladder tumour as a function of duration of continual exposure to benzidine (From data in Zavon *et al.*, 1973)



Benzene

Several epidemiological studies of benzene provide quantitative information on levels and duration of exposure. To begin, we consider the study of Rinsky *et al.* (1981), which essentially completes the follow-up by Infante *et al.* (1977) of leukaemia mortality in rubber hydrochloride workers. Lifetime risk of leukaemia mortality can be estimated from the data of Rinsky *et al.*: The excesses of leukaemia deaths observed in the two plants included in the study were similar and are thus combined to yield a relative risk of 7/1.26 = 5.6.

Unlike the situation for the cohort considered in the previous section, it was not possible to obtain estimates of the risk associated with continuous exposure. It is apparent, however, that risk is greatest among those with longest exposure, relative risks of approximately 2, 14 and 32 being observed for exposures of less than 5 years (2 cases), 5-9 years (2 cases) and 10+ years (3 cases), respectively. The relative risk associated with at least 5 years of exposure is thus likely to be a lower bound for risk associated with lifetime exposure at similar levels. For those with at least 5 years' exposure, 5 cases were observed compared with an expected number of 0.237, giving a relative risk of 21.1. Since the expected cumulative male adult lifetime (from 20 years to end of life, taken as age 75) probability of dying from leukaemia is approximately 7 per 1000 in the general population of the Untied States, then an observed relative risk of 21.1 would give an extra $(21.1 - 1.0) \times 7 = 141$ cases of leukaemia per 1 000 exposed population.

An alternative approach would be to suppose that relative risk increases linearly during continuous exposure. The overall relative risk of 5.6 found in the Rinsky study derives from leukaemia cases who had had an average of 8.5 years of exposure. Over a 45-year working lifetime, the excess relative risk would increase smoothly from 0 at the start to 4.6 x 45/8.5 = 24.4 after 45 years. The excess number of cases to be expected during life can then be obtained by applying these increasing values of the excess relative risk to the age-specific rates for leukaemia. This calculation gives a figure of 170 excess cases per 1000, over a lifetime. The two estimates, 140 and 170 per 1000 are close, the method of calculation for the former being clearly conservative, as explained.

With regard to the exposure levels associated with these cases, it appears reasonable to assume that the average level to which the cohort was exposed during the critical study period of 1940-1950 would fall in the range of 10 ppm to 100 ppm, although the methods employed at that time for measuring benzene concentrations in air, while reasonably accurate, were relatively less sensitive than those available today. Even in the face of these uncertainties in exposure estimation, assuming exposure was at the upper end of the range, then it is reasonable to postulate that a working lifetime exposure to 100 ppm of benzene would be likely to result in 140 to 170 cases of leukaemia per 1000 exposed workers.

Ott *et al.* (1978) described the mortality experience of 594 benzene-exposed workers. Levels of exposure reported by the authors ranged from 'very low exposure', to 1 ppm, up to a 'high exposure' of 30 ppm. The average duration of exposure of the cohort appeared to be approximately 8-9 years. The 3 cases of leukaemia observed gave a relative risk of 3.75 when compared with the 0.8 cases expected (based only on myelogenous leukaemia, with a lifetime rate of about 3 per 1000). These values are fairly consistent with the findings of the Rinsky *et al.* and Infante *et al.* study. Duration of exposure was about the same in both studies. However, the relative risk observed by Ott *et al.* was only two-thirds of that given by Rinsky *et al.*, and exposure was also somewhat lower, i.e., 1-30 ppm *versus* 10-100 ppm. (In addition, only 1/5 of the Ott cohort had had less than one year's exposure, as compared with 1/2 of the Rinsky-Infante cohort.)

The study of Vigliani (1976) suggests a relative risk of 20:1 for the heavily exposed workers (200-500 ppm), with a median tumour latency of 9 years. This also appears to be quantitatively compatible with the Infante-Rinsky values (4 times higher risk and a 2-5 times higher exposure rate).

The exposure levels of several hundred ppm observed by Aksoy (1977) and the interpretation of his study give a relative risk of the order of 25 (see section 3.3 of the monograph on benzene), which is also in agreement with the above calculations.

The other epidemiological studies did not contain sufficient data on cohort exposures to justify risk calculations.

Summary Remarks

In the quantitative estimates of cancer risk to man that are presented here, the Working Group restricted their analyses to data available in published form and kept extrapolations to a minimum. Although the quantitative estimates for benzidine and benzene are

relatively crude and reflect the present early stage of development of the field of risk estimation, the Working Group noted that several valuable points emerge from the exercise of estimating quantitative risk of cancer.

First, the review of the literature underscored the relative inadequacy of most published reports for quantitative risk estimation. Too few reports give sufficient detail on methods of exposure measurement, on variations in individual exposure over time, or on epidemiological methods. Clearly, it will be necessary in future epidemiological studies to collect the data that will permit more confident and complete estimation of quantitative risk. No longer can merely qualitative assessment of the carcinogenic potential of chemical agents be considered adequate.

At the same time, however, the Working Group found that quantitative estimation of the human cancer risk which is associated with exposure to benzidine or to benzene was more feasible than seemed initially to be the case. The Group was impressed that rather large amounts of quantitative information could be extracted from the published epidemiological studies and that reasonable risk estimations could then be based on that information.

Life-table analysis of data published for occupational exposure to benzidine resulted in estimates of 25% and 75% bladder cancer incidence after 15 years' and 20 years' exposure, respectively. These estimates and the estimated final cumulative risk of 100%, although based on small numbers, suggest that benzidine has a strong carcinogenic potency for the bladder, which, given the circumstances of occupational exposure, appears to have overcome any possible biological variability in host response, such that individual variability was expressed only in terms of variations in the length of induction-latency.

Major new observations emerged in the course of this exercise with regard to quantitative estimation of the human cancer risk associated with exposure to benzene. The Working Group noted that the magnitude of this risk was consistent across the several published epidemiological studies, but cautioned that the estimates are based on reported levels of exposure which were sustained decades ago and which were measured using analytical techniques which, while reasonably accurate, were less sensitive than those available today. The Group observed that exposure to benzene under the conditions of the workplace was associated with increased mortality from leukaemia, and that this mortality increased further with increasing duration of exposure. The Working Group declined to give a figure for the upper bound of estimated numbers of excess leukaemia cases following exposure over a working lifetime to the benzene levels reported in the published studies. Minimum estimates of 140 to 170 excess leukaemia deaths per 1000 exposed workers over a working lifetime were calculated for an exposure level of 100 ppm.

These two examples illustrate the utility of quantitative analysis of epidemiological data for assessing carcinogenicity. The Working Group recognized, however, that the methods used to arrive at these quantitative estimates are based on certain simplifying assumptions about levels of exposure and about mechanisms, as described in the first three sections of this annex. It thus recommended systematic review of the subject of risk estimation and creation of a monograph on the theory, methods and illustrative examples of quantitative estimation of cancer risk, including comparisons of human and animal studies.

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SUPPLEMENTARY CORRIGENDA TO VOLUMES 1—28

Corrigenda covering Volumes 1-6 appeared in volume 7; other appeared in Volumes 8,10-13,15-28.

Volume 10

p. 29	line 16	*replace "threo" by* thr

Volume 24

p. 163	after title	*add* These compounds were considered by a previous working group, in December 1974 (IARC, 1975). Since that time, new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.
p. 171	at end off page	*add* IARC (1975) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man,* Vol. 8, *Some aromatic azo compounds,* Lyon, pp. 117-123

CUMULATIVE INDEX TO IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Numbers in bold indicate volume, and other numbers indicate page. References to corrigenda are given in parentheses. Compounds marked with an asterisk (*) were considered by the working groups, but monographs were not prepared because adequate data on carcinogenicity were not available.

Composition, impression et façonnage
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