WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISK

OF CHEMICALS TO MAN

VOLUME 1

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

LYON

1972

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RISK OF CHEMICALS TO MAN

Volume 1

This publication is the outcome of the
meeting of the IARC Working Group on
the Evaluation of the Carcinogenic Risk
of Chemicals to Man, Geneva, 13-17 December 1971

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Geneva, 13-17 December 1971

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Note to the Reader

This is the first series of monographs on the evaluation of the carcinogenic risk of chemicals to man elaborated by the IARC Working Group which met in Geneva from 13 to 17 December 1971.

In the case of some of the substances under consideration, the Group was faced with the problem of interpreting animal data, in the absence of human data, in terms of possible human risk. Since there are no objective criteria for doing so, the Group did not express any opinion on the significance of such data to man and referred to decisions taken in this respect by certain WHO expert committees (see the section "Extrapolation from animals to man" in the introduction to this volume). Some of the members felt that an educated guess as to the degree of power and/or carcinogenic potential would have been feasible and desirable. IARC, with the help of experts, will attempt to elaborate some guiding principles for the extrapolation from animals to man. This can only be done with some definite cases in mind. Therefore, the compilation of monographs in the present form will continue in order to provide the necessary background material. However, these monographs may be revised at a later date in the light of such new principles.

IARC would welcome comments on these monographs.

CONTENTS

BACKGROUND AND PURPOSE OF THE IARC PROGRAMME ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO MAN

There has been a rapid increase in the number and quantity of chemicals in the environment. The possible adverse effect of these chemicals on human health is a matter of international concern, as reflected by the frequency with which IARC has been asked for its advice as to the carcinogenic risk from various chemicals. The Agency has in consequence considered means of obtaining international expert opinion on this subject.

In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended: "...that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented."

Governments have become increasingly aware of the importance of environmental pollution. Control measures must be at least as strong as those taken for microbiological hazards. With this in mind, the Governing Council of IARC at its Ninth Session considered a Resolution concerning the role of the Agency in providing government authorities with expert, independent scientific opinion on environmental carcinogenesis. As one means to this end, the Governing Council recommended that the Agency should continue to prepare monographs on the carcinogenic risk of individual chemicals to man.

The objective of this programme is to achieve a balanced evaluation of data through the deliberations of an international group of experts in chemical carcinogenesis and to put into perspective the present state of knowledge with the final aim of evaluating the data in terms of possible human risk, as well as to indicate the need for research efforts.

SCOPE OF THE MONOGRAPHS

The present volume is the first series of monographs to be published. These monographs summarize the evidence for the carcinogenicity of individual chemicals in a condensed uniform manner for easy comparison. The data were compiled, reviewed and evaluated by a working group of experts. No recommendations are given concerning preventive measures or legislation, since these matters depend on risk-benefit evaluation, which seems best made by individual governments and/or international agencies such as WHO and ILO.

As new data on chemicals for which monographs have already been written and new principles for evaluation become available, re-evaluation will be made at future meetings, and revised monographs will be published as necessary. Special meetings can be called to evaluate important compounds, for which the data are controversial and there seems to be an urgent need for action by public

health authorities or other governmental bodies. The monographs will be distributed to international and governmental agencies, will be available to industries and scientists dealing with these chemicals, and will form the basis of advice from IARC on carcinogenesis from these substances.

MECHANISM FOR PRODUCING THE MONOGRAPHS

As a first step, a list of chemicals for possible consideration by the Working Group was established. IARC collected pertinent references regarding physico-chemical characteristics, use and occurrence, as well as biological data on these compounds. Assistance in collecting data on use and occurrence was provided by WHO, ILO and the United States National Cancer Institute. The material was summarized by an expert consultant or an IARC staff member, who prepared the first draft monograph, which was then sent to another expert for comments. In some cases, a study group was organized to finalize the draft monograph, which was circulated to all members of the Working Group about two months before the meeting, at which further additions to and deletions from the data were agreed upon and a final version of comments and evaluation on each compound was adopted.

Priority for the Preparation of Monographs

Priority for consideration was given mainly to chemicals for which experimental evidence of carcinogenicity existed and/or for which there was evidence of human exposure. However, neither human exposure nor carcinogenicity could be judged until all the relevant data had been collected and examined in detail. The preparation of a monograph on a particular compound did not necessarily mean that the substance was carcinogenic. Equally, the fact that a substance had not yet been considered did not imply that it was non-carcinogenic.

Data on which the Evaluation was based

With regard to the biological data, only published articles or papers already accepted for publication were reviewed. Every effort was made to cover the whole literature, but the monographs were not intended to itemize all studies on carcinogenicity. Since some important data might have been missed, research workers who have completed studies that may change the comments on the data reported are invited to send their publications to the Unit of Chemical Carcinogenesis, International Agency for Research on Cancer, Lyon, France.

The Working Group

The members of the Working Group who participated in the meeting at Geneva are listed at the beginning of this publication. Each monograph bears a footnote

indicating the date of the meeting at which it was considered. The members of the Working Group were invited by IARC to serve in their individual capacities as scientists, and not as representatives of their governments or of any institute to which they were affiliated.

GENERAL REMARKS ON THE EVALUATION

Terminology

The term "chemical carcinogenesis" in its widely accepted sense is used to indicate the induction or enhancement of neoplasia by chemicals. It is recognized that, in the strict etymological sense, this term means the induction of cancer. However, common usage has led to its employment to denote the induction of various types of neoplasm. The terms "tumourigen", "oncogen" and "blastomogen" have all been used synonymously with "carcinogen", although occasionally "tumourigen" has been used specifically to denote the induction of benign tumours.

Response to Carcinogens

For present practical purposes, no distinction is made between the induction of tumours and the enhancement of tumour incidence, although it is noted that there may be fundamental differences in mechanisms that will eventually be elucidated.

The response to a carcinogen in experimental animals may be observed in several forms:

(a) as a significant increase in the frequency of one or several types of neoplasm, as compared with other than zero frequency in control animals;

(b) as the occurrence of neoplasms not observed in control animals;

(c) as a decreased latent period as compared with control animals;

(d) as a combination of (a) and (c).

Qualitative Aspects

The qualitative nature of neoplasia has been much discussed. Many instances of carcinogenesis involve the induction of both benign and malignant tumours. There are few, if any, recorded instances in which only benign tumours are induced; their occurrence in experimental systems is usually indicative of eventual malignancy.

In experimental carcinogenesis, the type of cancer seen is often the same as that recorded in human studies (e.g., bladder cancer in man, monkeys, dogs and hamsters with 2-naphthylamine). In other instances, however, a chemical will induce different neoplasms or neoplasms at different sites in different animal species (e.g., benzidine, which induces hepatic carcinoma in the rat, but bladder carcinoma in man).

Quantitative Aspects

Dose-response studies are important in the evaluation of human and animal carcinogenesis. On occasion, the only way in which a causal effect can be established is by the observation of increased carcinogenesis in relation to increased exposure. It is hoped that, eventually, dose-response data may be used for assessment in carcinogenesis in the same way that they are used in general toxicological practice.

Extrapolation from Animals to Man

In this first series of monographs, no attempt has been made to interpret the animal data in the absence of human data in terms of possible human risk, and no distinction has been made between weak and strong carcinogens, since no objective criteria are at present available to do so. These monographs may be reviewed if some such criteria should be elaborated. In the meantime, the critical assessment of the validity of the animal data given should help national and/or international authorities to make decisions concerning preventive measures or legislation in the light of WHO recommendations on food additives,[1] drugs[2] and occupational carcinogens.[3]

Evidence of Human Carcinogenicity

Evidence that a particular chemical is carcinogenic in man depends largely on epidemiological data, which may be in the main descriptive, retrospective or prospective.

Descriptive study may identify a cluster, or a change or difference in rates for a neoplasm in a sub-group of the population. Retrospective study - going into the histories of affected persons - has revealed such occupational or iatrogenic carcinogens as shale oil, chromates, asbestos, naphthylamine, benzidine, chlornaphazin, thorotrast and transplacental synthetic oestrogens.

Once a relationship is known or suspected between a chemical and human cancer, prospective (cohort) studies will identify more precisely the time relationship and the magnitude of the risk, among other details of cancer induction. Prospective studies are follow-up studies. Groups of persons are identified in respect of degree and duration of exposure to a suspected or known carcinogen.

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1 Wld Hlth Org. techn. Rep. Ser., 1961, No. 220, pp. 5, 18 and 19.

2 Wld Hlth Org. techn. Rep. Ser., 1969, No. 426, pp. 19, 21 and 22.

3 Wld Hlth Org. techn. Rep. Ser., 1964, No. 276, pp. 29 and 30.

In case-control studies, the comparison groups should be as similar as possible in all respects except exposure to the agent. Follow-up is then made to determine the occurrence of cancer in the various exposure categories (from none to heavy), and by comparison of results among them, the magnitude of the increased risk may be indicated. In addition, the analysis will reveal the relationship between cancer occurrence and age at first exposure, as well as the latent period. Care must be taken in the analysis to exclude the influence of variables other than the agent under suspicion in inducing the cancer under study (e.g., cigarette-smoking in the study of lung cancer among asbestos workers).

Finally, if man does develop cancer from a specific chemical, its removal from the environment should be followed by epidemiological evidence of a decline in the frequency of the neoplasm.

Mixtures and Groups of Carcinogens

Mixtures of chemicals are sometimes associated with the occurrence of cancers in man, but no information is available on the specific components. Continuing efforts should be made to elucidate the role of the various components, to assist in planning better preventive measures and to provide a basis for assessing similar hazards. There are situations where carcinogens may occur in groups in the human environment and where it is not yet possible to attribute the observed effects to individual substances. This is notably so in the case of the polycyclic aromatic hydrocarbons and certain aromatic amines. It may be necessary at a later stage, for practical purposes, to record also the effects of mixtures pending further elucidation.

EXPLANATORY NOTES ON THE MONOGRAPHS

In sections 1, 2 and 3 of each monograph, except for minor remarks, the data are recorded as given by the author, whereas the comments by the Working Group are given in section 4, headed "Comments on data reported and evaluation".

Title of the Monograph

The monograph has as its title the chemical name of the substance under consideration. For these names, the chemical abstract nomenclature is normally used.

Chemical and Physical Data (section 1)

Chemical and physical properties include data that might be relevant to carcinogenicity (for example, lipid solubility) and those that concern identification. Wherever possible, data on solubility, volatility and stability are indicated. All data except those for "Technical products and impurities" refer to the pure substances.

Use and Occurrence (section 2)

It must be borne in mind that the data recorded under "Occurrence" are dependent on the analytical method used. In some instances, the results are questionable quantitatively and sometimes even qualitatively because the methods employed are not satisfactory.

Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man (section 3)

As pointed out earlier in this introduction, the monographs are not intended to itemize all studies reported in the literature. Although every effort was made to review the whole literature, some studies were purposely omitted (a) because of their inadequacy, (b) because they only confirmed findings already reported or (c) because they were judged irrelevant for the purpose of the evaluation. The data recorded here are summarized as given by the author and critical comments by the Working Group are made in section 4("Comments on data reported and evaluation").

Carcinogenicity and related studies in animals (3.1)

Mention is made of all routes of administration by which the compound has been tested and all species in which the chemical has been investigated. In some cases where similar results were obtained by other authors and/or other laboratories, reference is made to a summary article. Quantitative data are given in so far as they will enable the reader to realize the order of magnitude of the effective dose. The doses are indicated as they appear in the original paper. In general, negative experiments of an inadequate standard are not summarized. In certain cases, however, it was felt that such data should be included since they would contribute to the total picture even if rigid requirements on the experimental conditions were not fulfilled (for example, at least 25 animals of each sex kept for at least 18 months in the case of mice and two years in the case of rats, and where there is an assurance of an adequate level of pathological examination).

Metabolism in animals and man (3.2)

The reporting of metabolic data is restricted to data that help to trace the metabolic pattern of the carcinogen in animals or man. Mention is made, whenever the information is available, as to whether the substance under consideration is carcinogenic per se, that is, in its final reactive form, or requires metabolic activation. Other metabolic information (e.g., absorption and excretion) helpful in interpreting quantitative data is also included.

Observations in man (3.3)

This sub-section includes summaries of reports of cases of cancer in man that have been related to possible exposure to the chemical. Epidemiological studies

are also summarized. These also are recorded as given by the author, and sometimes include his own comments on the study as well as comments by other experts.

Comments on Data Reported and Evaluation (section 4)

This section includes the critical view of the Working Group on the data reported. It is purposely kept as brief as possible since it should be read in conjunction with the data recorded.

Animal data (4.1)

The animal species mentioned are those in which the carcinogenicity of the substances was clearly demonstrated, irrespective of the route of administration. If some species was tested with negative results, this fact is also recorded. In the case of inadequate studies, comments to that effect are included. The route of administration used in experimental animals that is similar to the possible human exposure (ingestion, inhalation and skin exposure) is given particular mention. In most cases, tumour sites are also indicated. If the substance has produced tumours on pre-natal exposure or in single-dose experiments, this is also indicated. This sub-section should be read in the light of comments made in the section "Extrapolation from animals to man" of this introduction.

Human data (4.2)

In some cases, a brief statement is made here on the possible exposure of man, but details on this are found in section 2. The significance of epidemiological studies and case reports is discussed and, if possible, the data are interpreted in terms of possible human risk. Other supporting evidence, such as toxicity related to carcinogenicity or similarities in metabolism in animals and man, is also briefly mentioned.

THE MONOGRAPHS

INORGANIC SUBSTANCES

BERYLLIUM AND BERYLLIUM COMPOUNDS*

1. Chemical and Physical Data

Beryllium

1.1 Synonyms and trade names

Chem. Abstr. No.: 7440417

Glucinium

1.2 Chemical formula and molecular weight

Be At. wt: 9.01

1.3 Chemical and physical properties of the pure substance

(a) Description: A hard, non-corrosible grey metal

(b) Boiling-point: 2970°C

(c) Melting-point: 1284-1300°C

(d) Hardness: 60-125

(e) Density: 1.84-1.85

(f) Solubility: Soluble in acids or alkalis

(g) Chemical reactivity: For an authoritative account of the chemistry of beryllium, see Krejci & Scheel (1966).

Beryllium oxide

1.1 Synonyms and trade names

Chem. Abstr. No.: 1304569

Beryllia

1.2 Chemical formula and molecular weight

BeO Mol. wt: 25.01

1.3 Chemical and physical properties of the pure substance

(a) Description: An amorphous white powder

(b) Melting-point: 2530°C

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* Considered by the Working Group in Geneva, December 1971.

(c) Hardness: 9

(d) Density: 3.02

(e) Solubility: Soluble in acids and alkalis; insoluble in water. (The purified beryllium oxide used by Dutra & Largent (see below) was soluble in water to the extent of 0.7 µg per 100 ml.)

(f) Technical products and impurities: Spencer et al.(1965) reported that the toxicity of beryllium oxides varied according to the method of their preparation. Beryllium oxide prepared by calcining α-$Be(OH)_2$ for 10 hours at 500°C was more toxic and produced more fibrosis and more tumours in rats than did beryllium oxide prepared by calcining α-$Be(OH)_2$ for 10 hours at 1600°C. In general, as the calcining temperature and time were increased, the surface area decreased and the crystallinity and density increased.

Beryl

1.1 Synonyms and trade names

Chem. Abstr. No.: 1302529

1.2 Chemical formula and molecular weight

$3BeO.Al_2O_3.6SiO_2$ Mol. wt: 537.54

1.3 Chemical and physical properties of the pure substance

(a) Description: Hexagonal crystals

(b) Hardness: 7.5-8

(c) Density: 2.7

(d) Technical products and impurities: According to Wagner et al. (1969), the chemical composition of beryl ore samples is as follows: Be (4.14%), Al_2O_3 (18.1%), SiO_2 (65.5%), Fe_2O_3 (1.1%), MnO_2 (1.0%), MgO (1.1%), Na_2O (0.5%), NiO (0.5%).

Beryllium sulfate

1.1 Synonyms and trade names

Chem. Abstr. No.: 7787566

1.2 Chemical formula and molecular weight

$BeSO_4.4H_2O$ Mol. wt: 177.14

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless crystals

(b) Solubility: Insoluble in ethanol; soluble in water

(c) Stability: Loses 2 moles of water at 100°C and 4 moles of water at 250°C.

(d) Chemical reactivity: Krejci & Scheel (1966) discuss in detail the behaviour of beryllium salts in aqueous solution.

2. Use and Occurrence

(a) Use

According to Hueper (1966), beryllium has only found significant industrial use since 1920. At first, it was used mainly in Europe, but during the Second World War its industrial use grew rapidly in the USA.

Processes and products involving the use of beryllium include: (i) alloys of beryllium with copper, aluminium and nickel; (ii) ceramics and vitreous enamel; (iii) refractory crucibles; (iv) textile fibres; (v) gas mantles; (vi) atomic energy reactors; (vii) space vehicles and rocket motors.

The use of beryllium phosphors in fluorescent and neon tubes was discontinued in the USA and most other countries during the early 1950's. The main human hazard related, not to the manufacture of tubes, but to the breaking-up of faulty tubes. Hueper (1966) listed the occupations in which men have been exposed to beryllium-containing compounds as follows:

Processing Be from ore	BeO
Fluorescent powder manufacture	BeO and $ZnBeSiO_2$
Fluorescent lamp works (manufacture and salvage)	$ZnBeSiO_2$ and BeO
Machining Be	BeO
Alloying Be	BeO
Laboratory work	BeO
Ceramics	BeO
Crystal manufacture	BeO

It should also be noted that rocket exhaust fumes may contain beryllium oxide, fluoride and chloride in significant amounts (Robinson et al., 1968). Because of its toxicity, the commercial use of zinc beryllium silicate as a phosphor has now been largely discontinued (Krejci & Scheel, 1966).

(b) Occurrence

Beryl, the ore from which beryllium is refined, is mined in Argentina, Brazil, India, Madagascar and the USA. Beryl is a beryllium aluminium silicate ($3BeO.Al_2O_3.6SiO_2$).

Beryllium may also be obtained from other ores such as bertrandite, which contains less than 1% beryllium. Bertrandite ores contain a minimum of 15% free silica and have a high fluoride content.

The following table from Krejci & Scheel (1966) enumerates the natural sources of beryllium:

Natural Occurrence of Beryllium. Representative Minerals

Mineral	Composition	Geological occurrence	Geographical distribution
Beryl	$3BeO.Al_2O_3.6SiO_2$	Pegmatite	Widely distributed
Beryllonite	$NaBePO_4$	Pegmatite	Maine
Bertrandite	$Be_4Si_2O_7(OH)_2$	Pegmatite	Colorado, Maine, France, Bohemia
Bromellite	BeO	Veins	Sweden
Chrysoberyl	$Be(AlO_2)_2$	Pegmatite	Brazil, Ceylon, Urals, New York
Euclase	$BeHAlSiO_5$	Pegmatite	Brazil, Urals, Austrian Alps
Hambergite	$Be_2(OH)BO_3$	Pegmatite	Norway, Madagascar
Helvite	$Mn_4Be_3Si_3O_{12}S$	Pegmatite, veins	Iron Mountain, New Mexico; Norway, Russia, Australia, Canada, Brazil
Herderite	$CaBePO_4(OH,F)$	Pegmatite	Maine
Leucophanite	$(Ca,Na)_2BeSi_2(O,OH,F)$	Pegmatite	Norway
Phenacite	Be_2SiO_4	Pegmatite	Colorado, Urals, Vosges Mountains

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

3.1 Carcinogenicity studies in animals

(a) Inhalation studies

Rat: Schepers et al. (1957) reported the induction of malignant pulmonary neoplasms in rats exposed to an aerosol of beryllium sulfate for periods of up to six months and thereafter observed without treatment for periods of up to 18 months.

Reeves et al. (1967) exposed 75 male and 75 female rats continuously to the inhalation of beryllium sulfate aerosol at a mean concentration of

34 µg of beryllium per cubic metre for periods of up to 56 weeks. Sample animals were killed at 4-week intervals during the 56-week exposure period and also after the end of treatment until the 82nd week of age. Metaplastic changes of the alveolar epithelium were evident in rats killed at 20, 24, 28 and 32 weeks. Thereafter, changes classed by the authors as anaplastic were a constant finding. From the 40th week onwards, alveolar adenocarcinomas began to be found and tumours of this type were seen in all of 43 rats killed after the 56th week from the start of exposure. No lung tumours developed in 150 unexposed control rats.

Wagner et al. (1969) exposed rats for 6 hours a day on 5 days per week to an atmosphere containing 15 mg of bertrandite or beryl ore dust per cubic metre. By 17 months, 18 out of 19 rats exposed to beryl ore dust had pulmonary tumours of various types. Most of these tumours were of microscopic dimensions, but 4 were identified grossly at necropsy. No metastases were observed in any of the rats. Lung changes, including granulomatous lesions, but no tumours, were seen in rats exposed to bertrandite dust.

Hamster: Lesions that may have been pre-neoplastic or early neoplastic, but no definite neoplasms, were seen in hamsters exposed to either beryl ore or bertrandite for up to 17 months (Wagner et al., 1969).

Rabbit: Dutra et al. (1951) reported the occurrence of an osteogenic sarcoma in a rabbit exposed to beryllium oxide by inhalation.

Monkey: Schepers (1964) found a 3-mm diameter neoplasm - probably an alveolar carcinoma - in a female monkey of the Macacus mulatta species that died 82 days after the last of 10 daily exposures (6 hours per day) to an aerosol of beryllium phosphate ($BeHPO_4$) at a concentration of 373 µg per cubic foot. The monkey was one of 20 females of the same species exposed variously to beryllium sulfate, beryllium fluoride or beryllium phosphate. Most of the monkeys died or were killed early in the experiment. Chemical pneumonitis was a common cause of death. The author could not rule out the possibility that the tumour was spontaneous in origin.

Vorwald et al. (1966) gave each of 20 young Rhesus monkeys a single intrabronchial and/or bronchomural implantation of pure beryllium oxide (5% suspension in physiological saline). They exposed a further 10 monkeys intermittently over a prolonged period to an atmosphere containing a beryllium sulfate aerosol at a concentration of 35 $\mu g/m^3$. During the first 8 years of this study three of the monkeys exposed to beryllium oxide and two of those exposed to beryllium sulfate developed pulmonary cancers. In one of

the monkeys exposed to beryllium sulfate the tumour had metastasized to the liver and other abdominal organs. The cancers were predominantly anaplastic, but with both adenomatous and epidermoid patterns.

Wagner et al. (1969) exposed monkeys for 6 hours a day on 5 days per week to an atmosphere containing 15 mg of bertrandite or beryl ore dust per cubic metre. No pre-neoplastic or neoplastic lesions were seen in monkeys exposed to either ore.

(b) Other experimental systems

Intravenous administration: Cloudman et al. (1949) claimed to have produced malignant bone tumours in mice by the intravenous administration of zinc beryllium silicate via the tail vein (total dose of beryllium, 0.26 mg), but no details are given.

Gardner & Heslington (1946) were the first of many to report the induction of osteosarcomas in rabbits by the intravenous injection of zinc beryllium silicate or beryllium oxide. All of 7 rabbits that survived treatment with zinc beryllium silicate for seven or more months developed malignant osteosarcomas, often with multiple primary sites. Visceral metastases were present in 4 of the 7. The incidence of sarcomas in rabbits treated with beryllium oxide is not stated. The administration of 65 other different minerals in the same way to rabbits produced none of the above effects. The compounds that gave negative results included zinc silicate, zinc oxide and silicilic acid.

Hoagland et al. (1950) confirmed the findings of Gardner & Heslington (1946) by reporting 6 examples of the induction of osteosarcomas following the intravenous administration of zinc beryllium silicate to 10 rabbits. They also reported the induction of one tumour of the same type by the intravenous administration of beryllium oxide to a total of 9 rabbits. No tumours arose in 5 rabbits given a total of 1 g of beryllium phosphate by the ear vein.

Cloudman et al. (1949) produced radiographical bone changes and osteosarcomas in rabbits by the intravenous administration of zinc beryllium silicate but not by the intravenous administration of beryllium oxide.

Barnes et al. (1950) induced osteosarcomas in rabbits by the intravenous injection of non-radioactive zinc beryllium silicate or beryllium silicate. Seven out of 17 rabbits that survived the course of injections with zinc beryllium silicate developed osteosarcomas and 1 out of 11 survivors given beryllium silicate injections did so. Tumours arose between 39 and 83

weeks after the end of treatment. No tumours were seen among 4 rabbits that survived for between 61 and 120 weeks after intravenous injections of zinc silicate.

Barnes (1950) reported the induction of osteosarcomas in two rabbits given intravenous injections of beryllium metal particles.

Dutra & Largent (1950) studied the effects in rabbits of intravenously administered "highly purified beryllium oxide" and a "highly purified calcined phosphor comprised of beryllium oxide, zinc oxide and silica mixed in molar ratio 1:1:1". Four of the 6 animals treated with beryllium oxide and 2 out of the 3 treated with beryllium phosphor developed osteosarcomas.

The production of osteosarcomas in rabbits by the intravenous injection of insoluble beryllium salts has also been recorded, by Janes et al. (1954, 1956), Araki et al. (1954), Kelly et al. (1961), Yamaguchi (1963) and Komitowski (1968). Vorwald (1950) reported the induction of osteosarcomas in rabbits by the intravenous injection of beryllium phosphate. According to Janes et al. (1956), splenectomy before the intravenous injection of zinc beryllium silicate enhanced the carcinogenic effect of the latter in bone. However, their data were inadequate to prove this.

Higgins et al. (1964) reported the occurrence of a transplantable chondrosarcoma in a rabbit given a total of 3.3 g of beryllium, as zinc beryllium silicate, by a series of 20 twice-weekly intravenous injections.

Intramedullary administration: Tapp (1966, 1969) reported the induction of osteosarcomas in rabbits following a single intramedullary injection of zinc beryllium silicate into the tibia. No tumours arose at the site of intramedullary injection of 20 mg of zinc oxide into the opposite tibiae of the same rabbits.

3.2 Metabolism in animals and man

(a) Animals

Dutra & Largent (1950) reported on the distribution of beryllium in rabbits given beryllium oxide or a beryllium phosphor by intravenous injection a year or more before being killed or dying from osteosarcoma. Relatively high levels of the metal were found in the reticulo-endothelial cells of the liver and spleen. The lungs also contained high concentrations. The levels in bone, kidney and heart were much lower.

According to Spencer et al. (1965), the distribution of beryllium oxide in the body varies with the method of its preparation: after intratracheal instillation, an oxide prepared by calcining α-$Be(OH)_2$ for 10 hours at 500°C was found in high concentrations in the liver, kidneys and bones of rats, whereas an oxide prepared by calcining α-$Be(OH)_2$ for 10 hours at 1600°C was not distributed to these organs but remained mainly in the lungs.

In an experiment in which rats were exposed continuously to an atmosphere of 34 µg of Be per cubic metre in the form of an aerosol of beryllium sulfate, Reeves & Vorwald (1967) recorded a rate of accumulation of beryllium in the lungs that decreased with length of exposure. Beryllium left the lungs via the blood stream following solution in lung fluid or via lymph vessels. The latter route was more efficient in males than in females.

The ultimate site of accumulation of beryllium is the skeleton (Crowley et al., 1949; Van Cleave & Kaylor, 1955).

Reeves (1965) studied the absorption by rats of beryllium sulfate administered in the drinking water in daily doses ranging from 6.6 to 66.6 µg per rat per day. The majority of the beryllium was thought to be precipitated within the gut lumen as phosphate and to be lost in the faeces. At first a low concentration of beryllium was found in the urine, but after 6 to 9 weeks this decreased to a mere trace. Beryllium accumulated in the bones and to a lesser extent in the liver.

3.3 Observations in man

(a) Case reports

The occurrence of acute and chronic berylliosis in workers exposed to beryllium has been reported by De Nardi et al. (1953). In 20 cases of acute illness, which were followed up for 12 years, one pulmonary fibrosis with decreased vital capacity was observed.

(b) Epidemiology

Stokinger (1966) stated that "to date no human beryllium cancer has been identified, but the difficulties in attributing a lung, or other organ, cancer to beryllium are such that the beryllium source could well be overlooked; chemical carcinogens do not commonly remain at the site of the cancer, so the causative agent can be identified only through work histories."

Hardy et al. (1967), in their review of the United States Beryllium Case Registry data for the period 1952-1966, stated: "There is no Registry evidence as yet that beryllium causes cancer in humans." The fact that 3 cases

of bone cancer were seen as against between 0.05 and 0.1 cases expected is noteworthy, though uninterpretable at the present time.

Stoeckle et al. (1969) reported the long-term follow-up of 60 selected cases of chronic beryllium disease first diagnosed between 1944 and 1966. The nature of the exposure to beryllium varied. At the time of the report, 18 patients had died: 13 from cor pulmonale, 1 from respiratory insufficiency, 1 from cardiac arrest, 1 from virus pneumonia, 1 from renal insufficiency and 1 from an unstated cause. It is interesting that cancer had not been observed in this series up to 1969.

Mancuso & El-Attar (1969) studied the incidence of cancer in workers in two separate beryllium companies but could not conclude that cancer at any particular site could be correlated with the worker's exposure to beryllium.

Mancuso (1970) calculated that beryllium workers with a history of "chemical respiratory illness" experienced a higher risk of subsequently developing lung cancer than beryllium workers without such a history. The retrospective nature of this study and the fact that details of one worker's habits could not be taken into account render interpretation of this report difficult.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Experimental evidence for the carcinogenicity of beryllium, beryllium salts or beryl exists for three animal species. In particular, beryllium sulfate, beryl ore and bertrandite produce lung tumours in the rat following inhalation exposure; beryllium oxide and beryllium sulfate produce tumours in the monkey following intrabronchial implantation or inhalation; zinc beryllium silicate, beryllium metal and beryllium phosphate produce bone tumours in rabbits following i.v. administration.

4.2 Human data

Several epidemiological studies have been carried out on the possible relationship between exposure to beryllium compounds and the occurrence of cancer in man. These studies have not provided evidence of the existence of such a relationship.

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HAEMATITE AND IRON OXIDE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No. (iron oxide): 1309371

Iron oxide: Ferric oxide; Sesquioxide of iron

Iron ore: Haematite (hematite, red iron ore, blood stone); Limonite (brown hematite, brown iron ore, brown ironstone clay)

It should be noted that haematite (or hematite in the American spelling) may have two distinct meanings. It may be used to describe an iron plus silicon ore, to which miners are exposed (see Stewart & Faulds, 1934; Faulds & Stewart, 1956) or as a synonym for ferric oxide, Fe_2O_3 (see Saffiotti et al., 1968).

1.2 Chemical formula and molecular weight

Fe_2O_3 Mol. wt: 159.70

1.3 Chemical and physical properties of the pure substance

(a) Description

Iron oxide: A dense, dark red powder or lumps; after strong ignition it is steel grey.

Iron ore: The most important iron ore is haematite, which is a mineral found mainly in two forms: red haematite or red iron ore, which consists mainly of Fe_2O_3 and contains approximately 70% of iron; brown haematite or brown iron ore, which consists mainly of hydrated sesquioxide of iron (limonite) and contains approximately 42% of iron.

Less commonly occurring forms of haematite include: (i) brilliant lustre (a steel grey crystalline variety, known also as looking-glass ore, iron glance, or specular iron ore); (ii) micaeous iron ore consisting of thin scales or plates; (iii) speculous ore formed by the interaction of steam and ferric chloride in volcanic regions; (iv) soft red ore which is earthy; (v) red ochre and rouge.

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* Considered by the Working Group in Geneva, December 1971.

(b) Melting-point: Fe, 1535°C; Fe_2O_3, 1565°C

(c) Specific gravity: Fe, 7.85; Fe_2O_3, 5.12-5.24

(d) Solubility: Fe and Fe_2O_3 are soluble in acids and insoluble in water.

(e) Chemical reactivity: Iron is rapidly oxidized in damp or salty air (rust).

(f) Technical products and impurities: The absence of phosphorus in red haematite (red iron ore) makes it a valuable source of iron. Although haematite consists mainly of ferric oxide, the most commonly mined ores contain between 10% and 12% silica.

2. Use and Occurrence

(a) Use

Haematite is a source of iron. The very wide uses of iron ore are well known.

Hueper (1955) listed the following occupations as entailing the risk of inhalation of dust and fumes of iron and its various alloys and compounds: iron-ore miners; arc welders; grinders; polishers; silver finishers; metal workers. To this list boiler scalers should perhaps be added (Harding & Massie, 1951).

According to Stewart & Faulds (1934), haematite mining was judged to be a relatively healthy occupation until the introduction of pneumatic drills in 1913 into the West Cumberland mines and the great increase in airborne dust which their use entailed. Thereafter, the incidence of emphysema, bronchitis and tuberculosis among the iron-ore miners increased.

(b) Analytical methods

Iron was analysed by atomic absorption spectrophotometry in baking powder (Holak, 1970) and in alcoholic beverages (Meredith et al., 1970) and by the ring oven technique for airborne particulates (West, 1966).

(c) Occurrence

Deposits of haematite are found in: (i) parts of Lancashire, Cumberland and Cornwall in the British Isles; (ii) Northern Spain in the region of Bilbao; (iii) Minnesota, USA, near Lake Superior; (iv) Italy, in the Apuan Alps between Massa and Lucca; (v) Sweden; (vi) Ukraine in Krivoi Rog iron district and Kertch district.

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

3.1 Carcinogenicity studies in animals

(a) Inhalation and intratracheal administration

Mouse: Campbell (1940, 1942, 1943) reported a higher frequency of lung tumours in mice exposed by inhalation to ferric oxide steel grindings, to a mixture of aluminium oxide, ferric oxide and silicon dioxide or to a mixture of the oxides of aluminium, silicon, iron and calcium than in control mice. (These experiments must be regarded as inconclusive because of the genetic randomness of the mice used and the fact that the differences in tumour frequency observed were small.)

Hamster: Saffiotti et al. (1968) gave to 24 male and 24 female Syrian golden hamsters a series of 15 once-weekly intratracheal injections of 3 mg of "hematite" dust (ferric oxide) suspended in 0.2 ml of physiological saline. Although more than 50% of the animals survived for 1 year and a few for over 2 years, none of them developed tumours of the lung. (It is important to point out that the dust referred to by Saffiotti et al. as "hematite" was ferric oxide, Fe_2O_3, as supplied by Fisher Scientific Co., Fair Lawn, N.J., and not the haematite to which miners are exposed.)

Guinea-pig: Vorwald & Karr (1937, 1938) failed to increase the frequency of lung tumours in guinea-pigs by exposing them to haematite dust.

(b) Other experimental systems

Subcutaneous implantation: Gilman & Herchen (1963) and Herchen & Gilman (1964) saw no tumours in rats following the subcutaneous implantation in them of ferric oxide discs.

3.2 Metabolism in animals and man

Iron is a vital component of the body. Over 70% of the body's iron is normally present as haemoglobin, 3% as myoglobin and 16% as transport iron. Iron may be stored in two forms: ferritin - an iron-protein complex - and haemosiderin (see Lancet, 1963, for review). For the purpose of this monograph, iron metabolism in general will not be considered and attention will be confined to the fate of inhaled particles of haematite ore and ferric oxide.

(a) Animals

In the experiments both of Campbell (1940, 1942, 1943) and of Saffiotti et al. (1968), a proportion of haematite or iron oxide dust introduced into the lungs of experimental animals remained within the lungs of tracheo-bronchial lymph nodes throughout their remaining life-span. The particles appear to penetrate the walls of alveoli and respiratory bronchioles and remain in the connective tissues of the lung within macrophages which form clusters of various sizes (Saffiotti et al., 1968).

(b) Man

In haematite miners, deposits of dust that contain both iron and silica are found in the lungs (Faulds & Stewart, 1956). It is clear, therefore, that under conditions of heavy exposure to haematite dust, pulmonary clearance mechanisms may be overwhelmed. According to Faulds & Stewart (1956), the upper lobes and upper parts of the lower lobes tend to be more affected by fibrosis than the lower parts of the lower lobes. Also, the peripheries of the lungs tend to be more affected than the central regions.

3.3 Observations in man

(a) Iron-ore (haematite) miners

Stewart & Faulds (1934) reported 1 case of bronchial carcinoma and 11 of gross pulmonary tuberculosis among a group of haematite miners.

Faulds & Stewart (1956) reported that 17/180 (9.4%) haematite miners in the county of Cumberland, England, who came to necropsy had primary carcinoma of the lung. The frequency of lung cancer at necropsy in comparable males who were not haematite miners was 45 out of 2221 (2.0%). This latter total included a number of coal miners working in the same region. The incidence of lung cancer in haematite miners was apparently higher during 1948-1953 than during 1932-1948.

Higher concentrations of silica and iron were found in the lungs of haematite miners with fibrosis (associated or not with pulmonary tuberculosis) or with bronchial carcinoma than in the lungs of those with no such pathology; but the levels in those with carcinoma were not higher than the levels in those with fibrosis (accompanied or not by tuberculosis) but no carcinoma (Stewart & Faulds, 1934).

According to Faulds & Stewart (1956), the primary tumour usually arose in the area of the lung most affected by fibrosis due to sidero-silicosis.

Vorwald & Karr (1937, 1938) reported 3 cases of lung cancer in haematite miners. Braun et al. (1960), in a study of iron-ore miners in the Lorraine basin of France, compared the incidence of bronchogenic carcinoma in 1095 iron-ore miners and 940 non-miners (all males in both cases): a 3.3% incidence in the former is significantly higher ($P = 0.01$) than in the latter (1.5%). The authors comment, however, that the use of tobacco is common among their iron miners. A high proportion of the cancers in iron-ore miners were of the anaplastic histological variety.

Monlibert & Roubille (1960) also reported an excessive incidence of bronchial cancer among iron-ore miners of the Lorraine basin. They found 64 cases of the disease among 10 000 iron-ore miners as compared with 28 cases among 10 000 workers from an iron works in the same district. The co-existence of lung cancer and silicosis was noted in 10 ex-miners on pension.

Kraus et al. (1957) reported that a higher proportion of males with a diagnosis of cancer of the stomach admitted to the Roswell Park Memorial Institute in Buffalo, N.Y., USA, gave a history of occupational exposure to iron-containing dusts than did males admitted with other diagnoses. The difference was 2.7-fold between those exposed to iron dust for 10 or more years and controls. There seems to have been no corroboration of this finding to date.

A survey of 355 cases of pulmonary cancer occurring in the Krivoi Rog mining region of the USSR during the period 1958-1964 was reported by Gurevich (1967). High death rates for the disease were found among iron-ore miners (particularly those working underground), transport and smelter workers. The death rate from lung cancer among iron-ore workers was 10.7 times higher than that among non-miner residents in the region of the mines.

The most comprehensive epidemiological study is that by Boyd et al. (1970), who examined the death certificates of 5811 male residents of a haematite mining region of Cumberland, England, who died between 1948 and 1967. They found 36 lung cancer deaths among underground haematite workers as compared with about 21 expected on the basis of either local non-miner deaths or the national average. No excess mortality from lung

cancer was found among surface iron-ore miners, and for iron miners in general mortality from cancers of sites other than the lung was close to the national average.

(b) Foundry workers and metal grinders

Turner & Grace (1938) reported that foundry workers and metal grinders had a higher mortality from lung cancer than any other occupational group in the environs of Sheffield, England. A high mortality from lung cancer among metal grinders was also a feature of the study reported by Kennaway & Kennaway (1947). Instead of the 22 deaths from this cause expected in men of this occupation in England and Wales between 1921 and 1938, 39 were recorded, the observed to expected ratio being 1.76 to 1. McLaughlin et al. (1950) and McLaughlin & Harding (1956), also in studies of workers in the Sheffield region of England, reported a raised incidence of lung cancer in iron and steel foundry workers. Podhrázský (1957) reported that the mortality from lung cancer among male workers in a steel plant in Czechoslovakia was 6.6 per 10 000, as compared with the national figure of 4 per 10 000 for Czechoslovakian males generally.

(c) Welders and "hot metal" workers

Wynder & Graham (1951) and Breslow et al. (1954), in separate surveys, found that larger numbers of men with lung cancer than of men with other diseases could be described as "hot metal" workers (exposed to hot metal fumes or metal dusts) most of whom are likely to have worked with iron. A third study, by Doll (1953), failed to confirm this finding. Moreover, a survey by Doig & McLaughlin (1948) failed to reveal any cases of lung cancer among 300 welders and Doig & Duguid (1951) did not mention lung cancer in their survey of the world literature on welders. It would be inadvisable to regard iron as solely responsible for disease in welders, since such workers are exposed to many metals. Zinc, for instance, may be present in higher concentration than iron (Coulter, 1954).

(d) Silver finishers

Studies of silver finishers, who may be heavily exposed to iron oxide dust, have revealed no excessive incidence of lung cancer (McLaughlin et al., 1945; Barrie & Harding, 1947; Harding, 1948).

(e) Boiler scalers

Harding & Massie (1951) reported 3 cases of lung cancer among 12 autopsies on boiler scalers, who are exposed to silica, iron and soot.

(f) Shipyard workers

Stumphius & Meyer (1968), in a discussion of the relationship between the presence of asbestos bodies and the development of mesothelioma, suggested that exposure to iron oxide might be an important co-factor in the genesis of mesothelioma in shipyard workers. They thought it highly desirable that experiments should be undertaken on animals to verify this hypothesis. The basis of their suggestion that iron oxide may be acting as a co-factor was the occurrence of 17 cases of mesothelioma among some 3000 workers at the Royal de Schelde shipyard in Flushing (Netherlands), where there was exposure to both asbestos (insulation) and iron oxide (welding). Although the size of the population at risk is not precisely known, the authors felt that the incidence of mesothelioma was higher than would be expected on the basis of exposure to asbestos alone, especially since in no case was exposure to asbestos sufficient to give rise to pulmonary fibrosis.

(g) General considerations

Faulds & Stewart (1956) suggested that "since no known specific carcinogen exists in iron-ore it would appear that the tumour arises indirectly as a result of the irritating effects of silica, ferric oxide and chronic infection". Doll (1953) considered that the report of the Miners' Phthisis Medical Bureau (1936) that there was no excess of lung cancer in gold miners, despite the risk of silicosis in gold mines, provided convincing evidence that silicosis does not predispose to lung cancer. However, the suspicion that exposure to silica might in some way predispose to lung cancer in iron-ore miners remained (Monlibert & Roubille, 1960), and cases of combined sidero-silicosis and lung cancer continued to be observed. (A report by Wagner (1970) of the occurrence of tumours in a high proportion of rats exposed to silica dust by intrapleural injection suggests that the question of the carcinogenicity of silica per se is not closed.)

In a review published in 1958, Doll (1958) concluded that the evidence presented by Faulds & Stewart (1956) and by Hueper (1955) was in neither case conclusive. Miners are more likely than non-miners to come to necropsy because compensation might be payable if they were found to have silicosis or silico-tuberculosis (Turner & Martin, 1949). However, from their own survey, Boyd et al. (1970) concluded that haematite miners seem to suffer a lung cancer mortality 70% higher than normal. He and his colleagues suggested that the risk may be due either to radioactivity in the air of the mines (average radon concentration, 100 pCi/litre), to the carcinogenic effect of iron oxide, or to the combined effects of exposure to silicon dioxide and ferric oxide. In relation to the last of these possibilities, there is much evidence that silicosis per se does not predispose to and is not associated with the development of primary cancer of the lung (e.g., Miners' Phthisis Medical Bureau, 1936; Kennaway & Kennaway, 1947; Schoch, 1954).

4. Comments on Data Reported and Evaluation

4.1 Animal data

Ferric oxide given by inhalation or by intratracheal route has not been found to be carcinogenic in the hamster, the mouse or the guinea-pig.

4.2 Human data

On the basis of epidemiological evidence, exposure to haematite dust may be regarded as increasing the risk of lung cancer development in man. The risk is manifest in underground workers but not surface workers, and it is not known whether the excess risk is due to radioactivity in the air of mines, the inhalation of ferric oxide or silica, or to a combination of these or other factors. There is no evidence that iron-ore dust (haematite) or ferric oxide influences the incidence of cancers at sites other than the lungs.

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LEAD SALTS*

1. Chemical and Physical Data

Lead acetate

1.1 Synonyms and trade names

Chem. Abstr. No.: 6080564

Neutral or normal lead acetate; Sugar of lead; Salt of Saturn; Plumbous acetate

1.2 Chemical formula and molecular weight

$Pb(C_2H_3O_2)_2.3H_2O$ Mol. wt: 379.34

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless crystals or white granules or powder; slight acetic odour; slowly effloresces

(b) Melting-point: 75°C

(c) Density: 2.55

(d) Solubility: One gram dissolves in 1.6 ml of water, in 0.5 ml of boiling water and in 30 ml of ethanol. Freely soluble in glycerol. Aqueous solutions of lead acetate dissolve lead monoxide.

(e) Stability: At a little above 100°C, begins to lose acetic acid; decomposes completely above 200°C.

Lead subacetate

1.1 Synonyms and trade names

Lead monosubacetate; Monobasic lead acetate

1.2 Chemical formula and molecular weight

$Pb(C_2H_3O_2)_2.2Pb(OH)_2$ Mol. wt: 807.75

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* Considered by the Working Group in Geneva, December 1971.

1.3 Chemical and physical properties of the pure substance

(a) Description: A white, heavy powder

(b) Solubility: Soluble in 16 parts of cold and 4 parts of boiling water with alkaline reaction. On exposure to air, absorbs CO_2 and becomes incompletely soluble.

Lead arsenate

1.1 Synonyms and trade names

Chem. Abstr. No.: 7784409

1.2 Chemical formula and molecular weight

Approximately: $PbHAsO_4$ Mol. wt: 347.13

1.3 Chemical and physical properties of the pure substance

(a) Description: A white, heavy powder

(b) Decomposition temperature: At about 280°C, loses water and is converted into pyroarsenate.

(c) Density: 5.79

(d) Solubility: Insoluble in water; soluble in nitric acid and caustic alkalis

Lead carbonate

1.1 Synonyms and trade names

Basic lead carbonate; Lead subcarbonate; White lead; Flake lead; Ceruse; Cerussa

1.2 Chemical formula and molecular weight

Approximately: $(PbCO_3)_2.Pb(OH)_2$ Mol. wt: 775.67

1.3 Chemical and physical properties of the pure substance

(a) Description: A white powder

(b) Decomposition temperature: At 400°C, decomposes to form the monoxide.

(c) Density: 6.14

(d) Solubility: Insoluble in water or ethanol; soluble in acetic acid or dilute nitric acid with effervescence

Lead phosphate

1.1 Synonyms and trade names

Chem. Abstr. No.: 7446277

Lead orthophosphate; Normal lead orthophosphate; Plumbous phosphate

1.2 Chemical formula and molecular weight

$Pb_3(PO_4)_2$ Mol. wt: 811.59

1.3 Chemical and physical properties of the pure substance

(a) Description: A white powder

(b) Melting-point: 1014°C

(c) Density: 6.9

(d) Solubility: Insoluble in water and ethanol; soluble in dilute nitric acid

2. Use and Occurrence

(a) Use

According to Ziegfeld (1964), the main uses of lead in the USA in 1962 were: in storage batteries, in the manufacture of tetraethyllead used as an additive to gasoline (petrol), in construction, in paints and varnishes, in cables, ammunition and brass, in printing, colours, in car manufacture and in can manufacture. Over 1 300 000 tons were used in 1968 in the USA.[1] The use of lead that is responsible for most human exposure is its addition to motor fuels as an anti-knocking agent. As a result of combustion, it is converted to lead and lead salts. The level of lead in the atmosphere in Los Angeles was higher close to main highways than a few hundred yards away from them (Thomas et al., 1967). The level of lead in the air of Fleet Street in London in 1962-1963 was 3.2 µg/m^3 (Waller et al., 1965; Bullock & Lewis, 1968) and in Warwick, England, in 1965-66 was between 2.8 and 4.4 µg/m^3. According to Chow & Earl (1970), the level of atmospheric lead from petrol is rising at the rate of 5% per annum in one community in the USA.

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[1] Data from Chemical Information Services, Stanford Research Institute.

Lead phosphate is used as a stabilizer in styrene and casein plastics; lead acetate is employed as a drying agent in paints, driers and printing inks, in the manufacture of lead salts and lead colours (lead chromate) and, in small amounts, in the manufacture of medicinal aluminium acetate preparations used as astringents. In 1964, the total demand in the USA was reported to have been about 300 000 pounds, while the total world supply and demand probably does not exceed 500 000 pounds.[1] Lead arsenate is a constituent of various insecticides, being effective in destroying the larvae of the gypsy moth and boll weevil, etc. It is used against insect infestations of fruits and vegetables (Calvery, 1938), and has been used in veterinary medicine in the control of tapeworm infestations of cattle, sheep, and goats. Lead carbonate is used as a pigment in oil paints and water colours, in cements and for making putty and lead carbonate paper. Lead subacetate is used in sugar analysis (Horne's dry lead) to remove colourants and to decolour solutions of other organic substances.

(b) Analytical methods

Cholak (1964) recommends three different methods for the analysis of samples for lead: (i) the dithizone method; (ii) spectrographic procedures; (iii) a polarographic method. To this list, atomic absorption spectrometry should now be added (Portman, 1971).

(c) Occurrence

Isotopes of lead with the following atomic weights occur in nature: 204.0 (1.5%), 206.0 (23.6%), 207.0 (22.6%) and 208.0 (52.3%). Lead is the final radioactive disintegration product of uranium and thorium. Lead occurs naturally mainly in the form of galena, galenite or lead sulfide (PbS) in the USA, Australia, Mexico, Bolivia, Upper Silesia and Spain. Production was at one time considerable in Great Britain but is now negligible. The USA is now the largest producer. Lead also occurs as basic lead carbonate - $(PbCO_3)_2.Pb(OH)_2$ - in cerussite (white lead ore), as lead sulfate ($PbSO_4$) in anglesite and lanarkite, as the monoxide (PbO) in massicot and as basic lead chloride ($PbCl_2.PbO$) in matlockite. Large quantities of lead vanadate are found as vanadinite in the Transvaal and at Tsumeb.

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[1] Data from Chemical Information Services, Stanford Research Institute.

Boyland et al. (1962) stated that people living in cities in Europe absorb 20-30 µg of lead per day from the atmosphere and between 200 and 300 µg from food.

de Treville (1964) summarizes much collected data on the concentration of lead in various foodstuffs, beverages, soils, the atmosphere and water supplies. He suggests that the average concentration of lead is 16 ppm in rock soil and 350 ppm in garden soil. The average concentration in public water supplies in the USA is 0.01 ppm.

Patterson (1965) calculated that the average American ingests 400 µg of lead per day in food, air and water. This, he suggested, is about 30 times higher than the exposure to be expected under "natural" conditions and is giving rise to an average body burden of lead equal to 200 mg (per 70 kg), which is about 100 times the probable "natural" level. The natural level is calculated on the basis of geological and chemical data. These calculations, however, ignore the fact that only about 10% of ingested lead is absorbed.

According to Crawford & Crawford (1969), the lead content of the ribs of persons dying from accident or ischaemic heart disease in a soft-water region was much higher than that in persons dying from the same causes in a hard-water region.

The Joint FAO/WHO Expert Committee on Food Additives (World Health Organization, 1967) states in its tenth report:

> "Lead is a non-essential element that occurs naturally in foods and beverages and as a contaminant from the use of lead arsenate sprays and from contact with processing equipment and containers. There is evidence that these sources of contamination are decreasing. On the other hand, inhalation of lead from air pollution, such as exhaust fumes and industrial smoke, appears to be increasing; tobacco smoking may also contribute to lead intake.
>
> "Average daily intakes of lead from normal food and beverages probably lie between 0.0033 and 0.005 mg per kg body-weight, with approximately a further 0.0013 mg per kg body-weight per day from the atmosphere in urban environment. Total intakes of lead of this magnitude are known to cause accumulation of lead in the tissues with age, but there is no direct evidence that tissue accumulations at existing levels are harmful or potentially harmful to man. However, extrapolation from the

results of animal experiments suggests that present environmental exposures to lead may be harmful and points to the need to consider means of reducing the lead burden to which modern man is exposed.

"The maximum acceptable load of lead from food can tentatively be placed at 0.005 mg per kg body-weight per day."

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Van Esch & Kroes (1969) reported the induction of benign and malignant renal neoplasms in Swiss mice fed on diets containing 0.1% or 1.0% basic lead acetate.

Rat: Fairhall & Miller (1941) reported kidney changes (enlargement of cells, vesiculation of nuclei and accumulation of brown granules) but no tumours in 49 male rats fed diets containing 0.1% lead arsenate or 0.1% lead carbonate for 2 years.

Van Esch et al. (1962) observed benign and malignant kidney neoplasms in rats fed on diets containing 0.1% or 1.0% basic lead acetate for periods of up to 29 months and Boyland et al. (1962) reported the induction of renal tumours in rats fed on a diet containing 1% lead acetate.

Mao & Molnar (1967) reported the induction of renal tumours in 31 out of 40 rats subjected to the long-term feeding of a diet containing 1% basic lead acetate.

Zawirska & Medras (1968) reported the occurrence of benign and malignant kidney tumours and also of tumours of the testes, adrenals, thyroid, pituitary and prostate in 94 male and 32 female Wistar rats given a diet containing lead acetate (3 mg per rat per day for 2 months then 4 mg per rat per day for 16 months). Leydig-cell tumours were seen in 24% of treated rats.

Oyasu et al. (1970) and the same authors in earlier publications reported the occurrence of gliomas as well as renal tumours in male Sprague-Dawley rats fed on a diet containing 1% lead subacetate. Of 17 rats treated with the lead salt alone, 13 developed tumours of the renal cortex and 2 developed gliomas. Of 41 rats given 1.6% indole in the diet as well as 1% lead subacetate, 25 developed renal tumours and 3 developed gliomas. The

frequency of gliomas in control rats was 0.3%. The same authors reported the induction, in low frequency, of gliomas, but not of renal cortical tumours, in rats fed on a diet containing 2-acetylaminofluorene. Combined exposure to 2-acetylaminofluorene and lead subacetate did not appear to result in a higher frequency of either gliomas or renal cortical tumours than that seen in rats given only the lead salt. However, the rats given both substances did show a high frequency of tumours of the liver, urinary bladder, renal pelvis, forestomach, mammary gland and other sites attributable to exposure to 2-acetylaminofluorene.

Hamster: Van Esch & Kroes (1969) reported renal changes but no neoplasms in two groups of 22 and 24 male hamsters fed on a standard laboratory diet containing 0.1% or 0.5% basic lead acetate for up to 2 years.

(b) Subcutaneous administration

Rat: Zollinger (1953) reported the induction of renal tumours in rats given repeated subcutaneous injections of lead phosphate. In his experiments he exposed a total of 270 albino rats to lead and set aside a further 40 rats as untreated controls. Treatment was continued for up to 16 months, during which period the rats received between 40 mg and 760 mg of lead phosphate. Nineteen out of 29 rats that survived for 10 or more months from the start of treatment developed renal tumours. The total doses of Pb received by the rats that developed renal tumours ranged from 120 mg to 680 mg. The tumours were described as adenomas, papillomas or cystadenomas of the renal cortex. Two rats given injections of lead phosphate that died before 10 months were found to have tumours; one of these died less than 4 months after the start of treatment, having received just over 300 mg of Pb.

(c) Other experimental systems

Subcutaenous injections followed by intraperitoneal injections: Roe et al. (1965) saw renal tumours in rats given a total dose of 145 or 450 mg of lead phosphate by repeated subcutaneous and intraperitoneal injections, but none in 21 rats that survived for 300 days or more after a total dose of 29 mg.

3.2 Metabolism in animals and man

Calvery (1938) studied the effects of orally-administered lead and arsenic in rats and dogs. The concentrations of lead in the diet ranged from 0.61 mg to 2.64 mg per kg of diet; the substance was given either as lead arsenate or as lead acetate. There was definite storage of lead in all organs and tissues at all levels fed. In pregnant animals lead is transferred to the fetus. Lead also finds its way into the mother's milk. The concentrations of lead in the tissues of newborn animals were found to be the same as those in the mother's diets.

According to Kehoe (1964a, 1964b) there is a fairly good correlation between the degree of lead intoxication and the body burden of lead, the main exception being where there has been high exposure over a short period.

3.3 Observations in man

(a) Case reports

Portal (1961) described the occurrence of a cerebral tumour in a lead worker. This case was reported to illustrate the difficulty of distinguishing between intracranial tumour and lead encephalopathy.

Jecklin (1956) found concentrations of lead to be no higher in the lungs of 5 patients who died from lung cancer than in the lungs of 5 persons who died from other causes; in both groups levels of between 0.010 mg and 0.018 mg of lead per 10 g lung tissue were found.

(b) Epidemiology

Dingwall-Fordyce & Lane (1963) reported the results of a follow-up study of 425 persons who had previously been exposed to lead in an accumulator factory. They found "no evidence to suggest that malignant disease was associated with lead absorption", although there was an increased incidence of cerebrovascular catastrophes.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Lead acetate is carcinogenic in rats and mice; lead subacetate and lead phosphate are carcinogenic in the rat. Given orally, they produce benign and malignant tumours of the kidney. The observation that exposure of rats to lead subacetate may result in an increased frequency of gliomas needs confirmation, as well as the observation of a high frequency of tumours of the testis, adrenal, thyroid, pituitary and prostate, together with renal tumours, in rats

receiving lead acetate. No induction of tumours was reported to occur following exposure to lead arsenate or lead carbonate, but the evidence cannot be held as conclusive.

The pattern of absorption metabolism and storage of lead in the body seems to be similar in all animal species that have been studied. The kidney is a target from the point of view of toxicity in all animal species studied. Renal enlargement and the appearance of intranuclear inclusion bodies in the epithelial cells occur in all laboratory animal species and in man in the same way.

4.2 Human data

There is no evidence to suggest that exposure to lead salts causes cancer of any site in man. However, only one epidemiological study of the relationships between exposure to lead and the occurrence of cancer has been reported. It must be noted that the level of human exposure equivalent to the levels of lead acetate producing renal tumours in rats is 810 mg per day (550 mg Pb). This level appears to exceed by far the maximum tolerated dose for man.

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CHLORINATED HYDROCARBONS

CARBON TETRACHLORIDE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 56235

Methane, tetrachloro-; Tetrachloromethane; Perchloromethane; Benzinoform; Necatorina

1.2 Chemical formula and molecular weight

CCl_4 Mol. wt: 153.84

1.3 Chemical and physical properties

(a) Description: A colourless, non-flammable liquid with a characteristic odour

(b) Boiling-point: 76.7°C

(c) Density: 1.589 (25°C)

(d) Refractive index: n_D^{20} 1.4607

(e) Specific gravity: Liquid, 1.60; gas, 5.32

(f) Solubility and volatility: Miscible with most fat solvents; one part is soluble in 2000 parts of water; volatile

(g) Stability: In contact with various types of fire, decomposes into CO_2, HCl, phosgene and chlorine

(h) Chemical reactivity: Chemically not reactive; not easily hydrolysed

(i) Technical products and impurities: Reagent grade from one manufacturer contained chloroform as impurity (Butler, 1961).

2. Use and Occurrence

(a) Use

Preliminary data indicate that slightly over 1.0 billion pounds of carbon tetrachloride were produced in the USA in 1970. Approximately 700 million pounds (69%) of this was used in the manufacture of dichlorodifluoromethane and about

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* Considered by the Working Group in Geneva, December 1971.

260 million pounds (26%) was used to make trichlorofluoromethane. The remaining 5% of the production in the USA (about 50 million pounds) is used as: (i) grain fumigant (usually mixed with other chemicals); (ii) fire extinguishers (not in the home); (iii) anthelmintic; (iv) rodenticide; (v) solvent for oils, fats, rubber cements and resins, for reactants in organic chlorination processes, and in the recovery of tin from tin-plating waste; cleaning agent for machinery and electrical equipment; agent for degreasing metal fabricated parts (small); dry-cleaning agent (small). In Japan, the estimated demand will increase from 96 million pounds in 1971 to 140 million pounds in 1975. The total French production amounted to approximately 147 million pounds in 1969.[1]

When used as a grain fumigant, a typical rate of application is 1 gallon to 12 tons of grain (about 1 litre to 3 tons). Following fumigation, the compound is found as a residue in wheat (up to 50 ppm 1-5 months after fumigation) and flour,[2] but it has been claimed that it does not persist in the bread (Munsey et al., 1957). More sensitive and more selective methods of fumigant residue analysis have recently been developed which have been used to detect variations in the amount of the unchanged fumigant in certain foods after fumigation. The results confirm that the amount of residual unchanged fumigant continues to decline during storage, handling or processing, but indicate that, in some circumstances, carbon tetrachloride may still be detectable in food when offered for consumption. The Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticides (Joint FAO/WHO Meeting, 1972) indicated the following possible residues of carbon tetrachloride: in raw cereals, 50 ppm; in milled cereals, 10 ppm; and in bread and other cooked cereal products, 0.05 ppm. The residue of unchanged carbon tetrachloride in the food as offered for consumption was not expected to exceed an amount close to the limit of determination by present analytical methods (Joint FAO/WHO Meeting, 1972). The compound is used in veterinary medicine and concentrations of up to 3 ppm have

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[1] Data from Chemical Information Services, Stanford Research Institute.

[2] Joint FAO/WHO Expert Committee on Food Additives (1965) Evaluation of the hazards to consumers resulting from the use of fumigants in the protection of food (WHO/Food Add./28.65). This document can be obtained on request from: Food Additives, World Health Organization, Avenue Appia, 1211 Geneva, Switzerland.

been found in milk from cows recently treated (Cielszky et al., 1958). The threshold limit value is 10 ppm (American Conference of Governmental Industrial Hygienists, 1969).

(b) Analytical methods

Carbon tetrachloride as a residue in food was originally determined by colorimetry (Ramsey, 1957). A polarographic method permits rapid measurement of amounts as low as 10^{-5} moles (Berck, 1962). A gas chromatographic multi-detection method for the determination of microgram amounts of 34 fumigant gases including carbon tetrachloride has been described (Berck, 1965). In addition, an electron-capture gas chromatographic method for detecting residues in the ppb range is available (Bielorai & Alumot, 1966).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: At least 13 studies carried out up to 1960 (Hartwell, 1951; Shubik & Hartwell, 1957, 1969) clearly demonstrate that repeated oral administration of carbon tetrachloride leads to the formation of hepatomas in many strains of mice. A dose-response investigation by Eschenbrenner & Miller (1944) on strain A mice demonstrated that the magnitude of each dose and the interval between doses are important. Individual dose levels were 0.1, 0.2, 0.4, 0.8 and 1.6 ml/kg bw and the interval between consecutive doses was 1, 2, 3, 4 or 5 days. Each animal received 30 doses and the experiment was terminated at 150 days. No hepatomas were seen in the group given 30 doses of 1.6 ml/kg bw over a period of 30 days, whereas a significant number of tumours was observed in the groups receiving 30 doses of 0.1 ml/kg bw over a period of 90 days or more. No no-effect dose level was found in this study. In a later study with the same strain of mice in which the animals were killed at intervals and necrosis and repair were estimated, a correlation was found between the degree of necrosis and the incidence of hepatomas; but repeated liver necrosis and subsequent chronic regeneration are not necessary for tumour induction (Eschenbrenner & Miller, 1946).

Rat: In a few experiments carried out before 1960 on small numbers of animals followed for relatively short periods of time, no tumours were

seen (Hartwell, 1951; Shubik & Hartwell, 1957, 1969).

Hamster: Ten animals of each sex received 30 weekly doses of 6.25-12.5 µl carbon tetrachloride. Five animals of each sex that survived 10 or more weeks after the cessation of treatment had liver-cell carcinomas (Della Porta et al., 1961).

Dog: Twenty-five dogs that received 15 ml of a 33% solution of carbon tetrachloride twice weekly for only 3 years did not develop tumours (Bardawill & Gornall, 1952).

Trout: Rainbow trout were given diets containing 3200 and 12 800 ppm carbon tetrachloride. Four out of 44 of the animals at the lower dose level and 3 out of 34 at the higher dose level developed hepatomas after 20 months, whereas no tumours were found in the controls (Halver, 1967).

(b) Inhalation studies

Rat: A group of albino rats that had inhaled carbon tetrachloride (dose and schedule unspecified) for 7 months were killed 2-10 months after the cessation of treatment. Among 30 survivors, 12 had "adeno-cirrhosis" and 10 had liver nodules measuring up to 1 cm, histologically diagnosed as early or established liver carcinomas (Costa et al., 1963).

(c) Subcutaneous administration

Mouse: In an experiment on (C57L x A)F_1 mice which had received a single whole body exposure to fast neutrons (165-302 rad), a single s.c. injection of carbon tetrachloride (0.15 ml of a 40% solution) given 2-18 months after irradiation increased the frequency of hepatomas from 19% to 61%. No hepatomas appeared in the controls given carbon tetrachloride only (Cole & Nowell, 1964).

Rat: In a study designed for purposes other than the detection of carcinogenicity, 49 Wistar rats received 2-3 ml/kg bw of carbon tetrachloride twice weekly for about 25 weeks and were killed between 4 and 64 weeks after the cessation of treatment; hepatomas were found in 2 animals (Kawasaki, 1965).

In a study in which male rats were given subcutaneous injections of 1.3 ml/kg bw of a 50% solution of carbon tetrachloride twice weekly, 4/12 Wistar rats, 8/13 Osborne-Mendel rats and 12/15 Japanese rats that survived 70 or more weeks had hepatocellular carcinomas. No tumours

were found in the controls (12 rats of each strain) (Reuber & Glover, 1970). In a previous experiment on Buffalo rats in which the same schedule of treatment had been used, a low yield of hepatomas had been observed (Reuber & Glover, 1967).

(d) Other experimental systems

Intrarectal administration: Twenty-five C3H male mice received bi-weekly intrarectal administrations of 0.1 ml of a 40% solution of carbon tetrachloride in oil for 20-26 weeks and were killed 1-37 weeks later. A total of 13 mice had hepatomas (Confer & Stenger, 1965).

3.2 Metabolism in animals and man

It is assumed that the selective toxicity of carbon tetrachloride for the liver of animals depends on its metabolism by the liver (Slater, 1966; Recknagel, 1967). Liver tissue reduces carbon tetrachloride to chloroform, and it was suggested that homolytic cleavage of the carbon-chlorine bond yields free radicals which could then alkylate the sulfhydryl groups of enzymes (Butler, 1961). A link between the latter and peroxidative decomposition has been established (Recknagel, 1967). The detection of hexachloroethane (CCl_3-CCl_3) in tissues of rabbits following carbon tetrachloride intoxication is of some interest (Fowler, 1969).

3.3 Observations in man

(a) Case reports

Cases of hepatomas appearing in men several years after carbon tetrachloride poisoning have been reported (Tracey & Sherlock, 1968; Simler et al, 1964).

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Carbon tetrachloride has produced liver tumours in the mouse, hamster, and rat following several routes of administration, including inhalation and oral ingestion. The results in trout were of borderline significance. There

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

is no evidence of carcinogenicity for organs other than the liver, but most experiments were shorter than the life-span of the animals. No single-dose carcinogenic experiment with long follow-up has been reported.

4.2 Human data

Man is exposed to carbon tetrachloride from several sources involving a wide range of doses. No long-term follow-up studies on men exposed to carbon tetrachloride have been reported. The occasional case reports of liver tumours in man following acute intoxication are of doubtful significance, but cannot be disregarded.

5. References

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CHLOROFORM*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 67663

Methane, trichloro-; Trichloromethane; Methenyl chloride

1.2 Chemical formula and molecular weight

$CHCl_3$ Mol. wt: 119.39

1.3 Chemical and physical properties of the pure substance

(a) Description: A clear, colourless and mobile liquid with a characteristic odour and a burning sweet taste

(b) Boiling-point: 61-62°C

(c) Density: 1.484 (20°C)

(d) Refractive index: n_D^{20} 1.4459

(e) Solubility and volatility: One part is soluble in 200 parts of water; miscible with oils, ethanol, ether and other organic solvents. Vapour pressure is 246.0 mm Hg at 30°C and 739.6 mm Hg at 90°C.

(f) Stability: When exposed to air and light, breaks down to phosgene, HCl and chlorine; should be stored in dark bottles and kept cool.

(g) Chemical reactivity: Easily hydrolysed by aqueous alkali to formic acid

(h) Technical products and impurities: Reagent grade chloroform of several brands was reported to contain detectable amounts of CH_2Cl_2 and other chloromethanes (Butler, 1961). It is usually stabilized by the addition of 0.6-1% ethanol to avoid photochemical transformation to phosgene and hydrogen chloride.

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* Considered by the Working Group in Geneva, December 1971.

2. Use and Occurrence

(a) Use

In the USA, preliminary data for 1970 indicate that the production of chloroform amounted to almost 240 million pounds. It is estimated that 230 million pounds of this (over 96% of all the chloroform consumed) was converted into chlorodifluoromethane. The chief markets (an estimated 78% of total consumption in the USA) for this substance are as a refrigerant and an aerosol propellant; the remainder is used as a raw material for the synthesis of fluorinated resins, e.g., polytetrafluorethylene. The remaining 10 million pounds (4%) is used: (i) in pharmaceuticals and toiletries (in some widely used toothpastes, in hair-tinting and permanent waving formulations, in cough medicines, in liniments and salves, as a processing solvent in the manufacture of penicillin, and as an anaesthetic (small)); (ii) as an industrial solvent (in photographic processing, industrial dry cleaning, extracting essential oils and alkaloids, and removing rubber from machinery); (iii) as a heat transfer medium; (iv) as a fire extinguisher (in conjunction with carbon tetrachloride); (v) as a pesticide. In Japan, where chloroform-based fluorocarbon refrigerants are widely used for home window coolers, 10 800 tons were produced in 1969. Chloroform is produced in many European countries, but production and consumption figures are not available.[1] It is still used as an anaesthetic in veterinary practice (Hall, 1966).

(b) Analytical methods

Electron-capture gas chromatographic methods have been described (Bielorai & Alumot, 1966).

(c) Occurrence

Small amounts of chloroform have been found in tomatoes (Schormüller & Kochmann, 1969), muscat grapes (Stevens et al., 1966) and milk and cream (Wong, 1963; Wong & Patton, 1962). The origin of chloroform in these items is difficult to establish: it might derive from its use as a solvent for fat extraction of feed or as a pesticide, but in vegetables biosynthesis

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[1] Data from Chemical Information Services, Stanford Research Institute.

cannot be disregarded. The threshold limit value is 50 ppm (American Conference of Governmental Industrial Hygienists, 1969).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Groups of 5 different strains of mice of each sex were given 30 oral doses of 0.1, 0.2, 0.4, 0.8 and 1.6 ml/kg bw chloroform per dose in olive oil at 4-day intervals. Survivors were killed one month after the last treatment. All females at the highest doses and all males at the 3 highest doses died early in the experiment. Non-metastasizing hepatomas and cirrhosis were found in all of the 5 females given 0.8 or 0.4 ml/kg bw per dose that were alive at the end of the experiment. No hepatomas were observed at the two lowest dose levels or in the controls (Eschenbrenner & Miller, 1945). 0.1 ml of an oily 40% solution of chloroform given by stomach tube twice weekly for six months to 24 mice produced 3 hepatomas in 5 survivors (Rudali, 1967).

(b) Other experimental systems

Newborn mouse: An unspecified number of (C57 x DBA2 F1) mice received subcutaneously either a single dose of 200 µg chloroform in 0.02 ml of arachis oil when less than 24 hours old or 8 daily doses of 200 µg during the first week of life. They were killed when 77-80 weeks old. No evidence of carcinogenesis was obtained (Roe et al., 1968).

3.2 Metabolism in animals and man

Chloroform is rapidly absorbed and distributed in all organs, with relatively high concentrations in nervous tissue (Von Oettingen, 1964). After intraduodenal administration of ^{14}C- chloroform to rats, 70% chloroform was found unchanged in the expired air and 4% as $^{14}CO_2$. At least 75% of the radioactivity was excreted in 18 hours: the liver and, to a much lesser extent, the kidney were the main organs in which chloroform was metabolized (Paul & Rubinstein, 1963). Although it has been suggested that conversion to CO_2 is non-enzymatic (Butler, 1961), the observation that denatured liver slices do not catalyse this conversion and other findings support the hypothesis that an enzyme system is required (Paul & Rubinstein, 1963). Methylene chloride was found to be produced from chloroform by mouse liver tissues *in vitro* (Butler, 1961) but not by rat tissues (Paul & Rubinstein, 1963).

3.3 Observations in man

No data available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

The carcinogenicity of chloroform has been investigated only in mice in experiments involving a small number of animals at risk. Nevertheless among these the frequency of liver tumours was high. There is no evidence of carcinogenicity for organs other than the liver, but the experiments were shorter than the life-span of the animals. An experiment involving single or a few exposures of newborn mice gave negative results. An assessment of the carcinogenicity of chloroform awaits further experimental evidence.

4.2 Human data

Chloroform entails several sources of exposure for humans. No long-term follow-up studies in men exposed to chloroform have been reported.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

5. References

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AROMATIC AMINES

AURAMINE*

1. Chemical and Physical Data

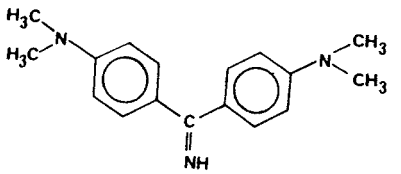
1.1 Synonyms and trade names

Chem. Abstr. No.: 492808; Name: C.I. solvent yellow 34

Aniline, 4,4'-(imidocarbonyl)-bis(N,N'-dimethyl)-; 4,4'-Imidocarbonyl-bis (N,N'-dimethyl)aniline; 4,4'-Dimethylaminobenzophenonimide; bis(p-Dimethyl-aminophenyl)methyleneimine; Tetramethyl-p-diamino-imido-benzophenone; Tetramethyldiaminodiphenylacetimine; Apyonine auramine base; Auramine N base; Auramine O base; Auramine SS; Auramine OO; Brilliant oil yellow; C.I. basic yellow 2 (free base); Fat yellow A; Yellow pyoctanine; Glauramine

1.2 Chemical formula and molecular weight

As base:



$C_{17}H_{21}N_3$ Mol. wt: 267.36

As hydrochloride: $C_{17}H_{22}ClN_3.H_2O$

1.3 Chemical and physical properties of the pure substance

(a) Description

Base: Yellow leaves

Hydrochloride: Yellow needles

(b) Melting-point: Base: 136°C (rapid heating)

(c) Solubility

Base: Insoluble in water; soluble in ethyl ether; very soluble in ethanol

Hydrochloride: Soluble in water, ethyl ether and glycerol; very soluble in ethanol and chloroform

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* Considered by the Working Group in Geneva, December 1971.

(d) Chemical reactivity: Decomposes at temperatures above 70°C; is a weak base that forms salts with HCl, H_2SO_4, etc.

(e) Technical products and impurities: Auramine is manufactured industrially from dimethylaniline and formaldehyde, which react to form Michler's base (tetramethyldiaminodiphenylmethane). This base is subsequently converted to auramine by heating it with sulfur and ammonium chloride in the presence of ammonia.

2. Use and Occurrence

(a) Use

The free base of auramine is used to prepare Solvent Yellow 34, a solvent-soluble yellow dye.

It has been reported in the United Kingdom that auramine has come into prominence as a powerful antiseptic for use in nose and ear surgery. Also, in the United Kingdom, a specially purified auramine, sold under the name of Glauramine, is used as an antiseptic in the treatment of gonorrhoea.[1]

Auramine and its hydrochloride are used in large quantities in the colouring of paper and cardboard and, to a lesser extent, of some textiles and leather. In the first-mentioned case, they are added during the processing of the raw material prior to manufacture. Auramine has been used in some countries as a food dye. It is also used as a smoke dye (Tatyrek, 1965).

(b) Analytical methods

Guidelines for the analysis of aromatic amines have been given (UICC, 1970). A method for the determination of auramine in certain smoke mixtures has been described by Ripley & Need (1961).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Thirty mice were given a diet containing 0.1% of commercial auramine for 52 weeks (total dose: 728 mg per animal). The animals were

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[1] Data from Chemical Information Services, Stanford Research Institute.

kept for their life-span. Seven hepatomas and 11 lymphomas were found as compared with 0 and 5, respectively, in 60 control animals. Two other tumours were also reported (Bonser et al., 1956). Thirty stock mice and 27 CBA mice of both sexes were given during 52 weeks 0.1% and 0.2%, respectively, of auramine dissolved in acetone in their diet (approximate total dose: 1820 mg and 3650 mg per mouse, respectively). In stock mice, 57% of the males and 30% of the females that survived to tumour-bearing age showed hepatomas. No cholangiomas were observed and the degree of cirrhosis was minimal. No hepatomas were seen in 16 control animals. In CBA mice, the frequency of hepatomas was over 50% in the treated group and only 7/90 in the control group. A few other tumours occurred in stock mice, but none in CBA mice (Williams & Bonser, 1962; Walpole, 1963).

Rat: Twelve male Wistar rats were given, during 87 weeks, a diet containing 0.1% of commercial auramine (estimated total dose: 10 g per rat). 92% of the animals (11/12) developed hepatomas between the 91st and the 122nd week following the start of treatment. A few other tumours were observed. Twelve control rats were tumour-free at death between 90 and 120 weeks (Williams & Bonser, 1962; Walpole, 1963).

Rabbit: In a preliminary comparative experiment, 9 rabbits were given auramine (purity not stated) orally to the limit of tolerance, and the treatment was continued until the onset of the final illness. Six animals were sacrificed in the first two years and three between 3 and 4 years. Metaplasia of the urinary tract epithelium, suggestive of precancerous change, was seen in 2 out of 5 rabbits examined (Bonser, 1962).

Dog: No abnormalities have been detected in dogs given auramine (purity not stated) orally, daily, for about seven years (total ingested amount: 66 g). (Walpole, 1963).

(b) Subcutaneous administration

Rat: Twenty-four male Wistar rats were given, 5 days per week for 21 weeks, subcutaneous injections of 0.1 ml per 100 g bw of a 2.5% suspension of commercial auramine in arachis oil (estimated total dose: 110-120 mg per animal). In 20 animals that survived 21 weeks of treatment, 11 fibrosarcomas (tumour yield: 55%) and 3 hepatomas (tumour yield: 15%) were observed. Three intestinal carcinomas were also reported (Williams & Bonser, 1962).

3.2 Metabolism in animals and man

No data available to the Working Group.

3.3 Observations in man

(a) Case reports

von Müller (1933) described two cases of bladder cancer in men occupied in auramine manufacture.

(b) Epidemiology

Case & Pearson (1954) showed a relatively high incidence of bladder tumours in workers engaged in the manufacture of auramine with a latent period ranging between 9 and 28 years. Overall, there were six death certificates where only 0.13 would have been expected from the whole male population of England and Wales ($P<0.005$). The morbidity was 9 cases. The average latent period from the start of exposure to the discovery of the disease was 19.3 years, i.e., similar to that found for benzidine and 2-naphthylamine.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Auramine is carcinogenic in the mouse and rat. Given orally, it has produced liver tumours in these two species. No tumours were obtained in the only experiment in the dog and in the rabbit. The purity of the auramine used in these experiments is not known.

4.2 Human data

One epidemiological study indicates that the open manufacture of auramine presents an occupational bladder cancer risk.

5. References

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4-AMINOBIPHENYL*

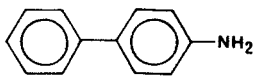
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 92671; Name: 4-Biphenylamine

p-Aminobiphenyl; p-Aminodiphenyl; 4-Aminodiphenyl; p-Biphenylamine; p-Phenylaniline; Xenylamine

1.2 Chemical formula and molecular weight



$C_{12}H_{11}N$ Mol. wt: 169.22

1.3 Chemical and physical properties of the pure substance

(a) Description: A colourless, crystalline compound that darkens on oxidation

(b) Boiling-point: 191°C (15 mm)

(c) Melting-point: 53°C

(d) Solubility: Very slightly soluble in cold water; soluble in hot water and non-polar solvents; soluble in lipids

(e) Chemical reactivity: A weak base that forms salts with HCl, H_2SO_4, etc.; can be diazotized to yield coloured coupling products; is oxidized by air and its amino group can be acetylated and alkylated

(f) Technical products and impurities: 2-Aminobiphenyl probably occurs as an impurity.

2. Use and Occurrence

(a) Use

At least in one country 4-aminobiphenyl was manufactured from 1935 to 1955 (Melick et al., 1955) and used as a highly efficient rubber antioxidant, but recent information suggests that it is no longer commercially produced.[1]

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* Considered by the Working Group in Geneva, December 1971.

[1] Data from Chemical Information Services, Stanford Research Institute.

(b) Analytical methods

Guidelines for the analysis of aromatic amines have been given (UICC, 1970).

(c) Occurrence

It was alleged to occur as an impurity in pre-1900 samples of aniline (Walpole et al., 1952) and is present in some samples of diphenylamine.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Two out of 12 mice surviving to 90 weeks developed bladder carcinoma as a result of the oral administration (gavage) of commercial 4-aminobiphenyl (1 mg per mouse per week) for 38 weeks. Hepatomas were found in both treated animals and controls in similar frequencies (Clayson et al., 1965). One bladder carcinoma was observed in 21 male mice as compared with none in 19 controls in mice of a different strain given 1.5 mg of 4-aminobiphenyl for 52 weeks. In this experiment the frequency of hepatomas in both male and female mice was significantly higher than that in the controls (Clayson et al., 1967).

Rabbit: Among 7 rabbits given commercial 4-aminobiphenyl orally (dose unstated), bladder papillomas were found in 1 and carcinomas in 3 animals. The earliest carcinoma was observed four years after the start of treatment (Bonser, 1962).

Dog: Two dogs fed 4-aminobiphenyl (b.p. 302^{o}C) in a gelatin capsule 6 times weekly for life (total dose per dog: 30, 34 g) developed carcinoma of the bladder in 33 months (Walpole et al., 1954). This was confirmed by similarly feeding capsules containing 4-aminobiphenyl (0.3 g per dog) three times weekly. Bladder carcinomas were observed after 21-34 months (total dose: 94.5-144.0 g per dog) (Deichmann et al., 1958). When the dose of 4-aminobiphenyl was reduced to 1.0 mg/kg bw and given to 6 dogs 5 times weekly for life (total dose: 5.5-7.0 g per dog), 2 bladder papillomatoses and 3 bladder carcinomas (transitional cell type) were observed (Deichmann et al., 1965). A single dose was not effective in inducing bladder tumours over a period of 5 years (Deichmann & MacDonald, 1968).

(b) Subcutaneous administration

Rat: 4-Aminobiphenyl (purified, b.p. 302°C) in arachis oil was injected daily into rats, to a total dose of 3.6-5.8 g/kg bw. The yield of mammary gland and intestinal tumours was significantly raised (Walpole et al., 1952).

(c) Other experimental systems

Newborn mouse: Three subcutaneous injections of 200 µg of 4-aminobiphenyl in 0.02 ml of aqueous gelatin produced hepatomas in 19/20 male and 6/23 female mice after 48-52 weeks (Gorrod et al., 1968).

3.2 Metabolism in animals and man

(a) Animals

In rats, 4-diphenylylacetamide yields the N-hydroxy derivative (Miller et al., 1961). In dogs, 4-aminobiphenyl is converted to 4-amino-3-biphenylyl hydrogen sulfate (Bradshaw & Clayson, 1955) and 4-amino-3-biphenylyl glucuronic acid (Gorrod, 1971).

(b) Carcinogenicity of metabolites

N-hydroxy-4-acetamidobiphenyl is the only metabolite to demonstrate carcinogenic activity by conventional testing techniques. In addition, 4-amino-4'-hydroxy-biphenyl, 4-hydroxylaminobiphenyl and 4-amino-3-hydroxy-biphenyl have enhanced the yield of hepatomas observed 48-52 weeks after injection into newborn mice (Gorrod et al., 1968). 3-Hydroxy-4-amino-biphenyl sulfate and N-acetyl-4-biphenylyl hydroxylamine were tested by bladder implantation, in the mouse, but only the former was active (Bonser et al., 1956; Boyland et al., 1964).

3.3 Observations in man

(a) Epidemiology

The major study of 4-aminobiphenyl carried out by Melick and co-workers appears to have been sufficient to prevent the widespread use of this chemical. A population of 171 male workers was stated to have been exposed to 4-aminobiphenyl between 1935 and 1955. Individual exposure times varied from 1.5 to 19 years. There were 19 cases (11.1%) of bladder tumours (Melick et al., 1955).

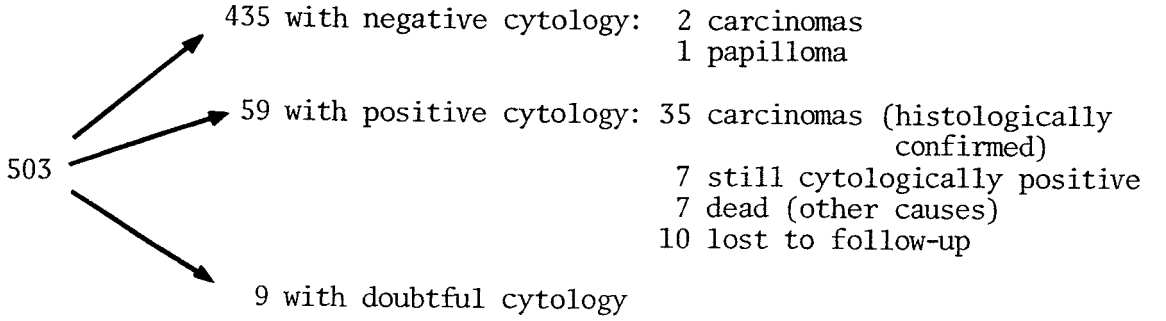
In a more recent study in which the number of male workers examined was extended to 315, Melick et al. (1971) found 53 men with bladder tumours. The exact exposure times could not be established. Melamed (1960) and

Koss et al. (1965, 1969), in their studies, found that in an exposed population of 503, histologically proved carcinoma of the bladder developed in 35 men. (The 315 workers in the study by Melick et al. (1971) are also included among the 503 workers studied by Koss et al. (1969).)

The schema below indicates the way in which tumours developed in the exposed population.

Development of Bladder Tumours among 503 Workers, 1960-68

(Koss et al., 1969)



4. Comments on Data Reported and Evaluation

4.1 Animal data

4-aminobiphenyl is carcinogenic in the mouse, rat, rabbit and dog. Following its oral administration it has produced bladder and liver tumours in mice and bladder papillomas and carcinomas in rabbits and dogs.

4.2 Human data

In the epidemiological studies, confined to one series of workers occupationally exposed to commercial 4-aminobiphenyl, a high incidence of bladder carcinomas was reported. In consequence one can say that bladder cancer was strongly associated with occupational exposure to 4-aminobiphenyl.

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BENZIDINE*

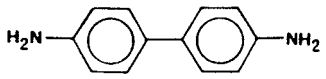
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 92875

Biphenyl, 4,4'-diamino-; 4,4'-Biphenyldiamine; C.I. azoic diazo component 112; 4,4'-Diaminobiphenyl; 4,4'-Diphenylenediamine; Fast Corinth base B; p-Diaminodiphenyl; 4,4'-Diaminodiphenyl

1.2 Chemical formula and molecular weight



$C_{12}H_{12}N_2$ Mol. wt: 184.23

1.3 Chemical and physical properties of the pure substance

(a) Description: A colourless, crystalline compound that darkens on oxidation

(b) Melting-point: 128°C (rapid heating)

(c) Solubility and volatility: Slightly soluble in cold water; more soluble in hot water and readily soluble in less polar solvents such as diethyl ether and ethanol; can be sublimed.

(d) Chemical reactivity: A weak base that forms insoluble salts with H_2SO_4; can be diazotized and oxidized, and its amino groups can be acetylated and alkylated.

(e) Technical products and impurities: Semidines and o,o- and o,p-benzidines are products of its manufacture from hydrazobenzene.

2. Use and Occurrence

(a) Use

In the USA, the production of benzidine amounts to many million pounds annually. According to the Colour Index, more than 250 dyes are derived from benzidine.[1]

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* Considered by the Working Group in Geneva, December 1971.

[1] Data from Chemical Information Services, Stanford Research Institute.

Benzidine and its salts are used in the manufacture of dyestuffs based on the coupling of tetrazotized benzidine with phenols and amines. It has been, and still may be to some extent, used in the rubber industry as a hardener. It has been used for specialized processes such as the manufacture of plastic films, but not after 1956, at least in one country. It is used clinically for the detection of blood. It is used for the detection of H_2O_2 in milk. It reacts with ink erasers to give coloured products and therefore has been used in security printing. It is also used as a laboratory agent for the detection of HCN and sulfate, for the quantitative determination of nicotine and as a spray reagent for sugars.

(b) Analytical methods

Methods for the determination of benzidine in the air, plant deposits and clothing have been described by Butt & Strafford (1956).

(c) Occurrence

It has been suggested that benzidine may be produced from 1,2-diphenylhydrazine (hydrazobenzene) by acidity in the stomach.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Cholangiomas and liver-cell tumours are reported in rats fed 0.017% of benzidine in the diet throughout the experiment, which lasted 424 days (Boyland et al., 1954).

Hamster: Benzidine and benzidine dihydrochloride given to hamsters in the diet at concentrations of 0.1% throughout their life-span induced cholangiomas, hepatomas and liver-cell carcinomas (Saffiotti et al., 1967; Sellakumar et al., 1969).

Dog: Seven dogs were given a total dose of 325 g in 5 years (200 and then 300 mg per day, 6 days a week). Three of the animals developed bladder carcinoma 7, 8 and 9 years after the start of treatment (Spitz et al., 1950; Bonser et al., 1956b).

(b) Subcutaneous administration

Mouse: Single weekly doses of 6 mg per animal were injected subcutaneously into 181 mice over a period of 8-13 months (total dose:

210-336 mg). Of the 46 mice that survived 15-28 months, 31 (67.4%) showed hepatomas. A parallel control was not included, but previous observations in the same colony of C_3HA mice showed a 1% hepatoma frequency in untreated animals (Prokofjeva, 1971). Benzidine in arachis oil suspension was injected subcutaneously twice a week for 52 weeks. The total dose given was 312 mg. A significant yield of hepatomas was observed in the benzidine-treated mice (the animals were badly affected by intercurrent disease) (Bonser et al., 1956b).

Rat: Commercial benzidine given subcutaneously (total dose: 1.28 g) was carcinogenic, inducing hepatomas in 9.8% male rats and acoustic gland carcinomas in over 20% of the animals. Purified benzidine and benzidine sulfate were also carcinogenic at a similar dosage (total dose: 0.96 and 0.94 g, respectively). The treatment was given throughout the life-span of the animals. Seven colonic tumours in 385 rats were also reported with benzidine. Only the male rats were affected. There were no liver-cell or acoustic duct carcinomas or intestinal tumours in the controls (Spitz et al., 1950). Hepatomas, cymbal gland tumours and sarcomas in the underlying fat at the site of injection of benzidine were reported in 70% of rats. Benzidine seems to have more toxic effects on females than on males (Pliss, 1964).

3.2 Metabolism in animals and man

(a) Animals

Bradshaw & Clayson (1955), Clayson (1959) and Fabre et al. (1960) have studied the metabolism of benzidine by paper chromatography in the mouse, rat, guinea-pig, rabbit and dog. In the dog, in contradistinction to the other species, no acetylation was observed. The distribution of the metabolites is shown in the accompanying table.

Urinary Metabolites of Benzidine

Compound	Dog	Dog*	Rat	Mouse	Rabbit	Guinea-pig
Benzidine	+	+	+	+	±	±
4'Acetamido-4-aminodiphenyl	0	±	+	±	±	±
3-Hydroxybenzidine (ether extracts)	+		+		±	
4,4'-diamino-3-diphenylyl hydrogen sulfate	+	+	+	±	0	0
4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	0	±	+	+	+	+
4'-Amino-4-diphenylyl sulfamic acid	0	0	±	0	+	±
N-Glucuronides	+	+	+	±	+	+
Acid-stable unknowns	1	1	3	3	3	0

* 4'-Acetamido-4-aminodiphenyl was administered.

The diacetyl derivative of benzidine was detected when high levels of benzidine were given in rats, rabbits and guinea-pigs (Fabre et al., 1960).

(b) Carcinogenicity of metabolites

The carcinogenic activity of benzidine metabolites has not been extensively investigated. Essentially negative results were obtained when 3-hydroxybenzidine hydrochloride, 4-amino-3-biphenylyl sodium sulfate and 4'-nitro-4-amino-3-hydroxybiphenyl hydrochloride were tested by bladder implantation (Bonser et al., 1956a, 1963).

3.3 Observations in man

Benzidine may enter the body by percutaneous absorption, by ingestion or by inhalation (von Ehrlicher, 1958; Meigs et al., 1954). The suspicion that benzidine induced bladder cancer in workers was reported before 1940 (Hueper, 1942). A high incidence of bladder tumours in workmen exposed to benzidine or benzidine and aniline in British chemical factories was observed, with a mean latent period of approximately 16 years. Overall, there were 10 death certificates where only 0.72 would have been expected from the whole male population

of England and Wales (P<0.001). The morbidity was 34 cases (Case et al., 1954). The bladder cancer patients with exposure to benzidine and 1-and 2-naphthylamine were identified on the basis of working histories (Case et al., 1954; Scott, 1952). In a further cohort study of a factory population it has been reported that benzidine had an "attack rate" of 237 per 100 000, which was lower than that of 2-naphthylamine (Mancuso & El-Attar, 1967). A retrospective survey of a single factory showed that 17 out of 76 workmen exposed to benzidine alone developed bladder tumours (Goldwater et al., 1965). Analysing a series of 100 cases of bladder cancer among workers in the dye industry, von Übelin & Pletscher (1954) concluded that benzidine and 2-naphthylamine were chiefly responsible for the induction of tumours of the bladder; the presence of primary tumours at other sites was noted in 6 of 19 autopsied cases in the benzidine-exposed group, but in none of 14 autopsied cases who had mainly been in contact with 2-naphthylamine. (The significance of the latter finding is controversial.) Along with bladder tumours induced in man by 2- and 1-naphthylamine, cases of benzidine-induced bladder cancer have been reported from several countries (Hueper, 1969).

4. Comments on Data Reported and Evaluation

4.1 Animal data

Benzidine is carcinogenic in the mouse, rat and hamster, and possibly the dog. Given orally, it has produced bladder carcinoma in the dog after a long latent period and liver tumours in the rat and hamster.

4.2 Human data

The epidemiological studies showed that occupational exposure to commercial benzidine alone was strongly associated with bladder cancer. In the same studies, exposure to 2-naphthylamine alone was similarly associated with bladder cancer. A number of case reports from several countries support the relationship between this neoplasm and occupational exposure to benzidine.

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3,3'-DIMETHYLBENZIDINE (o-TOLIDINE)*

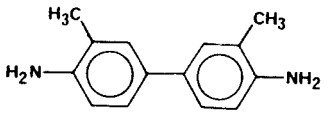
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 119937; Name: Benzidine, 3,3'-dimethyl-

4,4'-Diamino-3,3'-dimethylbiphenyl; 4,4'-Diamino-3,3'-dimethyldiphenyl; 3,3'-Dimethyl-4,4'-biphenyldiamine; 3,3'-Dimethyl-4,4'-diphenyldiamine; 3,3'-Dimethylbiphenyl-4,4'-diamine; 3,3'-Dimethyldiphenyl-4,4'-diamine; 3,3'-Tolidine; 4,4'-Bi-o-toluidine; 4,4'-Di-o-toluidine; Diamino-ditolyl; Fast dark blue base R; C.I. azoic diazo component 113

1.2 Chemical formula and molecular weight



$C_{14}H_{16}N_2$ Mol. wt: 212.28

1.3 Chemical and physical properties of the pure substance

(a) Description: White to reddish crystals or crystalline powder

(b) Melting-point: 129-131°C

(c) Absorption spectroscopy: The ultraviolet absorption spectrum in aqueous solution at different pH and in 2N hydrochloric acid is given by Pickett et al. (1950).

(d) Solubility: Slightly soluble in water; very soluble in ethanol and ethyl ether

(e) Chemical reactivity: A weak base that forms salts with HCl, H_2SO_4, etc.; can be tetrazotized to yield coloured coupling products, and oxidized; its amino groups can be acetylated.

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* Considered by the Working Group in Geneva, December 1971.

2. Use and Occurrence

(a) Use

Production data for o-tolidine are not available in recent years; however, in 1962 the production in the USA was reported as 243.0 thousand pounds. Imports of o-tolidine into the USA have ranged from a low of 2.3 thousand pounds in 1959 to a high of 134.6 thousand pounds in 1962. Imports in 1969 and 1970 were 80.6 thousand pounds and 97.8 thousand pounds, respectively.[1] o-Tolidine and its salts are widely used in the manufacture of dyestuffs and pigments based on the coupling of the tetrazotized base with phenols and amines. According to the Colour Index, more than 95 dyes are derived from o-tolidine. It is also used as a laboratory agent, e.g., for the detection of blood and for the colorimetric determination of chlorine in air and water.

(b) Analytical methods

Methods for the determination of o-tolidine in the working environment have been described by Meigs et al. (1954) and Ghetti et al. (1963). Guidelines for the analysis of aromatic amines have been given (UICC, 1970).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Twenty female Sprague-Dawley rats were given, by stomach tube, a suspension of o-tolidine in sesame oil up to a total dose of 500 mg per rat, fractionated in 10 doses at 3-day intervals. Sixteen rats were alive at the end of the observation period, i.e., nine months: three of these showed a total of four mammary carcinomas. In comparison, four out of five animals that survived after being given benzidine (50 mg per rat) showed a total of 17 mammary carcinomas. Among 132 rats that received the solvent only, 5 had a total of three mammary carcinomas, one fibroadenoma and five hyperplasias (Griswold et al., 1968). The purity of the material is not stated.

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[1] Data from Chemical Information Services, Stanford Research Institute.

Hamster: Commercial o-tolidine at a dietary level of 0.1% (3.0 g per animal per year) fed to groups of 30 male and 30 female hamsters throughout their life-span did not induce bladder or other tumours. Negative results were also obtained at a dietary level of 0.3%, which was the highest tolerated level. No tumours were observed with 1-naphthylamine, 2-naphthylamine, 3,3'-dichlorobenzidine or o-dianisidine at the 0.1% dose level. However, cholangiomas and liver-cell tumours were observed with benzidine and benzidine dihydrochloride at 0.1% and transitional cell carcinomas of the bladder and liver tumours were observed with 3,3'-dichlorobenzidine at 0.3% in the diet; 2-naphthylamine at a concentration of 1.0% in the diet also induced transitional cell carcinoma of the bladder (Saffiotti et al., 1967; Sellakumar et al., 1969).

(b) Subcutaneous administration

Rat: Commercial o-tolidine in olive oil was given as a weekly s.c. injection, at a dose level of 60 mg per rat per week (total dose: 5.5 g), to 105 Sherman rats. Forty-eight survived more than 300 days and were kept throughout their life-span. In contrast to rats given benzidine, no cirrhosis or hepatomas were observed among those receiving o-tolidine. Five rats developed cancer of the external auditory canal, all tumours appearing after the 354th day. No control group was run at the same time. Out of 56 tumours occurring among 578 untreated rats of the same colony, none was located in the external auditory canal (Spitz et al., 1950). Random-bred rats received weekly s.c. injections of purified o-tolidine in sunflower oil for 13 months, at doses of 20 mg per rat per week. Among 50 animals that survived for 8 months (time of occurrence of the first tumour), 30 developed a total of 41 tumours. Twenty carcinomas of Zymbal's gland, and tumours at other sites were seen. Two additional groups of rats (the first comprising 24 and the second 20 animals of each sex) received weekly a subcutaneous implant of a pellet containing 20 mg of purified o-tolidine and 10 mg of glycerol for 14 months. In the second of these groups the o-tolidine had been subjected to ultraviolet irradiation prior to the preparation of the pellet. The difference between the two groups was minimal. Out of a total of 68 animals that were alive at the time of appearance of the first tumour (11-12 months), 48 developed a total of 60 tumours. Among these were 27 Zymbal's gland carcinomas, and tumours at other sites (Pliss & Zabezhinsky, 1970). No control group was run at the same time as these

experiments, but in a preliminary report on these studies it was stated that rats from the same colony did not develop tumours of Zymbal's gland (Pliss, 1965).

3.2 Metabolism in animals and man

(a) Animals

Sciarini & Meigs (1961) after intraperitoneal injection of o-tolidine (70-100 mg/kg bw) into mongrel dogs recovered free o-tolidine from the urine to the extent of 4% and about 40% of a metabolite, probably the 5-ethereal sulfate of o-tolidine, within 3 days. These results are in agreement with the known fact that the dog is unable to acetylate aromatic amines.

(b) Man

Analysing the urine of workers manufacturing o-tolidine, Dieteren (1966) obtained positive evidence of the presence of diacetyl-o-tolidine and of a hydroxyamino metabolite, probably 5-hydroxy-o-tolidine. The possibility of the presence of monoacetyl-o-tolidine was left open.

3.3 Observations in man

o-Tolidine may enter the body by percutaneous absorption, by ingestion or by inhalation (Meigs et al., 1954). Some suspicion of carcinogenicity has been suggested (Scott, 1962), but supporting evidence is not available.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Purified o-tolidine is a systemic carcinogen in the rat when given subcutaneously. The oral experiment in the rat is of doubtful significance because of the small number of animals involved. In feeding experiments, the commercial product failed to produce tumours in hamsters.

4.2 Human data

No epidemiological studies are available.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

5. References

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UICC (1970) The quantification of environmental carcinogens (UICC Technical Report Series, Vol. 4)

N-NITROSO COMPOUNDS

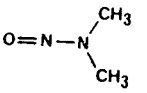
N-NITROSODIMETHYLAMINE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 62759; Name: Dimethylamine, N-nitroso-
Dimethylnitrosamine; N,N-Dimethylnitrosamine; DMN; DMNA

1.2 Chemical formula and molecular weight



$C_2H_6N_2O$ Mol. wt: 74.1

1.3 Chemical and physical properties of the pure substance

(a) Description: A yellow liquid of low viscosity

(b) Boiling-point: 149-150°C(755 mm); 50-52°C(14 mm)

(c) Density: D_4^{20} 1.0048

(d) Refractive index: n_D^{20} 1.4368

(e) Absorption spectroscopy: λ_{max} 230 nm; log ε 3.86
λ_{max} 332 nm; log ε 1.98 (in water)

(f) Identity and purity test: Identity and purity control is best done by thin-layer chromatography (Preussmann et al., 1964) or by gas chromatography (Foreman et al., 1970).

(g) Solubility and volatility: Miscible with water in all proportions; soluble in all common organic solvents and in lipids; very volatile

(h) Stability: Stable at room temperature for more than 14 days in aqueous solution at neutral and alkaline pH in the absence of light (Druckrey et al., 1967). Slightly less stable at strongly acid pH at room temperature. Light-sensitive, especially to ultraviolet light, so should be stored in dark bottles.

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* Considered by the Working Group in Geneva, December 1971.

(i) Chemical reactivity: Can be oxidized by strong oxidants to the corresponding nitramine or reduced by various reducing agents to the corresponding hydrazine and/or amine. Relatively resistant to hydrolysis. Photochemically reactive (Friedman et al., 1971).

2. Use and Occurrence

(a) Use

N-Nitrosodimethylamine (DMN) has been used as an industrial solvent and also in the synthesis of the rocket fuel 1,1-dimethylhydrazine. There are patents for its use as a solvent in the fibre and plastics industry, as an antioxidant, as a softener for copolymers, as an additive for lubricants and in condensers to increase the dielectric constant (see Daiber, 1966). DMN has also been used as a nematocide.

(b) Analytical methods

The analytical methods used until recently (UICC, 1970) for the trace analysis of nitrosamines have been unreliable. The results obtained with these methods, which include staining on thin-layer plates, polarography, colorimetric methods and gas-liquid chromatography with unspecific detection systems (thermal conductivity; flame ionization), need to be confirmed by the improved methods that have been developed recently. After sample extraction and adequate purification (steam distillation; liquid-liquid extraction; chromatography), the best available procedures for positive end determination are: (i) gas chromatography combined with spectrometry for direct identification of the nitrosamine (Heyns & Röper, 1971; Telling et al., 1971; Fazio et al., 1971a); (ii) derivative formation (Eisenbrand & Preussmann, 1970; Sen, 1970; Althorpe et al., 1970); (iii) gas-liquid chromatography with nitrogen-specific detector systems such as the alkali flame-ionization detector (Fiddler et al., 1971) or the Coulson electrolytic conductivity detector (Rhoades & Johnson, 1970). Results obtained with one or several of these methods can be considered as reliable at a concentration level of 5-10 ppb.

(c) Occurrence

With the older analytical methods (see (b) above), the presence of trace amounts of DMN was detected in nitrite-treated herring meal fed to sheep (Ender et al., 1964), the toxicity of which resembled the toxicity of DMN (Sakshaug et al., 1965); and in the fruit of Solanum incanum (Du Plessis et al., 1969), which is used as food by the Bantus in South Africa.

It was also claimed to be present in locally distilled spirits (kachusu) from Zambia (McGlashan et al., 1968), but this report was later attributed to a false positive analytical result (McGlashan et al., 1970). There is some indication that trace amounts of DMN may occur in wheat flour, cheese, smoked meat and fish, and other foodstuffs (Eisenbrand & Marquardt, 1969; Howard et al., 1970). Several types of Cantonese salt-dried fish were reported to contain 0.6-9.0 ppm DMN, as determined by thin-layer and gas-liquid chromatography (Fong & Walsh, 1971). As has been mentioned in (b) above, most of these results need to be confirmed by reliable modern analytical methods.

However, some reliable results, confirmed by mass spectrometry, are available. Fazio et al. (1971a) have demonstrated the occurrence of DMN, in concentrations of from 4 to 26 ppb (µg/kg), in samples of raw, smoked and smoked nitrite- and/or nitrate-treated fish (sablefish, salmon and shad). An investigation of 51 samples of various meat products showed 5 ppb of DMN in one sample of smoked ham confirmed by mass spectrometry (Fazio et al., 1971b), and indications of 1-5 ppb DMN in most of the other samples, which, however, were not confirmed by mass spectrometry. Evidence has been presented for higher concentrations (0.38-0.45 ppm) of DMN in one source of soya bean oil, confirmed by mass spectrometry (Hedler, 1971; Marquardt, 1971).

There is some evidence that DMN might be formed during the burning of dimethylhydrazine as a rocket fuel (Simoneit & Burlingame, 1971).

Nitrosamines may be formed in the stomach from ingested amines and nitrosating agents such as nitrite (Sander, 1967; Lijinsky & Epstein, 1970). Kinetic studies (Mirvish, 1970) have shown that the rate of nitrosation of dimethylamine is relatively low, owing to the strong basicity of the amine. However, simultaneous oral administration of high doses of nitrite and dimethylamine to mice induced acute liver damage and methylation of liver nucleic acids, indicating the formation of DMN in the stomach (Asahina et al., 1971). The formation of small amounts of DMN in the stomach of rats fed with dimethylamine and sodium nitrite has also been reported (Magee, 1968). The formation of nitrosamines has been demonstrated, under neutral conditions, from secondary amines and nitrate in the presence of certain bacteria present in the human digestive tract or in urinary tract infections (Alam et al., 1971a, 1971b; Hawksworth & Hill, 1971a, 1971b; Klubes & Jorndorf, 1971).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Several studies on different strains of mice have demonstrated that DMN produces haemangiomas, haemangioendothelial sarcomas, haemangioendotheliomas, adenomas and hepatocellular carcinomas of the liver, as well as adenomas and adenocarcinomas of the lung. In addition, renal adenomas have been observed in some strains (Takayama & Oota, 1963, 1965; Toth et al., 1964; Terracini et al., 1966; Clapp et al., 1968, 1971; Clapp & Toya, 1970; Den Engelse et al., 1970; Shabad & Savluchinskaya, 1971; Otsuka & Kuwahara, 1971). DMN in the drinking water, at a concentration of 0.005%, for one week was sufficient to induce tumours in the kidney and lung (Terracini et al., 1966). The lowest dose tested in long-term studies was a concentration in the drinking water that corresponds to a dose of 0.4 mg/kg bw/day (total dose, 89 mg/kg bw), and it produced tumours (Clapp & Toya, 1970). A feeding experiment with both DMN precursors, dimethylamine and nitrite, gave no evidence of carcinogenicity, probably because of the low nitrosation rate of the strongly basic amine (Greenblatt et al., 1971).

Rat: Carcinogenicity has been demonstrated in several laboratories on different strains of rat. Although differences have been seen in respect of target organs, a consistent observation has been that long-term treatment with doses compatible with a good survival rate leads to the development of liver tumours, whereas short-term treatment with high doses produces renal tumours (Magee & Barnes, 1956, 1962; Schmähl & Preussmann, 1959; Zak et al., 1960; Argus & Hoch-Ligeti, 1961; Terracini et al., 1967). One dose-response study is available; it concerns dietary concentrations ranging between 2 ppm and 50 ppm. At 2 and 5 ppm the frequencies of liver tumours among the survivors at 60 weeks were, respectively, 1/26 and 8/74; at 20 and 50 ppm liver tumours were observed in more than 66% of the animals. A concentration of 5 ppm daily in the diet for a year can be regarded as carcinogenic and corresponds to a total intake of 54 mg per animal (Terracini et al., 1967). In other studies, carcinogenic doses were: (i) 100 ppm in the diet for four weeks (Magee & Barnes, 1962); (ii) 0.4 mg per rat given by stomach tube five times weekly for 24 weeks (total intake, 144-240 mg/kg bw)

(Hoch-Ligeti et al., 1968); (iii) a small number of daily oral administrations (for example, 48 mg/kg bw over a period of six days) (Riopelle & Jasmin, 1969); and (iv) 30 mg/kg bw as a single dose or 20 mg/kg bw for one week (Magee & Barnes, 1959). Lung tumours were occasionally seen (Zak et al., 1960; Argus & Hoch-Ligeti, 1961).

Hamster: Administrations by stomach tube of 1.0-1.6 mg per hamster once or three times induced cholangiomas, cholangiocarcinomas and hepatocellular carcinomas, as well as haemangioendotheliomas of the liver (Tomatis & Cefis, 1967). Similar results were obtained by giving 0.0025% DMN in the drinking water for 11 weeks (Tomatis et al., 1964).

Guinea-pig: Male guinea-pigs given 1-2 mg/kg bw orally for 40 to 55 weeks developed papillary cholangiomas and liver-cell carcinomas (Le Page & Christie, 1969b).

Rabbit: Doses of 25 ppm and 50 ppm given to rabbits in the diet for 17 to 51 weeks resulted in hepatocellular carcinoma with lung metastases and benign papillary cholangiomas (Le Page & Christie, 1969a).

Rainbow trout: Doses of 300 ppm, 1200 ppm, 4800 ppm and 19 200 ppm given in the diet for more than six months induced adenomas and adenocarcinomas of the liver (Ashley & Halver, 1968).

(b) Subcutaneous administration

Mouse: Weekly injections of 0.15 mg of DMN for 1 to 25 weeks (total dose, 0.15-3.75 mg per mouse) induced haemangioendothelial sarcomas of the liver and other organs, and adenomas or adenocarcinomas of the lung (Otsuka & Kuwahara, 1971).

Hamster: Weekly injections of 0.5-1.0 mg of DMN for one-and-a-half to five months (total dose, 6-14 mg per hamster) caused haemangioendothelial sarcomas and cholangiocarcinomas of the liver and esthesioneuroepitheliomas of the nasal cavity (Herrold, 1967).

Mastomys: Twice-weekly injections of 0.1 mg per animal for 10 to 44 weeks induced cholangiomas and cholangiocarcinomas in this small rodent (Fujii & Sato, 1970).

(c) Intraperitoneal administration

Mouse: A single dose of 7 or 14 mg/kg bw to mice of the GR and CFW/D strains resulted in lung adenomas (Den Engelse et al., 1970; Frei, 1970).

Rat: Single injections of 18 mg/kg bw produced renal tumours (Murphy et al., 1966).

(d) Inhalation

Rat: Single or repeated inhalation exposures to DMN resulted in tumours of the nasal cavities and kidneys (Druckrey et al., 1967).

(e) Other experimental systems

Various injections: Rats given a single injection of 18 mg/kg bw, either intramuscularly, retroperitoneally or directly into the kidney, developed renal tumours (Murphy et al., 1966).

Newborn or suckling animals: Mice developed parenchymal cell and vascular tumours of the liver, as well as lung tumours (Terracini et al., 1966; Toth & Shubik, 1967; Vesselinovitch, 1969; Frei, 1970). A dose as low as 1 mg/kg bw was carcinogenic in mice (Toth et al., 1964). A dose of 125 µg of DMN per animal given to 24-hour and one-week-old rats induced tumours of the kidney and hepatocellular carcinomas (Terracini & Magee, 1964; Terracini et al., 1969).

Transplacental route: DMN induced renal tumours in low frequency in the offspring of pregnant rats treated during the last week of pregnancy (Alexandrov, 1968). In mice, single or repeated injections of DMN (12.5-75 mg/kg bw) during the last days of pregnancy resulted in lung adenomas and hepatomas in the offspring (Smetanin, 1971).

3.2 Metabolism in animals and man

DMN is metabolized in vivo and in vitro. The metabolism in vivo has been measured (Heath, 1962). Oxidative N-dealkylation in vitro with rat liver microsomal preparations was shown to form formaldehyde (Mizrahi & Emmelot, 1962). The rate of metabolism of DMN in vitro by slices of various organs of rats and hamsters has been measured by the production of labelled carbon dioxide (Magee, 1969; Montesano & Magee, 1971). A comparative in vitro study showed that DMN is metabolized in human liver slices at about the same rate as in rat liver slices (Montesano & Magee, 1970). Many biochemical effects have been described indicating that DMN probably requires metabolic activation to exert its toxic and carcinogenic effects (review: Magee & Barnes, 1967; Magee & Swann, 1969).

3.3 Observations in man

It is known that exposure to DMN gives rise to acute necrosis and, after two years, to cirrhosis in man (Freund, 1937); no information is at present available in respect of longer term chronic effects.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

N-Nitrosodimethylamine (DMN) is carcinogenic in all seven animal species tested. The main target organs are the liver and the kidney. It induces tumours following different routes of administration, including ingestion and inhalation. It is carcinogenic following prenatal exposure and in single-dose experiments. Similarities in metabolism in human and rat liver tissues have been reported.

4.2 Human data

DMN has been used in the chemical industry. The extent of such use at present is not known.

Many data on occurrence have been obtained by inadequate analytical methods and must await confirmation. Considerable progress has been made in the development of adequate and specific methods for trace analysis of nitrosamines, and reliable information is to be expected in the near future. Recent results, which have been confirmed by mass spectrometry, indicate that DMN does occur in certain food products at the 5-10 ppb level. There is some indication that DMN might be formed from ingested dimethylamine and nitrosating agents *in vivo*. Both precursors can occur in food.

No long-term follow-up studies of human subjects exposed to DMN are known.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

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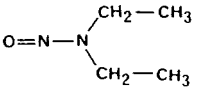
N-NITROSODIETHYLAMINE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 55185; Name: Diethylamine, N-nitroso-
Diethylnitrosamine; N,N-Diethylnitrosamine; DEN; DENA; DANA

1.2 Chemical formula and molecular weight



$C_4H_{10}N_2O$ Mol. wt: 102.1

1.3 Chemical and physical properties of the pure substance

(a) Description: A yellow, volatile liquid

(b) Boiling-point: 177°C (760 mm); 64-65°C (17 mm)

(c) Density: D_4^{20} 0.9422

(d) Refractive index: n_D^{20} 1.4386

(e) Absorption spectroscopy: λ_{max} 230 nm; log ε 3.86
λ_{max} 332 nm; log ε 1.98 (in water)

(f) Identity and purity test: Boiling-point, thin-layer chromatography (Preussmann et al., 1964) and gas chromatography (Foreman et al., 1970)

(g) Solubility and volatility: Solubility in water: approximately 10%; soluble in organic solvents and in lipids; volatile

(h) Stability: Stable at room temperature for more than 14 days in aqueous solution at neutral and alkaline pH in the absence of light (Druckrey et al., 1967). Slightly less stable at strongly acid pH at room temperature. Light-sensitive, especially to ultraviolet light, so should be stored in dark bottles.

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* Considered by the Working Group in Geneva, December 1971.

(i) Chemical reactivity: Can be oxidized by strong oxidants to the corresponding nitramine or reduced by various reducing agents to the corresponding hydrazine and/or amine. Relatively resistant to hydrolysis, but can be hydrolysed by HBr in acetic acid. Photochemically reactive (Friedman et al., 1971).

2. Use and Occurrence

(a) Use

There are patents for the use of N-nitrosodiethylamine (DEN) as a solvent in the fibre industry, as a softener for copolymers, as an additive for lubricants, in condensers to increase the dielectric constant and for the synthesis of 1,1-diethylhydrazine.

(b) Analytical methods

The analytical methods used until recently (UICC, 1970) for the trace analysis of nitrosamines have been unreliable. The results obtained with these methods, which include staining on thin-layer plates, polarography, colorimetric methods and gas-liquid chromatography with unspecific detection systems (thermal conductivity; flame ionization), need to be confirmed by the improved methods that have been developed recently. After sample extraction and adequate purification (steam distillation; liquid-liquid extraction; chromatography), the best available procedures for positive end determination are: (i) gas chromatography combined with mass spectrometry for direct identification of the nitrosamine (Heyns & Röper, 1971; Telling et al., 1971; Fazio et al., 1971); (ii) derivative formation (Eisenbrand & Preussmann, 1970; Sen, 1970; Althorpe et al., 1970); (iii) gas-liquid chromatography with nitrogen-specific detector systems such as the alkali flame-ionization detector (Fiddler et al., 1971) or the Coulson electrolytic conductivity detector (Rhoades & Johnson, 1970). Results obtained with one or several of these methods can be considered as reliable at a concentration level of 5-10 ppb.

(c) Occurrence

The occurrence of DEN in heated wheat flour has been reported (Marquardt & Hedler, 1966), denied (Thewlis, 1967) and confirmed (Hedler & Marquardt, 1968). The latter authors also claim to have detected trace amounts of DEN in wheat plant, wheat grain, milk and cheese.

Some of these results have been confirmed (Petrowitz, 1968). Sen et al. (1969b) found DEN in some samples of Cheddar cheese and pickled herring, but not in flour, frozen herring or spinach. No DEN was found in nitrite-bearing commercially bought spinach (Keybets et al., 1970). Using thin-layer and gas-liquid chromatography, Fong & Walsh (1971) found DEN at levels of 1.2-21.0 ppm (mg/kg) in Cantonese salt-dried fish. Practically all of these data need to be confirmed by improved analytical methods.

The formation of DEN from inactive precursors, diethylamine and nitrosating agents, has been investigated. Sander (1967) was able to detect the formation of DEN on incubating a 5-ml aliquot of human gastric juice with only 40 µg of sodium nitrite and a few crystals of diethylamine hydrochloride. This was confirmed by the in vitro formation that was demonstrated on incubation with gastric juices from rats, rabbits, cats, dogs and man. Increased synthesis was found in the more acid juices (man, rabbit) than in the less acid ones of the other animals (Sen et al., 1969a). The formation of DEN from diethylamine and methylnitrite, both present in tobacco smoke, could be demonstrated (Wynder & Hoffmann, 1967). The formation of nitrosamines has been demonstrated under neutral conditions from secondary amines and nitrate in the presence of certain bacteria present in the human digestive tract or in urinary tract infections (Sander, 1968; Alam et al., 1971a, 1971b; Hawksworth & Hill, 1971a, 1971b; Klubes & Jondorf, 1971).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Several studies on different strains have demonstrated carcinogenicity in this species. In the liver mainly haemangio-endotheliomas (Schmähl et al., 1963a; Takayama & Oota, 1965) but also hepatomas (Clapp & Craig, 1967; Clapp et al., 1970) are produced. In most of the studies mentioned squamous cell carcinomas of the oesophagus and forestomach were also found (see also Shvemberger, 1965; Clapp et al., 1971). An increased lung adenoma rate has also been observed (Clapp & Craig, 1967; Mirvish & Kaufman, 1970; Clapp et al., 1970).

The site and histological type of the tumours depend to a certain extent on the mouse strain used (Clapp et al., 1971). Tumour frequency was usually very high, approaching in many cases 100%, at a daily dosage of 2-13 mg/kg bw per day. Some indication of a dose-related tumour response has been reported by Schmähl & Thomas (1965b) and Clapp et al. (1970).

Rat: Following the first report on liver carcinogenesis by DEN (Schmähl et al., 1960), many laboratories have confirmed and enlarged these studies by using different strains and conditions (Thomas, 1961; Grundmann & Sieburg, 1962; Reid et al., 1963; Lacassagne et al., 1967). In most cases hepatocellular tumours have been observed, often with lung metastases. In some cases also cholangiomas have been described (Argus & Hoch-Ligeti, 1961; Hoch-Ligeti et al., 1964). In lifetime feeding studies using daily doses between 1 and 10 mg/kg bw, tumour yields often approaching 100% have been found. While most investigations have shown no sex difference, Reuber & Lee (1968) report an increased sensitivity of young females. The same authors also report a higher sensitivity of four-week-old animals as compared with older ones. Feeding for only 82 days increased the latent period and reduced the tumour yield as compared with lifetime feeding studies (Rajewsky et al., 1966).

At lower daily dosages (0.15-0.6 mg/kg bw) squamous cell carcinomas of the oesophagus have been obtained in addition to liver tumours (Druckrey et al., 1963a). This effect was increased by combined treatment with alcohol (Gibel, 1967). A single dose of 280 mg/kg bw or four weekly doses of 25 and 35 mg/kg bw respectively induced tumours of the liver, the oesophagus and the kidney (Druckrey et al., 1963c, 1964). A dose-response study is available (Druckrey et al., 1963a): given in the drinking water, daily doses ranged between 14.2 and 0.075 mg/kg bw in nine dosage groups. The total dose administered until death ranged between 965 and 64 mg/kg bw, the induction time between 68 and 840 days. All dosages higher than 0.15 mg/kg bw per day gave a tumour yield of 100%; 0.15 mg/kg bw per day gave a tumour yield of 27/30. At 0.075 mg/kg bw per day 20 rats lived for more than 600 days, and 11 of these animals had benign or malignant tumours of the liver, the oesophagus or the nasal cavity. The tumours diagnosed as benign were papillomas of the oesophagus and hepatomas. All four of the animals that lived longer

than 940 days at this dose level had tumours, 3 of which were hepatomas and 1 a liver carcinoma. A feeding experiment with both precursors, diethylamine and nitrite, gave no evidence of carcinogenicity (Druckrey et al., 1963b). The probable reason for this negative result is the strong basicity of diethylamine and the consequent low rate of nitrosation (Sander et al., 1968; Mirvish, 1970).

Hamster: The intragastric administration of 0.4 ml of a dilute aqueous solution of DEN (1: 250) twice weekly induced tumours of the trachea and lung (Dontenwill & Mohr, 1961; Dontenwill et al., 1962). In another study, malignant liver-cell tumours and tumours of the nasal cavity and bronchi were induced in addition (Herrold & Dunham, 1963).

Guinea-pig: DEN in the drinking water (5 mg/kg bw per day) induced hepatocellular and adenocarcinomas, some metastasizing into the lungs, in all treated animals. The median total dose applied was 1200 mg/kg bw (Druckrey & Steinhoff, 1962). Almost identical results were obtained at a dose of 3 mg/kg bw (Thomas & Schmähl, 1963). In another study, liver tumours were also induced as the main tumour type (Argüs & Hoch-Ligeti, 1963). In a dose-response study (Arcos et al., 1969), DEN was given in the drinking water for periods of 4, 8, 12 and 24 weeks. At an average daily intake of 1.2 mg per guinea-pig (total dose below 75 mg per animal) no tumours were observed after one year. At a higher daily intake, treatment for 12 weeks gave a 21% tumour yield and treatment for 24 weeks a 100% yield. The minimum effective cumulative dose was 86 mg per animal.

Rabbit: Daily doses of 3.4 mg/kg bw given continuously in the drinking water induced liver-cell carcinomas in two treated rabbits (Schmähl & Thomas, 1965a). In another experiment, all 13 animals receiving DEN continuously in the drinking water (0.04 µg/litre) for 6 days a week died with metastasizing hepatic carcinomas; one animal had an adenocarcinoma of the lung (Rapp et al., 1965).

Dog: The oral administration of 3 mg/kg bw for 10 months, followed a month later by 15 weekly s.c. injections (3 mg/kg bw per week), induced a large leiomyosarcoma of the liver (total dose of DEN: 565 mg/kg bw) (Schmähl et al., 1964).

Pig: Various tumours of the liver, 1 adenoma of the kidney and 1 squamous cell carcinoma of the ethmoid were induced by daily doses of 4.4 mg/kg bw (Schmähl et al., 1967). In another study, two pigs treated with 1.5 mg/kg bw for 11 months and then 3 mg/kg bw until death developed a hepatoma and a kidney adenoma respectively (total dose applied: 750 mg/kg bw) (Schmähl et al., 1969).

Monkey: By oral administration, beginning 12 hours after birth, at a dosage varying from 2 to 30 mg/kg bw per day, hepatocellular carcinomas were induced in 3/15 rhesus and cynomolgus monkeys (O'Gara & Kelly, 1965). Hepatocarcinomas were induced in 6/15 rhesus and cebus monkeys treated with varied doses of DEN for more than a year. Treatment of newborn or young animals began with 2 mg/kg bw and was gradually increased up to 25-50 mg/kg bw. The cumulative oral dosage ranged from 6 g to 24 g per monkey and the induction time varied between 15 and 26 months (Kelly et al., 1966).

Fish: Exposure of the aquarium fish, Brachydanio rerio, to 10-100 ppm DEN in the tank water for 8 weeks resulted in hepatomas or cholangiomas in 17/63 animals (Stanton, 1965).

(b) Skin application

Mouse: Twice weekly treatment of the skin with two drops of 0.2% DEN in acetone for 10 months induced squamous cell carcinomas of the nasal cavity in 17/24 animals. No local skin tumours were seen (Hoffman & Graffi, 1964a). Daily treatment with three drops of 0.2% DEN in acetone or twice weekly treatment with two drops induced squamous cell carcinomas of the nasal cavity in almost all the treated animals after the application of more than 8 mg per animal (Hoffman & Graffi, 1964b).

Hamster: Skin painting produced tumours of the trachea, the bronchi and the nasal cavity, and one liver tumour but no skin tumours (Herrold, 1964b). Intradermal injection produced tumours at the same sites and no local malignancies (Herrold, 1964a).

(c) Inhalation and intratracheal administration

Rat: Spray inhalation of a dilute aqueous solution of DEN (1:250) for four months produced a 50% yield of liver carcinomas (Dontenwill & Mohr, 1962).

Hamster: Spray inhalation of 1-2 mg DEN twice weekly for five months produced tumours of the trachea and the lungs (Dontenwill et al., 1962). The weekly intratracheal instillation of 0.05 ml of aqueous solution (1:14) for a period of up to 6 months induced tumours in the trachea and bronchi, but no liver tumours (Herrold & Dunham, 1963).

(d) Subcutaneous administration

Mouse: Doses of 50 mg/kg bw once or twice weekly, up to a total dose of 200 or 400 mg/kg bw, increased the frequency of lung adenomas significantly as compared with untreated controls (Hilfrich et al., 1971). Treatment of newborn animals with a single dose of 50 mg/kg bw also caused a significant increase in the number of lung adenomas. Most of the animals also developed hepatomas within six months (Gargus et al., 1969).

Hamster: Several experiments with Syrian golden hamsters have shown that DEN mainly produces carcinomas and papillomas of the upper and lower respiratory tract (nasal cavity; larynx; trachea; lungs) and, much less frequently, tumours of the liver (Dontenwill et al., 1962; Herrold, 1964a, 1964b, 1964c). A positive dose-response correlation for tumour induction in the upper respiratory tract, but not for that in the lower respiratory tract (where tumour frequency was low), was observed after 12 weekly doses of 4, 2, 1 or 0.5 mg; tumour yields in the nasal cavity and the larynx ranged from 17% to 72%, in the trachea from 88% to 100% (Montesano & Saffiotti, 1968). Six different single doses, ranging between 4 and 0.75 mg per animal, produced papillomas in the trachea while lower doses, down to 0.03 mg per animal, were without carcinogenic effect when the animals were killed at 25 weeks (Mohr et al., 1966b). A smaller dose (4 mg/kg bw) was found to give a 10% yield of tracheal papillomas in hamsters observed for their entire life-span (Dontenwill, 1968). A single dose of 0.15, 0.09, 0.03 and 0.015 mg given to newborn hamsters produced tumours of the upper respiratory tract in 30-65% of the animals, but very few tumours were observed in the lungs. Again a few liver-cell tumours were seen (Montesano & Saffiotti, 1970).

The consistent affinity of DEN for the respiratory tract was not observed in experiments with the Chinese hamster. A dose of 77 mg/kg bw once a week, given for up to 22 weeks, resulted in an 82% yield of

multiple papillomas of the forestomach and a 30% yield of papillomas of the oesophagus; in the respiratory tract, squamous metaplasia but no tumours were observed (Mohr et al., 1967).

Guinea-pig: Administration of total doses between 341 and 1310 mg/kg bw produced malignant liver-cell tumours and some benign tumours in the trachea and ethmoidal region (Lombard, 1965).

(e) Intraperitoneal administration

Mouse: Two injections of 100 mg/kg bw produced lung adenomas in 97% of the animals (Mirvish & Kaufman, 1970).

Rat: A daily dose of 0.55 mg per animal for 12 or 23 weeks produced hepatomas in more than 80% of the animals treated (Svoboda & Higginson, 1968).

Hamster: A dose of 2 mg per animal once a week for 4-7 months produced multiple squamous cell carcinomas of the trachea, epithelial papillomas and neuroepithelial tumours of the nasal cavity, a few squamous cell papillomas of the bronchi and hepatic carcinomas (Herrold, 1964a, 1964b).

Monkey: Two Cercopithecus (green) monkeys treated with 20-40 mg/kg bw every two weeks for 26 months developed hepatic cell carcinomas (Kelly et al., 1966). Hepatomas and hepatocellular carcinomas were produced by administration of 40 mg/kg bw once every two weeks for 15 months or longer; all 25 monkeys treated had malignancies, 17 of them with metastases (O'Gara et al., 1970).

(f) Other experimental systems

Intravenous administration: A single dose of 280 mg/kg bw given to 4 rats produced kidney tumours in all animals and one carcinoma of the ovary (Druckrey et al., 1963c, 1964).

Rectal administration: Twice weekly treatment of rats with 11.2 mg/kg bw for their lifetime produced hepatocellular carcinomas in all the treated animals (Schmähl et al., 1963b).

Intramuscular administration: Grass parakeets were injected once weekly with 100 mg/kg bw for 19 weeks and after that once every second week; 6 out of 9 birds that survived long enough died with malignant hepatic tumours (Schmähl et al., 1966).

Transplacental route: Pregnant mice were given DEN in doses of 80-240 mg/kg bw from the 15th to the 20th day of gestation; their offspring were reared and killed after 8 or 12 months. A significant increase (up to 63%) in the occurrence of multiple pulmonary adenomas was observed (Mohr & Althoff, 1965a).

DEN injection into C3HA mice in late pregnancy induced benign and malignant tumours of the lung, liver, oesophagus and forestomach in the offspring. Neoplasms in the young mainly developed during the second year of life (Likhachev, 1971).

Daily doses of 4 and 8 mg per animal were given s.c. to mother rats from the 10th to the 21st day of pregnancy; 14/26 of the mothers showed kidney tumours after 1 year, 5 of which were carcinomas; some kidney tumours were observed in the offspring at one year of age (Wrba et al., 1967). Under similar conditions, oral or s.c. administration to mother rats of varying high doses of DEN produced benign and malignant tumours in different organs, mainly thymomas and adenomas of the mammary gland. Treated mother rats died with carcinomas and adenomas of the kidneys and the liver (Thomas & Bollmann, 1968). Daily doses of 1 mg DEN given to mother rats before and during pregnancy up to a total dose of 60-90 mg per animal did not result in an increased tumour rate in the offspring during lifetime observation. However, in four of the mothers, directly treated with DEN, carcinomas of the kidney have been observed (Sydow, 1970).

A daily dose of 2 mg was given subcutaneously to hamsters for 1 to 7 days in the second half of the gestation period. Multiple tracheal papillomas were found in 42% of the young 25 weeks after birth and the first tumours appeared between 8 and 12 weeks of age (Mohr et al., 1965). The carcinogen appears to pass the placenta and is not transmitted through the mother's milk (Mohr & Althoff, 1965b). Of the treated mothers, 73% developed tracheal papillomas (Mohr et al., 1966a).

3.2 Metabolism in animals and man

DEN is metabolized in vivo and in vitro. In the rat, excretion, expiration, blood concentration and decomposition of DEN and metabolites have been measured (Heath, 1962). Oxidative N-dealkylation in vitro with rat liver microsomal preparations was shown to form acetaldehyde (Mizrahi & Emmelot, 1962, 1963). The rate of metabolism in vitro of DEN by slices of various organs of rat and hamster has been measured by the production of labelled

carbon dioxide, and a correlation between the degree of metabolism and the distribution of the induced tumours has been shown (Montesano & Magee, 1971). Many biochemical effects have been described (summary: Magee & Barnes, 1967; Magee & Swann, 1969). Reactions with cell constituents of reactive metabolites result in the ethylation of liver RNA and DNA to form 7-ethylguanine (Magee & Lee, 1964; Magee, 1969).

(a) Carcinogenicity of metabolites

No defined metabolites with retained nitrosamine structure have been identified. A possible metabolite ethyl-2-hydroxyethylnitrosamine is less carcinogenic in the rat than is the parent compound (Druckrey et al., 1967).

3.3 Observations in man

No data available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

N-Nitrosodiethylamine (DEN) is carcinogenic in all ten animal species tested, including sub-human primates. The main target organs are the nasal cavity, trachea, lung, oesophagus and liver. It induces tumours following different routes of administration, including ingestion, inhalation and skin painting. It is carcinogenic in single-dose experiments and following prenatal exposure.

4.2 Human data

Many data on the occurrence in the human environment have been obtained by inadequate analytical methods and must await confirmation. Considerable progress has been made in the development of adequate and specific methods for trace analysis and more information is to be expected in the near future.

The possibility of a formation of DEN from precursors, diethylamine and nitrosating agents, in vivo, must receive further attention. No long-term studies of human subjects exposed to DEN are known.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

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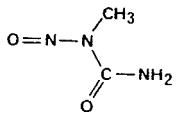
NITROSOMETHYLUREA*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 684935; Name: Urea, 1-methyl-1-nitroso-
N-Methyl-N-nitroso-urea; NMU; NMH

1.2 Chemical formula and molecular weight



$C_2H_5N_3O_2$ Mol. wt: 103.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Pale yellow crystals

(b) Melting-point: 124°C (Decomp.)

(c) Absorption spectroscopy: λ_{max} 231 nm; log ε 3.77 (in water)

(d) Identity and purity test: Melting-point, absorption spectroscopy and thin-layer chromatography (Preussmann et al., 1964)

(e) Solubility: Solubility in water: approximately 1.4% at room temperature; soluble in polar organic solvents

(f) Stability: Stability in aqueous solutions is pH-dependent (20°C) (Druckrey et al., 1967):

pH	4.0	6.0	7.0	8.0	9.0
half-life (hours)	125	24	1.2	0.1	0.03

The pure compound is sensitive to humidity and light, and should be stored at a low temperature (preferably below -10°C).

(g) Chemical reactivity: The compound is highly reactive (Garrett et al., 1965; McCalla et al., 1968). Reaction rates with various biologically important nucleophiles have been measured (Veleminsky et al., 1970). At alkaline pH it decomposes to diazomethane, a highly irritant and toxic gas.

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*Considered by the Working Group in Geneva, December 1971.

(h) Technical products and impurities: No data on technical products are available to the Working Group. Nothing is known about the decomposition products arising from prolonged storage.

2. Use and Occurrence

(a) Use

In a patent, the use of nitrosomethylurea (NMU) as a polymerization initiator is proposed. It is widely used in chemical laboratories for the synthesis of diazomethane. NMU and derivatives have been proposed for cancer chemotherapy. Some are already in use (Hansen et al., 1971).

(b) Analytical methods

Analytical methods have been described in a report by the UICC (1970). Since the publication of this report, colorimetric determination by denitrosation with dilute aqueous acids has been improved (Schaper, 1970). None of the available methods is suitable for trace analysis in complex mixtures, such as food, etc.

(c) Occurrence

An NMU derivative, streptozotocin, has been isolated from Streptomyces achromogenes.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Doses of 8 and 4 mg/kg bw per day in a lifetime feeding study produced squamous cell carcinomas of the forestomach at cumulative doses of 800-1400 mg/kg bw (Druckrey et al., 1961, 1967). Carcinomas of the forestomach were seen at a dosage of 10 mg/kg bw once every two weeks and of 20 mg/kg bw once every four weeks over a period of nine months. Malignant tumours of the brain (sarcomas, gliomas) and the peripheral nervous system (described as neurosarcomas) were observed in addition (Schreiber & Jänisch, 1967). Continuous administration in the drinking water in the presence of buffer (pH 6.8) produced tumours of the brain, mainly gliomas, and one neurinoma of the spinal cord, but no tumours of the stomach (Thomas et al., 1967). Stroobandt & Brucher (1968) have also observed only neurogenic malignant tumours. A single intragastric dose of 90 mg/kg bw produced malignancies of the kidney, the forestomach, the small and large intestine, the skin (keratoacanthomas) and the jaw (odontomas) (Leaver et al., 1969).

Hamster: Intragastric administration of 1 mg per animal twice a week for 4 months produced odontogenic tumours and epidermoid carcinomas of the oral cavity and adenocarcinomas of the small and large intestine, many metastasizing to lymph nodes (Herrold, 1968, 1969).

Guinea-pig: Doses of 2.5 mg/kg bw given in the drinking water produced carcinomas and sarcomas of the stomach, adenocarcinoma of the pancreas, malignant tumours of the ear duct and a neurinoma of the lumbar nerve and leukaemias (Druckrey et al., 1968) Similar results were obtained by Bücheler & Thomas (1971).

(b) Skin application

Mouse: One application of 50-100 mg/kg bw to newborn mice induced mainly lymphatic leukaemias in about 50% of the treated animals (Graffi & Hoffmann, 1966a). Topical application for 18 weeks produced malignant skin tumours (Graffi et al., 1967).

Rat: Application of a 0.5% solution in acetone three times a week (single dose, 1.75 mg) produced multiple epithelial and basal cell carcinomas after a latent period of 6-8 months in all animals (Graffi & Hoffmann, 1966b; Graffi et al., 1967).

Hamster: Treatment under the same conditions as with the rat produced malignant skin tumours in all experimental animals (Graffi & Hoffmann, 1966b; Graffi et al., 1967).

(c) Intratracheal administration

Hamster: The weekly intratracheal instillation of 0.5 mg per animal for two-and-a-half months produced epidermoid carcinomas in the nasopharyngeal tube, pharynx, larynx, trachea, bronchi, oesophagus and forestomach (Herrold, 1970).

(d) Subcutaneous administration

Mouse: Newborn animals receiving a single injection of 0.05 mg per mouse (33 mg/kg bw) developed a poorly differentiated lymphosarcoma involving thymus, myocardium, lung, spleen, lymph nodes, liver, kidney and bone marrow (Terracini & Stramignoni, 1967). The induction of leukaemias and pulmonary tumours after treatment of newborn mice was also reported by Kelly et al. (1968). Treatment of 12-week-old mice with 50 and 100 mg/kg bw produced a malignant lymphoma and one local subcutaneous sarcoma (Terracini & Stramignoni, 1967).

Hamster: Weekly injections of 0.5-1 mg per animal for 3-4 months induced sarcomas at the site of injection in all animals, most metastasizing to the lung or lymph nodes, and 20% of the animals had benign tumours of the forestomach, ovary and vagina (Herrold, 1966).

(e) Intraperitoneal administration

Mouse: Injection of various single doses into adult or newborn animals produced high yields of thymic lymphomas and pulmonary adenomas (Joshi & Frei, 1970a, 1970b; Frei, 1970). Fractionated dose schedules, ranging from 50 to 250 mg/kg bw (cumulative doses) resulted in a lymphoma yield of up to 93% (Joshi & Frei, 1970b). Terracini & Testa (1970) treated newborn and five-week-old mice with a single dose of 0.05 mg/g bw and found the newborn animals more susceptible to the induction of lymphosarcomas, lung adenomas and hepatomas. No difference was observed in the frequency of tumours of the forestomach.

Rat: Repeated weekly doses of 10 mg/kg bw produced malignant tumours in the peritoneal cavity, some of them being neurogenic tumours arising from peripheral nerves (Thomas et al., 1968). Newborn rats developed renal anaplastic tumours and forestomach tumours more readily than adult animals. No significant difference between adult and newborn rats was seen in the induction of lymphosarcomas, intestinal adenocarcinomas or mammary tumours (Terracini & Testa, 1970).

Hamster: Doses of 1 mg per animal per week for 4-5 months produced metastasizing adenocarcinomas of the large and small intestine (Herrold, 1969).

(f) Other experimental systems

Intravenous administration: In rats, repeated injections produced regularly and almost selectively malignant tumours of the brain, the spinal cord and the peripheral nervous system. Histologically the brain tumours were diagnosed as different types of gliomas, ependymomas, medulloblastomas and intracranial sarcomas; the tumours of the spinal cord were spongioblastomas, medulloblastomas and gliomas, and those of the peripheral nervous system were neurinomas. Dosage usually was 5-10 mg/kg bw per week, with total doses between 180 and 230 mg/kg bw and a median induction time of 300 days (Druckrey et al., 1964a, 1965; Fried & Fried, 1966; Jänisch et al., 1967; Weiss et al., 1970; Schiffer et al., 1970). The morphology of these tumours of the nervous system has been described in detail (Wechsler et al., 1969). Single doses of 70-100 mg/kg bw produced malignant and benign tumours

in various organs, including the stomach, large and small intestine, kidney and brain (Druckrey et al., 1963, 1964b).

In hamsters: three to four injections of 2.5 mg per animal produced adenocarcinomas of the small and large intestine, odontogenic tumours and epidermoid carcinomas of the oral cavity (Herrold, 1968, 1969).

Doses of 10 mg/kg bw given every two weeks to rabbits produced polymorphous gliomas and sarcomas in a tumour yield of 69%. No tumours of peripheral nerves were seen. Adenocarcinomas of the small intestine and vascular tumours of different organs were also found (Jänisch & Schreiber, 1967; Schreiber et al., 1969). The morphology of the malignant gliomas has been described (Kleihues et al., 1970).

Monthly injections of 20 mg/kg bw for 12-18 months produced brain tumours (sarcomas or multiform glioblastomas) in four out of ten dogs. Four other dogs developed sarcomas and haemangioendotheliomas of the lung, spleen and heart (Warzok et al., 1970).

Intracerebral injection: A single intracerebral injection of 0.2-0.4 mg per animal to newborn rats and mice induced no brain tumours, but gave rise to kidney fibrosarcomas and mammary gland carcinomas in the rats and leukaemias and pulmonary tumours in the mice (Kelly et al., 1968).

Transplacental route: In rats, Alexandrov (1969) observed tumours of the nervous system and kidney in the offspring and mammary tumours in the mothers after treatment during pregnancy.

In vitro transformation: Cultures of hamster lung tissue are transformed by NMU to altered growth behaviour indicative of malignancy (Sanders & Burford, 1967). The susceptibility of normal and transformed hamster cell cultures to the cytotoxic effect of NMU has been measured (Huberman et al., 1970).

3.2 Metabolism in animals and man

The high chemical reactivity of NMU makes it unlikely that enzymatic metabolism is involved in the activation of the compound. In vivo it is no longer detectable in blood after 15 minutes (Swann, 1968). The distribution in the rat after systemic administration has been investigated (Kleihues & Patzschke, 1971). No data on enzymatic metabolism in animals or carcinogenicity of metabolites are available. In vitro and in vivo NMU alkylates, for example, nucleic acids at the N-7 of guanine (Swann & Magee, 1968; Krüger et al., 1968, 1970) and at the O-6 of guanine (Loveless, 1969).

3.3 Observations in man

The compound may be formed *in vivo* from methylurea and nitrite or other nitrosating agents (Montesano & Magee, 1971; Mirvish, 1971).

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Nitrosomethylurea (NMU) is carcinogenic in all six animal species tested. It has a local as well as a systemic carcinogenic effect, producing tumours at different sites, including the nervous tissue. It induces tumours following different routes of administration, including ingestion. It is carcinogenic in single-dose experiments and following prenatal exposure.

4.2 Human data

The compound may be formed *in vivo* from methylurea and nitrite or other nitrosating agents. No data on direct human exposure are available.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

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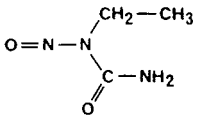
NITROSOETHYLUREA*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 759739; Name: Urea, 1-ethyl-1-nitroso-
N-Ethyl-N-nitroso-urea; ENU; ÄNH

1.2 Chemical formula and molecular weight



$C_3H_7N_3O_2$ Mol. wt: 117.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Yellow-pink crystals

(b) Melting-point: 103-104°C (Decomp.)

(c) Absorption spectroscopy: λ_{max} 233 nm; log ε 3.74 (in water)

(d) Identity and purity test: Melting-point, absorption spectroscopy and thin-layer chromatography (Preussmann et al., 1964)

(e) Solubility: Solubility in water: approximately 1.3% at room temperature; soluble in polar organic solvents

(f) Stability: Stability in aqueous solutions is pH-dependent (20°C) (Druckrey et al., 1967):

pH	4.0	6.0	7.0	8.0	9.0
half-life (hours)	190	31	1.5	0.1	~0.05

The pure compound is sensitive to humidity and light, and should be stored at a low temperature (preferably below -10°C).

(g) Chemical reactivity: The compound is highly reactive (Garrett et al., 1965; McCalla et al., 1968). Reaction rates with various

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* Considered by the Working Group in Geneva, December 1971.

biologically important nucleophiles have been determined (Veleminsky et al., 1970). At alkaline pH it decomposes to diazoethane, an irritant and toxic gas.

(h) Technical products and impurities: No data on technical products are available to the Working Group. Nothing is known about decomposition products arising from prolonged storage.

2. Use and Occurrence

(a) Use

Nitrosoethylurea is used in chemical laboratories for the synthesis of diazoethane.

(b) Analytical methods

Analytical methods have been described in a report by the UICC (1970). Since the publication of this report, colorimetric determination by denitrosation with dilute aqueous acids has been improved (Schaper, 1970). None of the available methods is suitable for trace analysis in complex mixtures, such as food, etc.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Ten-day-old rats were treated with single doses of 10, 20, 40 and 80 mg/kg bw and malignant neurogenic tumours in the brain, spinal cord and peripheral nervous system were induced in 75 out of 80 animals. Even at the lowest dose, the neurogenic tumour frequency was 21/23. At the highest dose, 9 out of 16 rats had nephroblastomas (Druckrey et al., 1970b).

(b) Subcutaneous administration

Rat: Newborn rats were injected with single doses of 5, 10, 20, 40 and 80 mg/kg bw and 10-day-old rats received single doses of 10, 20 and 40 mg/kg bw. Mainly tumours of the central and peripheral nervous system were produced. The lowest dose (5 mg/kg bw) gave a tumour yield of 32%. All higher doses gave tumour yields higher than 70%, in most cases near 100%. No difference in response was observed between the newborn and the 10-day-old animals (Druckrey et al., 1970b).

(c) Intraperitoneal administration

Mouse: Doses of 60 or 120 µg/g bw were injected into mice less than 24 hours, 15 days or 6 weeks old. Multiple types of tumours developed, including intracranial neurogenic and renal epithelial neoplasms. The multiplicity and the frequency of tumour types were dependent upon age at the time of treatment (Vesselinovitch & Lombard, 1971; Lombard & Vesselinovitch, 1971).

(d) Other experimental systems

Intravenous administration: Weekly doses of 10 mg/kg bw given i.v. to rats for 175 days produced leukaemias in 9 of 16 animals, gliomas of the brain in 4, a glioma of the spinal cord and an adenoma in the small intestine (Druckrey et al., 1967). Treatment of 30-day-old rats with a single dose of 20, 40 or 80 mg/kg bw predominantly produced tumours of the brain and nervous system. With 20 mg/kg bw the tumour yield was 63%. More tumours outside the nervous system (in the ovary, uterus and, especially, kidney) were seen in adult rats than in young or newborn animals (Druckrey et al., 1970b).

Transplacental route: Nitrosoethylurea, a standard compound for transplacental carcinogenesis, has been investigated very extensively since the first reports of Druckrey et al. (1966) and Ivankovic et al. (1966). Ivankovic & Druckrey (1968) gave single i.v. injections to pregnant rats, varying the dose and also the day of application during gestation. On the 15th day of gestation, 7 different single doses between 5 and 80 mg/kg bw were injected. Of 222 offspring, 193 died with malignant neurogenic tumours (87%). At the lowest dosage - 5 mg/kg bw (approximately 2% of LD_{50}) - 63% of the offspring developed tumours. Variation of the day of application showed that the yield of neurogenic tumours in the progeny was highest when the compound was administered during the last week of pregnancy. No tumours were observed when the mothers were treated before the 8th day of gestation. Similar findings have been observed in different strains of rat and when the compound was administered orally (Druckrey et al., 1970a; Koestner et al., 1971).

The response to the transplacental route in the hamster was similar to that of the rat (Ivankovic & Druckrey, 1968). The morphology and growth of the tumours induced have been described (Kleihues et al., 1968; Thomas & Kersting, 1968; Wechsler et al., 1969; Grossi-Paoletti et al., 1970). Mothers, treated during pregnancy, developed predominantly

malignant tumours in the uterus and vagina late in life (Ivankovic, 1969; Alexandrov, 1969). These tumours were not produced by treatment of adult females.

The offspring of pregnant mice, given single i.p. injections in doses ranging from 0.25 to 1.0 millimoles/kg bw between the 12th and the 19th day of gestation, were found to have pulmonary tumours at 12 weeks of age (Rice, 1969).

Adenomas of the sweat glands (adenoma hidradenoides) and papillomas of the skin have been produced in litters of pigs whose mothers were injected i.v. on the 20th and the 31st day of gestation (Kupfer et al., 1969).

The typical transplacental carcinogenic effects of nitrosoethylurea have also been observed after feeding ethylurea and sodium nitrite to pregnant rats, indicating the in vivo formation of the carcinogen from both precursors (Ivankovic & Preussmann, 1970).

3.2 Metabolism in animals and man

The high chemical reactivity of nitrosoethylurea makes it unlikely that enzymatic metabolism is involved in the biological effects observed. No data on metabolism are available. In vitro and in vivo, the compound reacts as an alkylating agent (Loveless, 1969; Swann & Magee, 1970).

3.3 Observations in man

The compound can be formed from ethylurea by nitrosation in vivo (Ivankovic & Preussmann, 1970; Alexandrov & Jänisch, 1971). The kinetics of the reaction have been determined (Mirvish, 1971).

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Nitrosoethylurea is carcinogenic in all four animal species tested. The main target organs are the kidney, nervous system and lympho-reticular system. Tumours have been induced following different routes of administration, including single oral doses. Prenatal exposure to the substance has been shown to be particularly effective in producing tumours of the nervous system.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

4.2 Human data

The compound can be formed from ethylurea by nitrosation *in vivo*. No data on direct human exposure are available.

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Druckrey, H., Ivankovic, S. & Preussmann, R. (1966) Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. Nature (Lond.), 210, 1378

Druckrey, H., Landschütz, Ch. & Ivankovic, S. (1970a) Transplazentare Erzeugung maligner Tumoren des Nervensystems. II. Äthyl-nitrosoharnstoff an 10 genetisch definierten Rattenstämmen. Z.Krebsforsch., 73, 371

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N-METHYL-N,4-DINITROSOANILINE*

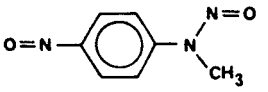
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 99809

N-Nitroso-N-methyl-4-nitroso-aniline; Methyl-(4-nitrosophenyl)nitrosamine; N,4-Dinitroso-N-methyl-aniline; Elastopar; Nitrozan K; N-Methyl-N-p-dinitrosoaniline

1.2 Chemical formula and molecular weight



$C_7H_7N_3O_2$ Mol. wt: 165.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Appears as green crystals on recrystallization from ethanol

(b) Melting-point: 101°C

(c) Solubility: Very slightly soluble in water

(d) Stability: Light-sensitive, so should be stored in the dark

(e) Technical products and impurities: Elastopar contains about 30% of the compound.

2. Use and Occurrence

(a) Use

The technical product with a content of about 30% has been used as a rubber additive (retarder with certain antioxidant and anti-flex-cracking properties). It is not known whether it is used any longer.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Weanling rats were given by gavage a commercial preparation

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* Considered by the Working Group in Geneva, December 1971.

containing 33% of N-methyl-N,4-dinitrosoaniline, at a dose of 0.1, 0.3, 1, 3, 10 or 30 mg per rat, 5 times a week for 52 weeks. The compound showed carcinogenic activity at 10 and 30 mg per rat. Tumours appeared in all groups in different organs, including breast, testicles, pituitary, thyroid and lung. The percentage of tumour-bearing animals at 10 and 30 mg per rat was approximately five times higher than that in the controls (Weisburger et al., 1966; Hadidian et al., 1968).

(b) Intraperitoneal administration

Rat: 5 mg/animal/week of the compound was administered i.p. once a week for 6 months to 24 rats (total dose, 600 mg/kg bw). Two local sarcomas, 1 hepatoma, 1 thymoma, 1 pancreatic adenoma and 1 pituitary tumour were seen; 1 hepatoma appeared also in the controls (Boyland et al., 1968).

3.2 Metabolism in animals and man

No data available to the Working Group.

3.3 Observations in man

No data available to the Working Group.

4. Comments on Data Reported and Evaluation

Although there is some evidence of carcinogenic activity of N-methyl-N, 4-dinitrosoaniline in the rat, the data available are considered insufficient for an evaluation.

5. References

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NATURAL PRODUCTS

AFLATOXINS*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Aflatoxin B_1 - Chem. Abstr. No.: 1162658; Name: Cyclopenta/c/furo/3', 2':4,5/furo/2,3-h/1/benzopyran-1,11-dione,2,3,6aα,9aα-tetrahydro-4-methoxy-

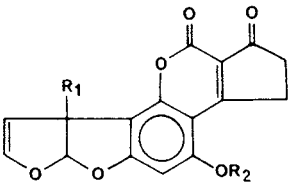
Aflatoxin B_2 - Chem. Abstr. No.: 7220817; Name: Cyclopenta/c/furo/3', 2':4,5/furo/2,3-h/1/benzopyran-1,11-dione,2,3,6aα,8,9,9aα-hexahydro-4-methoxy-

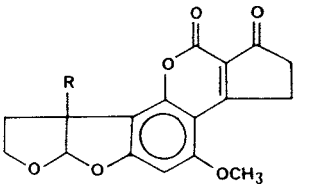
Aflatoxin G_1 - Chem. Abstr. No.: 1165395; Name: 1H,12H-Furo/3',2':4,5/furo/2,3-h/pyrano/3,4-c/1/benzopyran-1,12-dione,3,4,7a,10a-tetrahydro-5-methoxy-

Aflatoxin G_2 - Chem. Abstr. No.: 7241987; Name: 1H,12H-Furo/3',2':4,5/furo/2,3-h/pyrano/3,4-c/1/benzopyran-1,12-dione,3,4,7aα,9,10,10aα-hexahydro-5-methoxy-

Dihydroaflatoxin G_1

1.2 Chemical formula and molecular weight





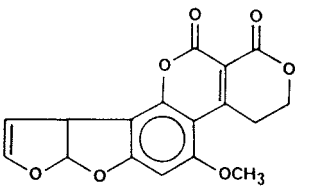
B_1: R_1=H; R_2=CH_3 Mol. wt: 312

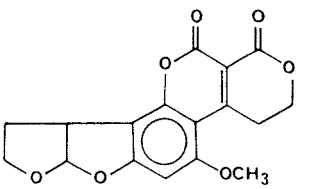
M_1: R_1=OH; R_2=CH_3 Mol. wt: 328

P_1: R_1=H; R_2=H Mol. wt: 298

B_2: R=H Mol. wt: 314

M_2: R=OH Mol. wt: 330





G_1 Mol. wt: 328

G_2 Mol. wt: 330

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* Considered by the Working Group in Geneva, December 1971.

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless to pale yellow crystals. Intensely fluorescent in ultraviolet light, emitting blue or yellow-green fluorescence, from which the designations "B" and "G" were derived.

(b) Melting-point; absorption spectroscopy: Data relating to these are given in the table below.

Aflatoxin	Mol. formula	Mol. wt	Melting-point (oC)	Ultraviolet absorption maxima (nm)	($\varepsilon x10^{-4}$)	Fluorescence emission max. (nm)
B_1	$C_{17}H_{12}O_6$	312	268-9	223 265 363	2.56 1.34 2.18	425
B_2	$C_{17}H_{14}O_6$	314	286-9	222 265 363	1.70 1.10 2.08	425
G_1	$C_{17}H_{12}O_7$	328	244-6	243 264 363	1.15 1.00 1.61	450
G_2	$C_{17}H_{14}O_7$	330	237-9	214 265 363	2.81 1.16 2.09	450
M_1	$C_{17}H_{12}O_7$	328	299	226 265 357	2.31 1.16 1.90	425
M_2	$C_{17}H_{14}O_7$	330	293	221 264 357	2.00 1.09 2.10	425
P_1	$C_{16}H_{10}O_6$	298	-	267 362	- -	-

(c) Identity and purity test: Purity can conveniently be determined by visual examination under ultraviolet light of fluorescence on chromatograms. Picogram quantities can be discerned under optimum conditions.

(d) Solubility: Very slightly soluble in water (10-20 µg/ml) and insoluble in non-polar solvents. Freely soluble in moderately polar organic solvents (e.g., chloroform and methanol), and especially in dimethylsulfoxide.

(e) Stability: Relatively unstable to light and air, particularly in solutions in highly polar solvents. Fluorescent and non-fluorescent degradation products appear upon brief exposure of chromatograms to light. Chloroform solutions are stable for years if kept in the dark and cold.

(f) Chemical reactivity: The lactone ring is susceptible to alkaline hydrolysis. Aflatoxins in natural products are only partially destroyed under ordinary cooking conditions, but can be totally destroyed by drastic treatment such as autoclaving in the presence of ammonia or by treatment with hypochlorite.

2. Use and Occurrence

(a) Analytical methods

Chemical assay methods are available for the detection and quantification of aflatoxins in various foods and foodstuffs at concentrations of 1-5 ppb or higher (Pons, 1969). Bioassays, mainly for confirmation of chemical assay results, have also been devised (Legator, 1969).

Modifications of methods originally developed for peanuts have been devised for use on other commodities, such as cottonseed meal, corn, etc. Methodology for determining aflatoxin M_1 in milk is under development.

(b) Occurrence

Aflatoxin-producing fungal strains appear to be ubiquitously distributed. Therefore, virtually every foodstuff or food product is potentially susceptible to contamination under conditions (particularly of moisture and of temperature) favouring fungal growth, which may occur at any stage of food production or subsequent processing. Some samples of nearly every major dietary staple have been found to contain some aflatoxin at one time or another. Adequate control measures, involving especially rapid post-harvest drying of crops and storage at moisture contents of less than 10% can virtually eliminate contamination.

Under inadequate conditions, contamination occurs in a given locality with great variability with regard to types of food affected, frequency of contamination and levels of aflatoxin present. However, the following generalizations are possible: (i) Aflatoxin B_1 is most frequently present in contaminated samples; B_2 and G_1 are present much less frequently and almost never in the absence of B_1; (ii) Dietary surveys in Uganda and in Thailand and Swaziland have revealed that peanuts, beans and corn were the principal vectors of aflatoxins, with many samples (up to 50% of market samples of peanuts) containing 0.1 ppm to 1 ppm aflatoxins; other grains such as rice were rarely contaminated (Alpert et al., 1971; Keen & Martin, 1971; Shank et al., 1972c).

In different regions of Murang'a (Kenya) mean aflatoxin levels of 0.121-0.351 µg per kg of food and of 0.05-0.167 µg per litre of beer have been detected (IARC, 1972).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Feeding of aflatoxin B_1 at 1 ppm to random-bred and inbred mouse strains for 70 weeks failed to induce tumours (Wogan, 1969a).

Rat: Since the first report of hepatoma induction in rats by peanut meal involved in the original aflatoxicosis episodes (Lancaster et al., 1961), many studies in rats have demonstrated the carcinogenic potency of aflatoxins for the liver of rats.

A linear dose-response relationship was observed in rats fed diets containing various amounts of aflatoxins for 294 to 384 days; 0.005 ppm failed to induce hepatomas (Newberne, 1965). When a diet containing 5 ppm aflatoxin B_1 was fed to rats for 1 to 9 weeks early in their lifetime, the liver tumour frequency rose from 0 to 100% in groups of 6-12 animals. In male rats fed 0.5 or 0.1 ppm aflatoxin for their lifetime, the frequency of liver tumours was 100% and 50% respectively for groups of 15-35 animals; the frequency was lower in females (Butler & Barnes, 1968).

In studies involving the feeding of purified aflatoxins to rats, it has been reported that aflatoxin B_1 at levels of 15 ppb added to a purified diet induced liver-cell carcinomas in 25/25 animals surviving for 68 to 80 weeks. Higher dietary levels induced tumours after shorter periods of treatment (Wogan & Newberne, 1967). In another experiment of similar design,

aflatoxin B_1 was fed at levels of 250, 500 and 1000 ppb to rats for 147 days; the animals were then kept without further treatment for their life-span. Yields of liver tumours were 62%, 72% and 86% in the three treated groups, respectively (Epstein et al., 1969). When aflatoxin B_1 was administered in the drinking water, it produced liver tumours in 19/30 rats given a total of 2 mg each and in 3/10 animals receiving a total dose of 1 mg each (Butler et al., 1969).

Aflatoxin B_1 caused mainly hepatocellular carcinomas in the above experiments. However, there are suggestions that it may also induce (in very low incidence) carcinomas of the glandular stomach (Butler & Barnes, 1966) and mucinous adenocarcinomas of the colon (Wogan & Newberne, 1967). One rat strain (Wistar) also displayed a high yield of renal epithelial neoplasias in response to highly purified aflatoxin B_1 (Epstein et al., 1969).

Aflatoxin G_1 is less potent than aflatoxin B_1 as a hepatocarcinogen for rats dosed orally and also induces kidney tumours at significant incidence (Butler et al., 1969; Wogan et al., 1971).

Aflatoxin B_2 is weakly active in inducing liver tumours in rats at doses more than 100 times higher than an effective dose of aflatoxin B_1 (Wogan et al., 1971).

Hepatocarcinogenesis caused in rats by aflatoxin feeding is enhanced by dietary deficiency of lipotropic agents (Rogers & Newberne, 1969, 1971) and by cirrhosis (Newberne et al., 1966). However, rats are partially or totally protected from aflatoxin carcinogenesis by simultaneous administration of diethylstilbestrol (Newberne & Williams, 1969) or phenobarbitone (McLean & Marshall, 1971), by dietary protein deficiency (Madhavan & Gopalan, 1968) or by hypophysectomy (Goodall & Butler, 1969).

Trout: Aflatoxin B_1 is hepatocarcinogenic to rainbow trout at very low dietary levels (Sinnhuber et al., 1968b). A linear dose-response curve exists over the range of dietary levels of 0 to 1.5 ppb of aflatoxin B_1 fed continuously for 20 months. The minimal effective dose was calculated to be about 0.1 ppb for a 10% tumour yield; no tumours occurred in control animals (Halver, 1969).

The response of rainbow trout to aflatoxins B_1 and G_1 is enhanced by the simultaneous feeding of cyclopropenoid fatty acids (Sinnhuber et al., 1968a). These acids have little, if any, modifying effect on the aflatoxin response in rats (Friedman & Mohr, 1968; Lee et al., 1969).

Aflatoxins G_1 and B_2 are carcinogenic to rainbow trout, but are less potent than B_1 (Ayres et al., 1971).

Duck: Continuous feeding of a diet containing 30 ppb aflatoxins derived from contaminated peanut meal induced liver tumours in 8/11 ducks after 14 months (Carnaghan, 1965).

Monkey: Few long-term feeding studies have been done in monkeys. In one such experiment, aflatoxin levels from 0.07 to 1.8 ppm failed to induce liver tumours in 6 males and 2 females that survived for 3 years. However, histological evidence of toxicity was observed in the livers of the survivors (Cuthbertson et al., 1967). In another study, a mixed aflatoxin preparation was given orally to Rhesus monkeys once per week at a level of 62 µg/kg bw. After 2 years, 5 surviving animals showed histological evidence of liver damage, but no tumours (Deo et al, 1970).

(b) Intratracheal administration

Rat: A mixture (crystalline) of aflatoxins B_1 and G_1 was suspended in peanut oil and administered intratracheally to rats in doses of 300 µg in 30 µl. Each of 6 rats was dosed twice weekly for 30 weeks and then held without further treatment up to 100 weeks. Three of the 6 animals developed squamous cell carcinomas of the trachea within 37 to 62 weeks, and 4/6 animals also developed hepatomas (Dickens et al., 1966).

(c) Subcutaneous administration

Mouse: Mice injected twice weekly with 10 µg of a mixture of aflatoxins B_1 and G_1 suspended in oil developed sarcomas at a frequency of 15/17 animals over a 76-week period (Dickens & Jones, 1965).

Rat: Injection of a mixture of aflatoxins B_1 and G_1 twice weekly at a dose of 2 µg per injection induced sarcomas in 5/6 rats in 44-69 weeks. Pure B_1, injected according to the same schedule at 20 µg per dose, induced sarcomas in 6/6 rats within 18-37 weeks; G_1 was less potent (Dickens & Jones, 1965).

(d) Intraperitoneal administration

Mouse: Administration of aflatoxin B_1 to strain A mice in 12 thrice-weekly doses up to a total average dose of 5.6 mg per animal produced an average of 5.6 primary pulmonary adenomas in 14/14 animals in 24 weeks after the first treatment; no tumours occurred in control animals (Wieder et al., 1968).

Rat: Aflatoxin B_1 dissolved in dimethylsulfoxide was as potent in inducing hepatocellular carcinomas in rats when injected intraperitoneally as when administered by stomach tube (Wogan et al., 1971).

(e) Other experimental systems

In vitro studies: Studies on the effects of aflatoxins on human embryo and adult liver cells in vitro have demonstrated that the order of toxicity is $B_1 > G_1 > B_2$. Aflatoxin B_1 is considerably less toxic to adult than to embryo cell lines (Sullman et al., 1970). Transformation in vitro by aflatoxin B_1 in newborn rat liver cells has been demonstrated (Toyoshima et al., 1970).

3.2 Metabolism in animals and man

(a) Animals

Several features of the metabolism of aflatoxins in animals have been described (Wogan, 1969b). Two types of metabolic transformation are known to occur and their products have been chemically identified.

Aflatoxins M_1 and M_2, resulting from ring hydroxylation, were isolated and chemically identified from sheep urine (Holzapfel et al., 1966) and subsequently from cows' milk (Masri et al., 1967). M_1 appears in the urine of all species after dosing with B_1, in general accounting for about 1-4% of the administered dose in 24 hours (Wogan, 1969b).

Aflatoxin P_1, the 0-demethylated derivative of B_1, was recently identified as the major urinary metabolite of B_1 in Rhesus monkeys, in which it accounts for about 20% of an injected dose in 24 hours. It is present mainly as glucuronide or sulfate conjugates (Dalezios et al., 1971).

(b) Man

Aflatoxin M_1 is also present in the urine of humans who ingest aflatoxin-contaminated foods (Campbell et al., 1970).

(c) Carcinogenicity of metabolites

Aflatoxin M_1 is carcinogenic for rainbow trout, and has approximately the same potency as B_1 in this species (Sinnhuber et al., 1970). Although its carcinogenicity to rats has not yet been evaluated, M_1 is equally toxic to B_1 in this species (Pong & Wogan, 1971).

No evidence is available on the carcinogenicity of aflatoxin P_1.

3.3 Observations in man

(a) Case reports

A number of episodes have occurred in which circumstantial evidence suggests the possibility of aflatoxin involvement in acute toxicoses in humans. Several of the earlier reports have been summarized by Kraybill & Shimkin (1964).

A fatal case was recently reported in which acute hepatic disease with histological changes in the liver that were identical with those seen in aflatoxin poisoning in monkeys, and where circumstantial evidence suggested aflatoxin involvement (Serck-Hanssen, 1970).

More recently, it was found that the tissues and body fluids of Thai children dying from an acute syndrome of unknown etiology contained substantial quantities of unmetabolized aflatoxin B_1. Although a causal relationship was not established, pathological findings in the liver and other tissues resembled those induced in monkeys by aflatoxin B_1 (Shank et al., 1971).

Two recent reports suggest that aflatoxins may play a role in an acute toxicity syndrome in Thailand (Bourgeois et al., 1971) and may contribute to the development of cirrhosis in Indian children with kwashiorkor (Amla et al., 1971). (In both instances agents other than aflatoxin may have been responsible for the observed effects.)

In none of the above cases is the information adequate to estimate effective doses for humans.

(b) Epidemiology

Published epidemiological studies have consisted of estimates of aflatoxin intake by populations in which the incidence or prevalence of primary liver cancer was simultaneously determined.

In one such study in Uganda, the frequency of aflatoxin contamination of market food samples was associated with liver cancer incidence in localized population groups (Alpert et al., 1971).

A study in Thailand showed large differences in aflatoxin intake (estimated by direct measurement in food samples as eaten) to be correlated with incidence in populations in which there was a threefold difference in liver cancer incidence (Shank et al., 1972a).

A preliminary report of a current survey of three areas of different altitude in the Murang'a district in Kenya relates the mean amount of aflatoxin ingested to the incidence of primary liver cancer in these areas (IARC, 1972). The results are given in the accompanying table.

Altitude sub-area	High		Middle		Low	
Sex	M	F	M	F	M	F
Mean aflatoxin ingested* (µg/kg bw per day)	4.88	3.46	7.84	5.86	14.81	10.03
Primary liver cancer cases (≥16 years old; 1967-70)	1	-	13	6	15	9
Incidence rates (per 10^5 per annum)	3.11	0.00	10.80	3.28	12.12	5.44

* Calculated on the assumption of a 2-kg intake of food and a 2-litre intake of beer (men only) per day and an average adult body-weight of 70 kg.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Aflatoxins B_1 and G_1 are carcinogenic in four animal species, inducing tumours of the liver and some other organs following several routes of administration, including oral exposure. In the only feeding study in the mouse, this species appeared to be resistant to the carcinogenic effect observed in other species. No tumours have been observed in the monkey following several years of dosing: experiments are still in progress.

Aflatoxin M_1 produced liver tumours in the trout and aflatoxin B_2 in the rat, but only at doses more than 100 times higher than B_1.

4.2 Human data

Considerable evidence is now available to indicate that market samples of some food commodities in some countries often contain aflatoxins. Increased frequency of liver cancer has been recorded in populations consuming diets contaminated by aflatoxins and possibly other mycotoxins, but no causal relationship has been established.

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CYCASIN*

The biologically active part of cycasin is its aglycone methylazoxymethanol. Data on this metabolite are given in section 3.2 (b). The chemically related methylazoxymethanol acetate has been synthesized and tested (see Appendix).

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 14901087; Name: Glucopyranoside, (methyl-ONN-azoxy)-methyl-β-D-(Methyl-ONN-azoxy)-methyl-β-D-glucopyranoside; β-D-Glucosyloxy-azoxymethane; Methylazoxymethanol-β-D-glucoside; β-D-Glucosyloxy-azoxymethase

1.2 Chemical formula and molecular weight

$CH_3-\underset{\underset{O}{\downarrow}}{N}=N-CH_2OC_6H_{11}O_5$ $C_8H_{16}N_2O_7$ Mol. wt: 252.23

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless long needles

(b) Melting-point: 144-145°C (Decomp.) (Nishida et al., 1955)
154°C (Decomp.) (Riggs, 1956)

(c) Optical rotation: $[\alpha]_D^{18}$ - 46.5° (Wells et al., 1968)

(d) Absorption spectroscopy: λ_{max} 218 nm; log ε 3.86
Inflexion 278 nm; log ε approximately 1.7 (Riggs, 1956)

(e) Solubility: Readily soluble in water and dilute ethanol; sparingly soluble in absolute ethanol; insoluble in benzene, acetone, chloroform, and ethyl acetate (Nishida et al., 1955)

(f) Chemical reactivity: Easily hydrolysed, especially under alkaline conditions, to yield nitrogen, formaldehyde and methanol among other products.

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* Considered by the Working Group in Geneva, December 1971.

2. Use and Occurrence

(a) Analytical methods

A method for the quantitative determination by gas-liquid chromatography after cilylation is described by Wells et al. (1968). Other methods are a chromotropic acid method (Matsumoto & Strong, 1963) and a paper chromatographic modification of the latter (Dastur & Palekar, 1966).

(b) Occurrence

Cycasin occurs in the seeds, roots and leaves of cycad plants (family Cycadaceae), which are found in the tropical and subtropical regions of the world, and was identified by Nishida et al. (1955) in the seeds of the Japanese cycad Cycas revoluta Thunb., and by Riggs (1956) in the seeds of Cycas circinalis L., a cycad indigenous to Guam (Mariana Islands). The amount of cycasin present in cycad nuts depends on the species of cycad. In ground and dried nuts of Cycas circinalis, 0.02% and 2.3% cycasin was found depending on the drying method used, 0.02% being obtained by air drying and 2.3% by rapid vacuum drying. Nuts prepared in Guam (leached with water, then sun-dried) contained only 0.02% (Matsumoto & Strong, 1963). A 2.3% level was also found in unwashed vacuum-dried nuts (Campbell et al., 1966). Using different extraction methods, 1% was detected after boiling with water for 20 minutes and 0.6% by washing for 8 days prior to boiling for 20 minutes. No cycasin was detected in chips of dried kernels of cycad nuts prepared in Guam and used as food by Guamanians. The sensitivity of the method used was of the order of 100 ppb (Palekar & Dastur, 1965). The wide range of levels found is due to the fact that boiling (during the extraction procedure) diminishes the cycasin-destroying enzyme (emulsin) which results in a higher cycasin yield. Washing and soaking, on the other hand, activates the enzyme (Dastur & Palekar, 1966).

Starch making from cycad nuts is both a home and a commercial industry. On Oshima (Ryukyu Islands), the annual harvest of cycad seeds for starch making in 1925 was estimated at 230 tons; 90% of this was consumed on the island and the remainder was shipped to Okinawa and Japan. Cycad starch from nuts of Cycas circinalis and Cycas revoluta is used on the Mariana Islands, on the Ryukyu Islands (Japan), and in Indochina, India and Africa. In the same geographical areas the seeds are prepared in different ways and used as medicine both externally and internally (Whiting, 1963).

The biologically active part of cycasin is its aglycone, methylazoxymethanol, which is identical with the aglycone of macrozamin (β-primeverosyloxyazoxymethane), isolated by Cooper (1941) from the seeds of an Australian cycad, Macrozamia spiralis. Macrozamin occurs in the seeds of a number of Australian cycads (Riggs, 1954).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Adult animals received by stomach tube single doses of 0.3, 0.5 and 1.0 mg/g bw: 4 mice out of 35 that survived more than 4 months developed tumours of the liver (hepatoma), lung (adenoma), kidney (adenoma) and a fibroma of the back; no mention is made of control animals (Hirono et al., 1969).

Rat: Long-term feeding of 1-3% cycad meal in the diet mainly induced benign and malignant tumours in the liver (hepatocellular carcinoma and reticulo-endothelial tumours) and kidney adenomas. One lung adenoma and 2 adenocarcinomas of the large intestine were also observed (Laqueur et al., 1963). Feeding of 200 and 400 ppm of pure cycasin or of cycad meal containing 2.3% cycasin for 6-9 months produced tumours in the same organs. Intestinal carcinomas were also produced by short-term exposure (2-21 days) to the same material (Laqueur, 1965). Long-term feeding of unprocessed cycad husk, fresh or dried, at 0.5% and 1% in the diet, produced kidney tumours, hepatomas and liver carcinomas (Yang et al., 1968). Higher concentrations (5% and 10%) of fresh and dried cycad husk in the diet for 10-250 days produced carcinomas, sarcomas and Wilms' tumours of the kidney and cholangiomas and hepatomas of the liver (Hoch-Ligeti et al., 1968). Feeding for 23 months of a home-made Guamanian cycad flour, intended for human consumption, in concentrations of 1.5%, 5% and 10% of flour in the diet did not induce a significant increase in the tumour rate after 715 days as compared with untreated controls. Fifteen animals were used in each dose group (Yang et al., 1966).

Single-dose experiments with weanling rats produced tumours of the kidney, intestine, liver, lung and brain in that order of frequency in animals that survived more than 6 months. Tumour yields were: at 100 mg/kg bw, 4/13; 250 mg/kg bw, 13/3; 500 mg/kg bw, 6/6; 750 mg/kg bw, 0/1 and 1000 mg/kg bw, 2/3 (Hirono et al., 1968). A preliminary report describes the induction of mammary cancer with 4 mg/kg bw daily (Kawaji et al., 1968). The morphology of cycasin-induced kidney tumours has been described (Gusek et al., 1966, 1967; Gusek & Mestwerdt, 1969).

Forty-one germ-free male rats received 200 mg per 100 g bw of cycasin in a germ-free diet for 20 days, after which they were returned to normal diet. Five animals were killed on the 21st day; 26 animals survived longer than one year, the oldest being 772 days old at autopsy. Seven animals showed neoplasms, but according to the authors they were not related to the treatment. No parallel control animals were kept (Laqueur et al., 1967).

The flour prepared from nuts of Encephalartos hildebrandtii, a plant of the family Cycadaceae, produced liver, kidney and lung tumours when fed to rats. The acute toxicity as well as the carcinogenic activity observed was very similar to that produced by cycasin (Mugera & Nderito, 1968a, 1968b; Mugera, 1969). (Although cycasin was not identified, it is likely that it or another methylazoxymethanol glycoside is responsible for the above-mentioned effects.)

Hamster: Adult animals received by stomach tube single doses of 0.15 and 0.1 mg/g bw. Eighteen out of 41 hamsters that survived more than 150 days developed tumours of the liver (adenoma, bile-duct carcinoma and haemangioendothelial sarcoma), the lung (adenoma), the intestine and the kidney. Some malignant lymphomas were observed. Those receiving the larger single dose had about twice as many tumours. Feeding of 2 to 4 doses of 0.1 mg/g bw produced tumours in 17 out of 37 animals. No difference in tumour frequency between single and repeated administration was seen (Hirono et al., 1971).

Guinea-pig: Repeated feeding of 5% cycad meal in the diet for two to three 5-day periods produced hepatocellular carcinomas and bile-duct tumours (Spatz, 1964).

Fish: Feeding with cycad meal or addition of cycasin to the tank water produced malignant neoplasms of the liver in the aquarium fish, Brachydanio rerio (Stanton, 1966).

Chicken: Feeding of 0.5% or 1% kernel and husk for 28 and 68 weeks did not produce tumours attributable to cycasin treatment (Sanger et al., 1969).

(b) Skin application

Mouse: Multiple applications with aqueous extract of cycad nut on an artificially ulcerated skin produced tumours of the liver (haemangiomas, hepatomas), adenomas of the kidney and one subcutaneous haemangioma of the site of application (O'Gara et al., 1964).

(c) Other experimental systems

Newborn animals: Subcutaneous administration of single doses of 0.5 and 1.0 mg/g bw to newborn mice produced lung tumours in more than 80% and liver tumours in 40-60% of the animals that survived longer than 150 days (Hirono et al., 1969; Hirono & Shibuya, 1970). Intraperitoneal injection of a single dose of 2.5 mg per animal into newborn rats produced tumours of the kidney, the liver, the intestine, the lung and the brain in 83.6% of the animals (Hirono et al., 1968). Newborn hamsters received single s.c. injections of 0.2, 0.4 and 0.6 mg/g bw. Out of 73 animals that survived longer than 150 days, 24 developed tumours which were almost exclusively confined to the liver (Hirono et al., 1971).

Transplacental route: Crude cycad meal containing 3% cycasin was fed to pregnant rats at various stages of gestation. The overall tumour incidence in the offspring was 18.5%. Frequent sites of neoplasia were the brain and jejunum, organs rarely involved in feeding experiments. Five out of 9 mothers who stayed alive also developed tumours, mainly in the kidney, but also in the liver, colon, ovaries, thymus and retroperitoneum (Spatz & Laqueur, 1967).

3.2 Metabolism in animals and man

(a) Animals

Cycasin is carcinogenic in adult rats only when given orally. The bacterial flora of the intestine determines the toxicity and carcinogenicity of cycasin by splitting it with bacterial β-D-glucosidase to form the proximate carcinogen methylazoxymethanol (Kobayashi & Matsumoto, 1965; Laqueur & Spatz, 1968). Enzymatic hydrolysis to methylazoxymethanol is

also possible in the subcutaneous tissue of newborn rats, which contains a glucosidase in early post-natal life that disappears after the 25th day of life (Spatz, 1968).

Alkylation of RNA and DNA by methylazoxymethanol in vitro was described by Matsumoto & Higa (1966). Nucleic acids have also been alkylated in vivo by cycasin (Shank & Magee, 1967).

(b) Carcinogenicity of metabolites

Methylazoxymethanol

Oral administration: Feeding of a lipid-soluble fraction of dried cycad nut, reported to contain methylazoxymethanol, to 3 rats for 9 months produced hepatomas in 2 of the animals (Matsumoto & Strong, 1963).

Subcutaneous administration: Repeated injections of 2, 4 and 6 mg of methylazoxymethanol once a week resulted in a variety of rat neoplasms, principally of the liver, kidney and intestinal tract (Laqueur & Matsumoto, 1966). In hamsters, repeated injections of 20 mg/kg bw methylazoxymethanol produced adenocarcinomas of the gall-bladder and multiple carcinomas of the colon (Spatz et al., 1969).

Intravenous administration: A single injection of 20 mg/kg bw methylazoxymethanol into hamsters produced multiple cystadenomas and other tumours of the liver and adenomas and adenocarcinomas of the colon (Spatz et al., 1969).

3.3 Observations in man

(a) Epidemiology

The mortality rate from cancer (1961 to 1966) in the Miyako Islands, Okinawa, where during 1959 the natives subsisted mainly on cycads, shows no significant difference in hepatoma death rates in comparison with the interior of Japan. The death rate from cirrhosis was higher, but results from each district of the island were not related to high cycad intake in 1959. However, the natives used this plant as food long before 1959 (Hirono et al., 1970).

4. Comments on Data Reported and Evaluation

4.1 Animal data

Cycasin is carcinogenic in 5 animal species, inducing tumours in various organs. Following oral exposure, it is carcinogenic in the rat, hamster, guinea-pig and fish. By this route, the data in the mouse is of borderline significance and the negative experiment in chickens only lasted 68 weeks. It is active in single-dose experiments and following prenatal exposure. The carcinogenicity of its metabolite, methylazoxymethanol, has been demonstrated in the rat and the hamster and that of a closely related synthetic substance, methylazoxymethanol acetate, in the rat (see Appendix).

4.2 Human data

Nuts prepared in Guam in the usual way (leached with water and sun-dried) were reported still to contain 160 ppb of cycasin. In another report, chips of dried kernels of cycad nuts did not contain cycasin. The epidemiological study in the Miyako Islands involved a follow-up after heavy exposure in 1959 for the years 1961 to 1966, which may have been too short for a carcinogenic effect to be observed. However, there was also chronic exposure prior to 1959 in this population. It is noteworthy that an increased mortality from cirrhosis was observed. This negative result concerning cancer in the only epidemiological study performed to date is insufficient to exclude a possible carcinogenic effect of cycasin on man.

Appendix

Methylazoxymethanol Acetate

A. Chemical and physical data

(a) Synonyms and trade names

MAM acetate

(b) Chemical formula and molecular weight

$CH_3-N=N(\rightarrow O)-CH_2OCOCH_3$ $C_4H_8N_2O_3$ Mol. wt: 132.12

(c) Chemical and physical properties of the pure substance

Description: A colourless liquid

Boiling-point: 191°C

Absorption spectroscopy: λ_{max} 215 nm; log ε 3.929

Inflexion 270 nm (Kobayashi & Matsumoto, 1965)

Chemical reactivity: Easily hydrolysed, especially under alkaline conditions, to yield nitrogen, formaldehyde and methanol among other products.

B. Biological data

(a) Oral administration

Rat: In germ-free rats, treatment with methylazoxymethanol acetate for 2-3 weeks with a cumulative dose of between 12.5 and 13.7 mg per rat produced carcinomas of the colon and rectum, bile-duct adenomas, liver-cell adenomas, hepatomas and sarcomas of the liver and kidney tumours (adenomas, nephroblastomas, interstitial tumours) (Laqueur et al., 1967).

(b) Subcutaneous administration

Rat: A total dose of 12.5 mg methylazoxymethanol acetate given over 21 days produced malignant tumours of the intestine and the liver in germ-free rats; no subcutaneous tumours were reported (Laqueur et al., 1967).

(c) Intraperitoneal administration

Rat: Four intraperitoneal injections over a period of 21 days (total dose, 12.5 mg of methylazoxymethanol acetate) produced predominantly tumours of the intestine and the liver in germ-free rats (Laqueur et al., 1967).

(d) Other experimental systems

Intravenous administration: A single dose of 35 mg/kg bw methylazoxymethanol acetate given to rats produced intestinal and liver tumours after 6-7 months (Zedeck et al., 1970).

(e) Metabolism

Nucleic acids were alkylated *in vivo* by methylazoxymethanol acetate (Nagata & Matsumoto, 1969).

5. References

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SAFROLE, ISOSAFROLE AND DIHYDROSAFROLE*

1. Chemical and Physical Data

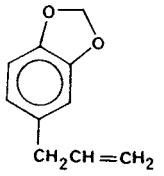
Safrole

1.1 Synonyms and trade names

Chem. Abstr. No.: 94597; Name: Benzene, 4-allyl-1,2-(methylenedioxy)-

4-Allyl-1,2-(methylenedioxy)-benzene; Allylcatechol methylene ether; Allyldioxybenzene methylene ether; 1-Allyl-3,4-methylenedioxybenzene; m-Allylpyrocatechin methylene ether; 4-Allyl-1,2-methylenedioxybenzene; 4-Allylpyrocatechol formaldehyde acetal; Allylpyrocatechol methylene ether; 3,4-Methylenedioxy-allylbenzene; Shikimol; Shikimole; Safrol; Safrole MF

1.2 Chemical formula and molecular weight



$C_{10}H_{10}O_2$ Mol. wt: 162.18

1.3 Chemical and physical properties of the pure substance

(a) Description: A colourless or slightly yellow liquid, with an odour of sassafras

(b) Boiling-point: 232-234°C; solidification ca. 11°C

(c) Density: 1.096 (20°C)

(d) Refractive index: n_D^{20} 1.5383

(e) Solubility: Insoluble in water; very soluble in ethanol; miscible with ether and chloroform

Isosafrole

1.1 Synonyms and trade names

Chem. Abstr. No.: 120581; Name: Benzene, 1,2-(methylenedioxy)-4-propenyl-

3,4-Methylenedioxy-1-propenyl benzene; 4-Propenyl-1,2-methylenedioxy-benzene; 1,2-Methylenedioxy-4-propenylbenzene

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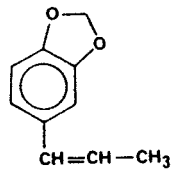
* Considered by the Working Group in Geneva, December 1971.

1.2 Chemical formula and molecular weight



$C_{10}H_{10}O_2$ Mol. wt: 162.18

1.3 Chemical and physical properties of the pure substance

(a) Description: A colourless liquid, with an odour of anise

(b) Boiling-point: 127-128°C (15 mm)

(c) Density: 1.122 (20°C)

(d) Refractive index: n_D^{20} 1.5777

(e) Solubility: Insoluble in water; miscible with many organic solvents

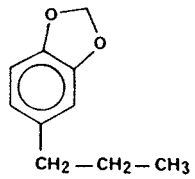
Dihydrosafrole

1.1 Synonyms and trade names

Chem. Abstr. No.: 94586; Name: Benzene, 1,2-(methylenedioxy)-4-propyl-

1,2-(Methylenedioxy)-4-propylbenzene; Safrole, dihydro-;
4-Propyl-1,2-(methylenedioxy) benzene

1.2 Chemical formula and molecular weight



$C_{10}H_{12}O_2$ Mol. wt: 164.18

1.3 Chemical and physical properties of the pure substance

(a) Description: An oily liquid

(b) Boiling-point: 228°C

(c) Density: 1.0695 (20°C)

(d) Refractive index: n_D^{25} 1.5187

(e) Solubility: Miscible with ethanol, ether, acetic acid and benzene

2. Use and Occurrence

(a) Use

Safrole and isosafrole have been used in perfumery, in soap manufacture, as a flavouring agent in drugs and in the manufacture of heliotropin. Data on the extent of these uses at present are not available to the Working Group. Safrole, isosafrole and dihydrosafrole have been used as flavouring agents in soft drinks and root beer. In the latter up to 26.7 ppm of safrole was found (Wilson, 1959). Its use for such a purpose has been prohibited in the USA since 1960.

(b) Analytical methods

Sensitive and specific analytical methods for determining the safrole content of biological materials are available. These utilize either combined liquid and gas-liquid chromatography (e.g., Russell & Jennings, 1969) or spectral properties of the compound (e.g., Wilson, 1959).

(c) Occurrence

Safrole is a constituent of several essential oils. Sassafras oil contains up to 93% safrole (Gemballa, 1958), while it is present in lesser quantities in essential oils from nutmeg, mace, ginger, star anise, cinnamon and black pepper. Its content in the latter is usually in the range of <1% to 10% of the oil (Bejnarowicz & Kirch, 1963; Cook & Howard, 1966; Itty & Nigam, 1966; Furia & Bellanca, 1971).

Isosafrole occurs naturally as a principal component of essential oil of star anise and also at lower quantities in essential oils of other spices. The distribution is generally similar to that of safrole. No data on the occurrence of dihydrosafrole are available to the Working Group.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: When administered to inbred mice by stomach tube (464 mg/kg daily) for 4 weeks and then in the diet at 1112 ppm for 78 weeks, safrole induced hepatomas in 27/33 mice. Isosafrole administered at lower doses (215 mg/kg then 517 ppm) induced hepatomas in 6/34 mice, and dihydrosafrole at a dosage of 464 mg/kg and then 1400 ppm induced them in 10/34 animals. No liver tumours occurred in control animals (Innes et al., 1969).

Rat: Liver adenomas were induced in rats fed 0.1% or 1.0% safrole in a diet deficient in riboflavine, tocopherol and protein (Homburger et al., 1961). Fourteen out of 50 rats fed for two years a diet containing 0.5% safrole developed malignant liver tumours, and rats fed 0.1% safrole also developed liver tumours, but at a lower frequency (Long et al., 1963). These findings were confirmed (Hagan et al., 1965). Under comparable conditions dihydrosafrole induced tumours of the oesophagus, but not a significant number of liver tumours (Long & Jenner, 1963; Hagan et al., 1965). Isosafrole fed at 0.5% in the diet induced liver tumours in a few, but insignificant number of animals.

(b) Subcutaneous administration

Mouse: Infant Swiss albino mice were injected subcutaneously with a suspension of safrole in tricaprylin on days 1, 7, 14, and 21 after birth. In males that received a total dose of 0.66 mg, 6/12 animals developed hepatomas within 49-53 weeks; at a total dose of 6.6 mg, 18/31 animals developed hepatomas. The latter animals also developed pulmonary adenomas and pulmonary adenocarcinomas (16%). The frequency of lung tumours in controls was 0%. The hepatoma frequency in 78 solvent-injected controls was 5-6% (Epstein et al., 1970).

3.2 Metabolism in animals and man

(a) Animals

Metabolism of safrole, isosafrole and dihydrosafrole by rats has been investigated by thin-layer chromatography.

Metabolites were excreted in bile and urine with a slow and prolonged pattern (Fishbein et al., 1967). In recent attempts to identify the carcinogenic form of safrole, a new metabolite (1'-hydroxy-safrole) was identified in rat urine (Borchert et al., 1971). Safrole, isosafrole and dihydrosafrole have been shown experimentally to be active synergists for pyrethrum and 1-naphthyl methyl carbamate (Sevin) in common with other methylene-dioxyphenyl compounds. As a class, these substances act as competitive inhibitors of microsomal mixed-function oxidases in houseflies (Esaac & Casida, 1969).

3.3 Observations in man

No data available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Safrole and isosafrole are liver carcinogens for the mouse and the rat when administered orally or subcutaneously. Dihydrosafrole is carcinogenic for the oesophagus of the rat following oral administration.

4.2 Human data

Man may ingest small amounts of safrole and isosafrole through essential oils in which they occur.

No epidemiological data are available on the effects of human exposure.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

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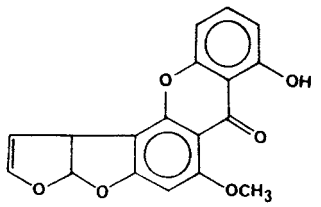
STERIGMATOCYSTIN*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 10048132; Name: 7h-Furo[3',2':4,5]furo[2,3-c]xanthen-7-one,3a,12C-dihydro-8-hydroxy-6-methoxy-

1.2 Chemical formula and molecular weight



$C_{18}H_{12}O_6$ Mol. wt: 324

1.3 Chemical and physical properties of the pure substance

(a) Description: Pale yellow needles

(b) Melting-point: 246°C (Decomp.)

(c) Absorption spectroscopy: λ_{max} 205, 233, 246 and 325 nm (log ε 4.40, 4.49, 4.53 and 4.21 respectively)

(d) Solubility: Insoluble in water and strong aqueous alkali; sparingly soluble in most organic solvents; readily soluble in chloroform, pyridine and dimethylsulfoxide

(e) Chemical reactivity: Forms deep yellow colour with aqueous NaOH and dark green-brown colour with H_2SO_4. Emits orange-red fluorescence in ultraviolet light.

2. Use and Occurrence

(a) Analytical methods

Chemical assay methods based on extraction have been reported and clean-up followed by quantitation by fluorescence on thin-layer chromatoplates (Holzapfel et al., 1966; Vorster & Purchase, 1968).

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* Considered by the Working Group in Geneva, December 1971.

(b) Occurrence

Produced in laboratory cultures of some strains of Aspergillus versicolor (Vuillemin) Tiraboshi, A. nidulans (Eidam) Wint., and a Bipolaris (Holzapfel et al., 1966). Although fungi are widely distributed on food grains, studies to detect the substance in food gave negative results, but they were not published.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: When sterigmatocystin was administered by stomach tube or in the diet at doses of 0.15-2.25 mg per rat per day for 52 weeks, 39/50 animals that survived to week 42 eventually developed hepatocellular carcinomas (Purchase & Van der Watt, 1970).

(b) Subcutaneous administration

Rat: Sterigmatocystin suspended in peanut oil was injected twice weekly into groups of 6 rats; dosing continued for 24 weeks. Each rat received a total dose of 24 mg (0.5 mg per injection). Three of 6 animals had developed sarcomas at the injection site by 65 weeks, the earliest tumour appearing at 47 weeks. One animal also developed a hepatoma and one a cholangioma. No tumours occurred in control animals (Dickens et al., 1966).

3.2 Metabolism in animals and man

No data available to the Working Group.

3.3 Observations in man

Comparison was made between sterigmatocystin-produced lesions in the rat and the pathology of hepatitis in Africans of Mozambique. Many similarities were observed, but it is difficult to attribute liver disease in the Bantu to any single agent in their environment. The authors concluded that their observations "are not incompatible with the theory that mycotoxins may be involved". They stated that it was not known whether sterigmatocystin was present in the Bantu diet (Torres et al., 1970).

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Sterigmatocystin is carcinogenic in the rat. It has produced liver tumours following oral administration and local sarcomas following subcutaneous administration.

4.2 Human data

Sterigmatocystin-producing fungi are known to be present in food grains, but surveys of human foods have not produced evidence of human exposure.

5. References

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

MISCELLANEOUS

N-[4-(5-NITRO-2-FURYL)-2-THIAZOLYL]ACETAMIDE*

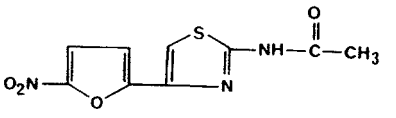
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 531828; Name: Acetamide, N-[4-(5-nitro-2-furyl)-2-thiazolyl]-

2-Acetamido-4-(5-nitro-2-furyl)thiadiazole; 2-Acetamido-4-(5-nitro-2-furyl)thiazole; Thiazole, 2-acetamido-4-(5-nitro-2-furyl)-; 2-Acetamino-4-(5-nitro-2-furyl)thiazole; Thiazole, 2-acetamino-4-(5-nitro-2-furyl)-; 2-Acetylamino-4-(5-nitro-2-furyl)thiazole; Thiazole, 2-acetylamino-4-(5-nitro-2-furyl)-; Furathiazole; Furium; NFTA

1.2 Chemical formula and molecular weight



$C_9H_7O_4N_3S$ Mol. wt: 253.22

1.3 Chemical and physical properties of the pure substance[1]

(a) Description: A dark yellow crystalline powder

(b) Melting-point: Between 270°C and 280°C

(c) Solubility: Insoluble in water; very slightly soluble in ethanol; moderately soluble in methanol; soluble in dimethylformamide and dimethylacetamide

(d) Absorption spectroscopy: Peaks at 226, 250 and 273 nm in methanolic solution and a peak at 385 nm in dimethylformamide solution; in thin-layer chromatography, has an Rf of 0.5.

(e) Technical products and impurities: Commercial N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA) was obtained from the manufacturer; no impurities were detected by infra-red spectroscopy, ultraviolet absorption spectroscopy or paper chromatography (Ertürk et al., 1970a).

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* Considered by the Working Group in Geneva, December 1971.

[1] Data from the manufacturer.

2. Use and Occurrence

(a) Use

NFTA has been marketed as an antibiotic for infections of the urinary tract. Dosages recommended by the manufacturer range between 100 and 400 mg per man per day for periods of several days. Its use is believed to be small.[1]

(b) Analytical methods[2]

NFTA in dimethylformamide and methanol solution (1 to 9) can be determined by chromatography on silica gel using benzene ethyl acetate (1 to 1) as eluent and a 0.5% solution of sodium fluorescein as developer under UV max. 385 nm. Quantitation can be done spectrophotometrically: $\varepsilon^{1\%}_{1cm} = 570$.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: A diet with 1000 ppm NFTA for 14 weeks produced generalized lymphosarcomas (lymphocytic leukaemias) in at least 50% of the survivors in Swiss, BALB/c, RF and C3H mice. Several tumours of the forestomach were also seen, particularly in Swiss mice. No tumours were seen in the controls except in 3/16 RF mice. In a dose-response study Swiss mice were given dietary concentrations of 100, 250, 500 and 1000 ppm NFTA for 14 weeks (total doses: 60, 160, 350 and 630 mg per mouse respectively); leukaemia occurred in 7/14 at the lowest and in 8/9 at the highest dose level. The frequency of stomach tumours was highest (6/9) at 1000 ppm (Cohen et al., 1970).

Rat: Female rats were given 1990 ppm NFTA in the diet (100-220 mg/kg per day) for 46 weeks followed by 20 weeks of control diet. Among 56 animals that survived for 16 or more weeks, 52 developed 67 tumours, including 24 benign and 23 malignant mammary tumours, 6 salivary gland adenocarcinomas, 7 alveolar cell carcinomas of the lung and 7 tumours at other sites. The first mammary tumour was detected at 16 weeks. Hyperplasia of the epithelium of the renal pelvis was seen in many animals and

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[1] Data from Chemical Information Services, Stanford Research Institute.

[2] Data from the manufacturer.

2 of them developed transitional cell carcinoma. No tumours were seen among 40 untreated controls (Ertürk et al., 1970b).

Dog: Two dogs given NFTA at a dosage of approximately 50 mg/kg per day for 30 months and observed for 3-5 additional months developed both gall-bladder adenomas and mammary fibroadenomas. Hyperplasia of the transitional epithelium of the renal pelvis was also recorded. No tumours were seen in one control dog (Ertürk et al., 1970a).

3.2 Metabolism in animals and man

NFTA is probably enzymatically reduced prior to binding to SH-groups of protein (Wang et al., 1971), but it did not react with glutathione in the presence of rat liver homogenate (Boyland & Speyer, 1970).

3.3 Observations in man

No data available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

N-/4-(5-nitro-2-furyl)-2-thiazolyl/acetamide (NFTA) given orally produced tumours of the forestomach in mice and mammary gland, lung and other tumours in rats. Two dogs given NFTA orally developed tumours.

4.2 Human data

The major source of exposure is its use in therapeutics. There is no information on the long-term effects of NFTA in humans.

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[1] See also the section "Extrapolation from animals to man" in the introduction to this volume.

5. References

Boyland, E. & Speyer, B.E. (1970) Enzyme-catalysed reactions between some 2-substituted 5-nitrofuran derivatives and glutathione. Biochem. J., 119, 463

Cohen, S.M., Ertürk, E. & Bryan, G.T. (1970) Production of leukemia and stomach neoplasms in Swiss, RF, BALB/c and C3H female mice by feeding N-/4-(5-nitro-2-furyl)-2-thiazolyl/acetamide. Cancer Res., 30, 2320

Ertürk, E., Atassi, S.A., Yoshida, O., Cohen, S.M., Price, J.M. & Bryan, G.T. (1970a) Comparative urinary and gallbladder carcinogenicity of N-/4-(5-nitro-2-furyl)-2-thiazolyl/ formamide and N-/4-(5-nitro-furyl)-2-thiazolyl/ acetamide in the dog. J. nat. Cancer Inst., 45, 535

Ertürk, E., Cohen, S.M. & Bryan, G.T. (1970b). Carcinogenicity of N-/4-(5-nitro-2-furyl)-2-thiazolyl/ acetamide in female rats. Cancer Res., 30, 936

Wang, C.Y., Ansfield, F.J. & Bryan, G.T. (1971) Enzymatic reduction of N-/4-(5-nitro-2-furyl)-2-thiazolyl/ acetamide (NFTA) into water-soluble metabolite and its binding to protein in vitro. Proc. Amer. Ass. Cancer Res., 12, 6