WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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

Some anti-thyroid and related substances,
nitrofurans and industrial chemicals

VOLUME 7

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
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OF CHEMICALS TO MAN:

Some anti-thyroid and related substances, nitrofurans and industrial chemicals

Volume 7

This publication represents the views of two
IARC Working Groups on the
Evaluation of the Carcinogenic Risk of Chemicals to Man
which met in Lyon,
4-11 February 1974 and 18-24 June 1974

IARC WORKING GROUP ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO MAN: SOME ANTI-THYROID AND RELATED SUBSTANCES

Lyon, 4-11 February 1974

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Note to the reader

Every effort is made to present the monographs as accurately as possible without unduly delaying their publication. Nevertheless, mistakes have occurred and are still likely to occur. In the interest of all users of these monographs, readers are requested to communicate any errors observed to the Unit of Chemical Carcinogenesis of the International Agency for Research on Cancer, Lyon, France, in order that these can be included in corrigenda which will appear in subsequent volumes.

As stated in the preamble, great efforts are made to cover the whole literature, but some studies may have been inadvertently overlooked. Since the monographs are not intended to be a review of the literature and contain only data considered relevant by the Working Group, it is not possible for the reader to determine whether a certain study was considered or not. However, research workers who are aware of important published data which may change the evaluation are requested to make them available to the above-mentioned address, in order that they can be considered for a possible re-evaluation by a future Working Group.

CONTENTS

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

The International Agency for Research on Cancer (IARC) initiated in 1971 a programme on the evaluation of the carcinogenic risk of chemicals to man. This programme was supported by a Resolution of the Governing Council at its Ninth Session concerning the role of IARC in providing government authorities with expert, independent scientific opinion on environmental carcinogenesis. As one means to this end, the Governing Council recommended that IARC should continue to prepare monographs on the carcinogenic risk of individual chemicals to man.

In view of the importance of this programme and in order to expedite the production of monographs, the National Cancer Institute of the United States has provided IARC with additional funds for this purpose.

The objective of this programme is to elaborate and publish in the form of monographs a critical review of carcinogenicity and related data in the light of the present state of knowledge, with the final aim of evaluating the data in terms of possible human risk, and at the same time to indicate where additional research efforts are needed.

SCOPE OF THE MONOGRAPHS

The monographs summarize the evidence for the carcinogenicity of individual chemicals and other relevant information. The data are 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.

The first volume[1] covers a number of substances not belonging to a particular chemical group; the second[2], third[3], fourth[4], fifth[5] and sixth[6] volumes contain monographs on: some inorganic and organometallic compounds; certain polycyclic aromatic hydrocarbons and heterocyclic compounds; some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents; some organochlorine pesticides; and some

sex hormones, respectively. The present volume is devoted to some antithyroid and related substances, nitrofurans and industrial chemicals.

As new data on chemicals for which monographs have already been written and new principles for evaluation become available, re-evaluations will be made at future meetings, and revised monographs will be published as necessary. The monographs are being distributed to international and governmental agencies and will be available to industries and scientists dealing with these chemicals. They also 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 is established. IARC then collects pertinent references regarding physico-chemical characteristics, production and use*, occurrence and analysis, and biological data** on these compounds. The material is summarized by an expert consultant or an IARC staff member, who prepares the first draft, which in some cases is sent to another expert for comments. The drafts are circulated to all members of the Working Group about one month before the meeting, during which further additions to and deletions from the data are agreed upon, and a final version of comments and evaluation on each compound is adopted.

Priority for the Preparation of Monographs

Priority is given mainly to chemicals belonging to groups for which at least some suggestion of carcinogenicity exists from observations in animals and/or man and for which there is evidence of human exposure. However, neither human exposure nor potential carcinogenicity can be judged until all

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* Data provided by Chemical Information Services, Stanford Research Institute, Menlo Park, California, USA

** In the collection of original data reference was made to CBAC profile sheets and to the publications "Survey of compounds which have been tested for carcinogenic activity" [7,8,9,10,11,12] and to a bibliography provided by the Franklin Research Institute, USA.

the relevant data have been collected and examined in detail, and the inclusion of a particular compound in a monograph does not necessarily mean that the substance is considered to be carcinogenic. Equally, the fact that a substance has not yet been considered does not imply that it is without carcinogenic hazard.

Data on which the Evaluation is Based

With regard to the biological data, only published articles and papers already accepted for publication are reviewed. Every effort is made to cover the whole literature, but some studies may have been inadvertently overlooked. The monographs are not intended to be a full review of the literature, and they contain only data considered relevant by the Committee. Research workers who are aware of important data (published or accepted for publication) which may influence the evaluation are invited to make them available to the Unit of Chemical Carcinogenesis of the International Agency for Research on Cancer, Lyon, France.

The Working Group

The tasks of the Working Group are five-fold: (1) to verify that as far as feasible all data have been collected; (2) to select the data relevant for the evaluation; (3) to determine whether the data, as summarized, will enable the reader to make his own judgement concerning the adequacy of the experiment and the effect observed; (4) to judge the significance of the experimental results; and (5) to make an evaluation.

The members of the Working Group who participated in the consideration of particular substances are listed at the beginning of each publication. The members of the Working Group serve in their individual capacities as scientists, and not as representatives of their governments or of any organization with which they are affiliated.

GENERAL PRINCIPLES FOR THE EVALUATION

The general principles for the evaluation which are listed below were elaborated by previous Working Groups and were also applied to the substances listed in this volume.

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 neoplasms. 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 purposes, in general, 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 in experimental animals to a carcinogen may take several forms:

(1) a significant increase in the incidence of one or more of the same types of neoplasms as found in control animals;

(2) the occurrence of types of neoplasms not observed in control animals;

(3) a decreased latent period as compared with control animals.

Purity of the Compounds Tested

In any evaluation of biological data with respect to a possible carcinogenic risk, particular attention must be paid to the purity of the chemicals tested and to their stability under conditions of storage or administration. Information on purity and stability is given, when available, in the monographs.

Qualitative Aspects

The qualitative nature of neoplasia has been much discussed. In many instances, both benign and malignant tumours are induced by chemical carcinogens. There are so far few recorded instances in which only benign

tumours are induced by chemicals that have been studied extensively. Their occurrence in experimental systems has been taken to indicate the possibility of an increased risk of malignant tumours also.

In experimental carcinogenesis, the type of cancer seen can be the same as that recorded in human studies (e.g., bladder cancer in man, monkeys, dogs and hamsters after administration of 2-naphthylamine). In other instances, however, a chemical can induce other types of neoplasms or neoplasms at different sites in various species (e.g., benzidine 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. The confidence with which a carcinogenic effect can be established is strengthened by the observation of an increasing incidence of neoplasms with increasing exposure. Such studies are the only ones on which a minimal effective dose can be established. The determination of such a dose allows a comparison with the reliable data on human exposure.

Comparison of potency between compounds can only be made if and when substances have been tested simultaneously.

Animal Data in Relation to the Evaluation of Risk to Man

At the present time no attempt can be made to interpret the animal data directly in terms of human risk since no objective criteria are available to do so. The critical assessment of the validity of the animal data given in these monographs is intended to assist national and/or international authorities to make decisions concerning preventive measures or legislation. In this connection, attention is drawn to WHO recommendations in relation to food additives[13], drugs[14] and occupational carcinogens[15].

Evidence of Human Carcinogenicity

Evaluation of the carcinogenic risk to man of suspected environmental agents rests on purely observational studies. Such studies require sufficient variation in the levels of human exposure to allow a meaningful relationship between cancer incidence and exposure to a given chemical to be established. Difficulties in isolating the effects of individual agents

arise, however, since populations are exposed to multiple carcinogens.

The initial suggestion of a relationship between an agent and disease often comes from case reports of patients who have had similar exposures. Variations and time trends in regional or national cancer incidence, or their correlation with regional or national 'exposure' levels, may also provide valuable insights. Such observations by themselves, however, cannot in most circumstances be regarded as conclusive evidence of carcinogenicity. The most satisfactory epidemiological method is to compare the cancer risk (adjusted for age, sex and other confounding variables) among groups or cohorts, or among individuals exposed to various levels of the agent in question and among control groups not so exposed. Ideally this is accomplished directly, by following such groups forward in time (prospectively) to determine time relationships, dose-response relationships and other aspects of cancer induction. Large cohorts and long observation periods are required to provide sufficient cases for a statistically valid comparison.

An alternative to prospective investigation is to assemble cohorts from past records and to evaluate their subsequent morbidity or mortality by means of medical histories and death certificates. Such occupational carcinogens as nickel, β-naphthylamine, asbestos and benzidine have been confirmed by this method. Another method is to compare the past exposures of a defined group of cancer cases with those of control samples from the hospital or general population. This does not provide an absolute measure of carcinogenic risk but can indicate the relative risks associated with different levels of exposure. The indirect means (e.g., interviews or tissue residues) used to measure exposures which may have commenced many years before can constitute a major source of error. Nevertheless such "case-control" studies can often isolate one factor from several suspected agents. The carcinogenic effect of this substance could then be confirmed by cohort studies.

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".

Chemical and Physical Data (section 1)

The most important chemical synonyms and trade names are recorded in this section. The trade names are listed separately.

Chemical and physical properties include data that might be relevant to carcinogenicity (for example, lipid solubility) and those that concern identification. Where applicable, data on solubility, volatility and stability are indicated. All chemical data in this section refer to the pure substance.

Production, Use, Occurrence and Analysis (section 2)

With regard to the data on use and occurrence of chemicals presented in the monographs, IARC has collaborated with the Stanford Research Institute, USA, with the support of the National Cancer Institute of the United States, in order to obtain production figures of chemicals and their patterns of use. These data more commonly refer to the United States and Western Europe than to other countries, solely as a result of the availability to the Working Group of more data from these countries than from others. It should not be implied that these nations are the sole sources or even the major sources of any individual chemical. In the case of drugs, mention of the therapeutic uses of such chemicals in this section does not necessarily represent presently accepted therapeutic indications, nor does it imply judgement as to their clinical efficacy.

In some countries, there are also legal restrictions on the conditions under which certain carcinogens, suspect chemicals and pesticides can be handled. Examples of these are given in section 2.2 when such information was available to the Working Group, but no attempt was made to be all-inclusive.

It is hoped that in future revisions of these monographs, more information on use can be made available to IARC from other countries.

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 consider 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, as judged from previously described criteria[16,17,18,19] (e.g., too short a duration, too few animals, poor survival or too small a dose); (b) because they only confirmed findings which have already been fully described; or (c) because they were judged irrelevant for the purpose of the evaluation. However, in certain cases, reference is made to studies which did not meet established criteria of adequacy, particularly when this information was considered a useful supplement to other reports or when it may have been the only data available. This does not, however, imply acceptance of the adequacy of experimental designs in these cases.

In general, the data recorded in this section are summarized as given by the author; however, certain shortcomings of reporting or of experimental design are also mentioned, and minor comments by the Working Group are given in brackets.

The essential comments by the Working Group are made in section 4, "Comments on Data Reported and Evaluation".

Carcinogenicity and related studies in animals: Mention is usually made of all routes of administration by which the compound has been tested and of all species in which relevant tests have been carried out. In most cases the animal strains are given; general characteristics of mouse strains have been reported in a recent review[20]. Quantitative data are given in so far as they will enable the reader to realize the order of magnitude of the effective doses. In general, the doses are indicated as they appear in the original paper; sometimes conversions have been made for better comparison, and these are given in parentheses.

Other relevant biological data: The reporting of metabolic data is restricted to studies showing the metabolic fate of the chemical in animals and man. Comparison of animal and human data is made when possible. Other

metabolic information (e.g., absorption, storage and excretion) is given when the Working Group considered that it would enable the reader to have a better understanding of the fate of the compound in the body. When the carcinogenicity of known metabolites has been tested, this also is reported.

Some LD_{50}'s are given and other data on toxicity are included occasionally, if considered relevant.

Observations in man: Epidemiological studies are summarized. Clinical and other observations in man have been reviewed, when relevant.

Comments on Data Reported and Evaluation (section 4)

This section gives the critical view of the Working Group on the data reported. It should be read in conjunction with the General Remarks on substances considered.

Animal data: The animal species mentioned are those in which the carcinogenicity of the substances was clearly demonstrated, irrespective of the route of administration. In the case of inadequate studies, when mentioned, 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 "Animal Data in Relation to the Evaluation of Risk to Man" of this introduction.

Human data: In some cases, a brief statement is made on the possible exposure of man. The significance of epidemiological studies and case reports is discussed, and the data are interpreted in terms of possible human risk.

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GENERAL REMARKS ON ANTI-THYROID AND RELATED SUBSTANCES

The role of various factors and precipitating agents in the induction of thyroid tumours as well as the morphology of these tumours have been reviewed by Christov & Raichev (1972), Doniach (1970) and Money & Rawson (1968). Thyroid tumours have been induced by various anti-thyroid substances, by low iodine diets, by chemical carcinogens, by internal (e.g., $^{125,131}I$) and external ionizing irradiation and after subtotal thyroidectomy.

In the case of their induction in animals by continuous feeding of a low-iodine diet, causation is attributed to hypersecretion of thyroid-stimulating hormone (TSH) acting upon the hypofunctional thyroid. The mechanism here is a disturbed synthesis of thyroid hormone due to decreased availability of inorganic iodine.

This finding clearly indicates the existence of an indirect mechanism leading to thyroid carcinogenesis, the ultimate cause being a hormonal imbalance of the hypothalamo-pituitary-thyroid system. Induction of thyroid tumours by anti-thyroid substances is a result of the suppression of the rate of synthesis of thyroxine, thus also leading to a hormonal imbalance.

There exists an inverse relationship between the level of iodine in the diet and the dose of thyroid-active substances required for induction of a hypothyroid state. Consequently, a low iodine intake makes the animals more prone to the development of a hyperthyroid state when they are exposed to a given dose of the anti-thyroid substance. In most of the papers reviewed the exact iodine intake has not been specified, and an appropriate evaluation of the thyroid tumour-inducing potency of the compounds tested is not possible.

An additional problem which faced the Working Group was the lack of well-delineated morphology of hyperplastic, pre-neoplastic and neoplastic lesions arising in various species. Also, the malignant nature of experimentally induced thyroid tumours has not been fully defined (i.e., some authors mention the existence of thyroid-like tissue in the lung as an indication of malignancy, while others use the same argument in support of the non-malignant nature of the lesion). Other workers utilize successful

growth of the transplants into isogenic, but TSH-stimulated, hosts as the criterion of the neoplastic nature of the primary lesion.

In general, administration of anti-thyroid agents to animals leads to development of thyroid neoplasms by an indirect mechanism as indicated above. The outcome will depend, however, upon the interplay of iodine intake, rate of thyroxin synthesis and the functional state of the hypothalamo-pituitary system. The true nature of the thyroid lesions, both biological and morphological, needs to be elucidated in order to assign their proper significance and to evaluate the carcinogenic risk arising from exposure to anti-thyroid agents.

Relative biological activities

On the basis of the decrease in iodine concentration and the increase in weight of thyroid glands of rats fed different anti-thyroid compounds, and assigning an arbitrary activity of 1 to thiouracil, the biological activity of thiourea is found to be 0.12; that of methylthiouracil, also 1; and that of propylthiouracil, 11 (Astwood et al., 1945). No information appears to be available concerning the relative activity of amitrole.

General analytical methods

Chromatographic (Ebing, 1972; Geldmacher-v. Mallinckrodt & Schmidt, 1970), colorimetric (Barrette & Scheuneman, 1973) and combined chromatographic and colorimetric (Onley & Yip, 1969; Soerensen, 1971; Wills, 1966) methods which have been developed for amitrole have been applied generally to analysis of commercial formulations or of crop residues, where interest is mainly in the herbicidal properties of the compound. For measurements of thiouracil and of thiourea in body fluids and tissue extracts, colorimetric procedures using the same reagent have been developed (Gerfast, 1966; Williams & Kay, 1945; Williams et al., 1944); while for propylthiouracil in serum a colorimetric method using a different reagent has been proposed (Ratliff et al., 1972). Other procedures (colorimetric, spectrophotometric, fluorimetric, UV absorption) which have been described for thiouracil, methylthiouracil, propylthiouracil and thiourea were developed for the analysis of purified compounds or of tablet formulations (Bartos, 1970; Berg, 1971; Bruno, 1963; Pinzauti et al., 1973).

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GENERAL REMARKS ON THE 5-NITROFURANS CONSIDERED

5-Nitrofurans are a group of nitroheterocyclic compounds that have been widely used as human and veterinary medicinals or as food preservatives for about 30 years. Use of these chemicals began after the report of Dodd & Stillman (1944) that many derivatives of 5-nitrofurans had antibacterial activity. Since that time, several thousand 5-nitrofuryl derivatives have been synthesized, and many have eventually been used commercially. They probably represent the largest group of nitro compounds available as drugs at the present time. The chemistry, biology and clinical applications of nitrofuran derivatives have been reviewed by Dunlop & Peters (1953), Miura & Reckendorf (1967) and Paul & Paul (1964, 1966).

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THE MONOGRAPHS

ANTI-THYROID AND RELATED SUBSTANCES

AMITROLE*

Amitrole is the common name approved by the International Standards Organization for 3-amino-1,2,4-triazole, except in Denmark, France, UK and the USSR, where it is known as aminotriazole.

1. Chemical and Physical Data

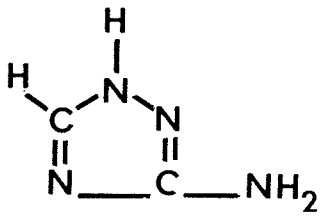
1.1 Synonyms and trade names

Chem. Abstr. No.: 61-82-5

3-Amino-s-triazole; 3-amino-1,2,4-triazol; aminotriazole; aminotriazole; 3-aminotriazole; 3-amino-1,2,4-triazole; 3-amino-1H-1,2,4-triazole; 2-amino-1,3,4-triazole; AT; 3AT; 3,A-T; ATA; ATZ; triazolamine; 1H-1,2,4-triazol-3-amine

Aminotriazol Spritzpulver; Amitril; Amitril T.L.; Amitrol; Amitrol 90; Amitrol-T; Amizine; Amizol; Amizol D; Amizol DP NAU; Amizol F; Azaplant Kombi; Campaprim A 1544; Cytrol; Cytrole; Diurol 5030; Domatol; Domatol 88; Elmasil; Emisol; Emisol 50; Emisol F; ENT 25445; Fenamine; Fenavar; Kleer-Lot; Orga-414; Radoxone TL; Ramizol; Solution Concentrée T271; Vorox; Vorox AA; Vorox AS; Weedar ADS; Weedar AT; Weedazin; Weedazin Arginit; Weedazol; Weedazol GP2; Weedazol Super; Weedex Granulat; Weedoclor; X-All Liquid

1.2 Chemical formula and molecular weight



$C_2H_4N_4$ Mol. wt: 84.1

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* Considered by the Working Group in Lyon, February 1974

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless crystals (from water, ethanol or ethyl acetate). Bitter taste

(b) Melting-point: 159°C

(c) Absorption spectroscopy: No specific absorption maximum in the visible and ultraviolet regions (200 to 650 nm)

(d) Identity test: Identified by a colour reaction with nitroprusside reagent or by diazotization and coupling with H-acid or N-(1-naphthyl)ethylenediamine (see 2.3)

(e) Solubility: Soluble in water (28% at 25°C) and ethanol (26% at 75°C); slightly soluble in chloroform, methylene chloride, acetonitrile, ethyl acetate; insoluble in ether, acetone and hydrocarbons

(f) Volatility: Sublimes undecomposed under reduced pressure

(g) Stability: Not decomposed by heating at 100°C for 2 hours. Although the triazole ring is stable to a variety of reagents, it is cleaved by free-radical generating systems. Carbon dioxide is liberated by Fenton's reagent, and urea and cyanamide were identified as reaction products. Irradiation of an aqueous solution at 200 nm, or daylight illumination in the presence of riboflavin, form similar products and possibly also polymeric compounds.

(h) Reactivity: Aqueous solutions are neutral, but the compound acts as a weak base and forms salts with acids. It forms chelates with metals such as iron and copper. The primary amino group reacts with acylating agents to give mono and diacyl derivatives, yields aldimines or ketimines with aldehydes and ketones and can be diazotized like a typical aromatic amine.

1.4 Technical products and impurities

Amitrole is available in the United States as a technical grade product containing 90% minimum active ingredient. It is formulated into

soluble powders, liquids and aerosol sprays (Frear, 1972; Meister, 1973). It is also available in combinations with an activator, ammonium thiocyanate (as Amitrole-T), and with a variety of other herbicides, e.g., atrazine (as Fenamine), bromacil (as Fenavar), linuron (as Kleer-Lot), simazine (as Amizine and X-All Liquid) and 2,3,6-trichlorobenzoic acid (Crafts, 1961; Frear, 1972).

2. Production, Use, Occurrence and Analysis

Two review articles on amitrole have been published (Crafts, 1961; Kröller, 1966).

2.1 Production and use[1]

A method for amitrole synthesis was first reported in 1946 (Allen & Bell, 1946). Its use as a herbicide was first patented in the US in 1954 under the number 2,670,282. It is believed to be produced commercially by the reaction of formic acid with aminoguanidine in an inert solvent at 100-120°C.

Amitrole is not produced in the US. Total US imports in 1972 were reported to have been 336,000 kg, 56% coming from France, 30% from the Federal Republic of Germany and the remainder from Japan and Canada (US Department of Commerce, 1973a). Total US imports of amitrole in the first nine months of 1973 were reported to have been 406,000 kg, with 53% from France, 34% from the Federal Republic of Germany, almost 13% from Japan and a very small amount from Sweden (US Department of Commerce, 1973b).

The major (and possibly the only commercial) use for amitrole in the US has been as a herbicide. It was first introduced in this respect for the defoliation of cotton but found limited use in this application because of its relatively high price (US Department of Agriculture, 1960). By 1958 it was being used to control weeds on other crops and in non-cropland applications; but in 1959 it became the centre of a major controversy,

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

when the feeding of amitrole to rats was found to induce thyroid tumours. Subsequently, amitrole residues were found in marketed cranberries, and sales of cranberries and cranberry products from the 1958 and 1959 crops were prohibited (House et al., 1967).

One survey of US farm use of pesticides reported that a total of 134,000 kg of amitrole was used by farmers in 1964. Approximately two-thirds of this was used on crops and the remainder for control of weeds along fences and ditch banks (US Department of Agriculture, 1968a). House et al. (1967) reported that amitrole and its combination with ammonium thiocyanate have also been used in non-cropland management for the control of such weeds and brush as bermuda grass, Canada thistle, cattails, poison oak, poison ivy, quackgrass, tules and water hyacinth.

As of August 31 1968, no tolerances for permissible residues of amitrole on crops where it was used for weed control had been established,pending a review by a US Department of Agriculture advisory committee. At that time, amitrole was approved for agricultural use in the control of weeds around the following crops: alfalfa, apples, clover, corn, grains (including its use as a chemical fallow before planting), grapes, legumes, pastures, pears, peas and soybeans (US Department of Agriculture, 1968b). However, in 1968, the US Department of Agriculture issued a notice of proposed cancellation for the registered uses of amitrole on crops. A subsequent review committee recommended cancellation, and the cancellation order took effect in July 1971 (Anon., 1971).

Amitrole usage in California, a major agricultural state, was reported to have been nearly 82,000 kg (30% was used on state highways and 14% on grapes) in 1970 (California Department of Agriculture, 1971) and over 64,000 kg (32% on state highways but none on grapes) in 1972 (California Department of Agriculture, 1973).

Although in 1959 it was found to have anti-thyroid activity (Goodman & Gilman, 1970), there is no evidence that amitrole has been used in human medicine in the US. It has been reported that amitrole has found use as a reagent in photography (Merck & Co., 1968), but this could not be verified.

Two companies were reported to be offering amitrole and amitrole formulations for sale in Japan in 1973 (Japan Chemical Week, 1973). Whether these companies are actual producers is not known. In Israel, the use of amitrole is forbidden at distances less than 120 metres from populated areas and less than 200 metres from citrus plantations (Hirsch, 1971).

2.2 Occurrence

Amitrole does not occur in nature. It may be found in soil for several weeks after application and has been found to persist in water for more than 200 days (Pimentel, 1971).

2.3 Analysis

Amitrole can be determined, after extraction with dimethylformamide, by adding 0.5 N acid and back-titrating the excess acid with 0.5 N sodium hydroxide. Weinmann & Finger (1971) described a potentiometric precipitation titration method using silver nitrate and silver/silver chloride- or silver/mercurous sulphate-electrode. The method can be used for the determination of amitrole in its formulations or in the presence of triazines, substituted urea herbicides or plant growth regulators such as bromacil and ammonium thiocyanate. Residue methods involve extraction with water and colour reaction with nitroprusside in alkaline solutions (Sund, 1956; Sund et al., 1960), or diazotization and coupling with either N-(1-naphthyl)ethylenediamine dihydrochloride (Storherr & Burke, 1961) or H-acid* (Agrawal & Margoliash, 1970; Herrett & Linck, 1961). Modifications of these methods, and other radiometric procedures for field use in analyses of contaminated creek and canal waters, as well as soils, have been described (Demint et al., 1970; Groves & Chough, 1971; Marston et al., 1968; Nearpass, 1969). A TLC method for its routine identification has been presented by Ebing (1972). For assaying urine samples, Geldmacher-v. Mallinckrodt & Schmidt (1970) separated amitrole by paper chromatography using phenol saturated with water, or a mixture of n-butanol:water (15:1) and propionic acid:water (7:6), and identified by spraying with a solution

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* 8-amino-1-naphthol-3,6-disulphonic acid, monosodium salt

of p-dimethylaminobenzaldehyde in acetic acid or hydrochloric acid. Aldrich & McLane (1957) described a paper chromatographic method for its detection in plant tissues (sensitivity, 0.1 µg). (See also the section, "General Remarks on Anti-thyroid Substances", p. 23).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: In a study reported as a preliminary note, 18 male and 18 female mice of two hybrid strains, (C57BL/6xC3H/Anf)F_1 and (C57BL/6xAKR)F_1, were administered 1000 mg/kg bw/day amitrole (AT) in distilled water (maximum tolerated dose) by stomach tube when the animals were 7 days of age. The same absolute amount was then administered each day until the mice were 4 weeks of age (the dose was not readjusted according to weight gain during this period). Subsequently, 2192 ppm of the compound (maximum tolerated dose) were mixed with the diet and fed ad libitum until the end of the observation period (53-60 weeks). Of the 72 mice from both strains which were necropsied, 64 were reported to have carcinomas of the thyroid. Liver tumours, broadly classified as hepatomas, were observed in 16/18 male and in 18/18 female (C57BL/6xC3H/Anf)F_1 hybrids and in 16/18 male and in 17/18 female (C57BL/6xAKR)F_1 hybrid mice. Liver tumours occurred in 8/166 and 6/172 controls (Innes et al., 1969). [Although metastasizing hepatic-cell tumours were reported to be rare in this study, the authors state that the term "hepatoma", in the present context, should not be considered as implying that these tumours are benign.]

Rat: In rats administered 10, 50 or 100 ppm AT in the diet for 104 weeks, thyroid adenomas developed in 1/10, 2/15 (1 "adenocarcinomatous") and 17/26 (4 "adenocarcinomatous") rats treated at the three dose levels. Except for a cystic follicle, no tumours were found in 5 controls examined (Jukes & Shaffer, 1960).

In rats administered diets containing 0, 10, 50 or 100 ppm AT for 104 weeks, those receiving the 100 ppm level showed a high incidence of thyroid

adenomas (15/27 rats examined). The incidence was lower (1-3/27 rats) in the groups given 10-50 ppm AT. Mammary and other tumours were distributed in a random fashion (Food Protection Committee, 1959, quoted in Hodge et al., 1966). [Control data were not reported.]

A high incidence of thyroid and liver tumours was observed in white stock rats of both sexes following oral administration of AT in drinking-water (20-25 mg/day/rat) or in the food at 2 dose levels (250 or 500 mg/day/rat) for lifespan, which varied from 10-32 months. In the groups receiving AT in the drinking-water and in the diet, 55 and 49 animals were alive at the time the first tumour of the thyroid was detected; by the end of the experiment a total of 28/55 and 26/49 thyroid tumours were found. At the time the first liver tumour was detected, 44 and 52 animals were alive, and of these 15/44 and 33/52 developed benign and malignant liver tumours. The latter included hepatocellular and hepatocholangiocellular carcinomas and were found in 5/44 and in 17/52 rats, respectively. Concurrent daily injections of 2.5 µg/100 g bw thyroxine and oral administration of 300 mg/day/rat AT in food for the length of the experiment resulted in the development of only one thyroid adenoma but of 9 liver tumours in 12 males. AT alone induced thyroid tumours in 7/22 and liver tumours in 12/23 male rats of the same age. No liver tumours but 2 thyroid cystic adenomas were observed in 51 control animals (Napalkov, 1969).

Dog: Dogs (2-4 per group) were administered diets containing 0, 10, 50, 100 or 500 ppm AT for 52 weeks. No tumours were detected in the thyroids or in other organs (Hazleton Laboratories, unpublished data, quoted in Hodge et al., 1966). [No further details were given, and the test cannot be evaluated.]

Fish: No hepatomas were seen by gross examination in 48 and 57 livers of rainbow trout fed 1200 and 4800 ppm AT in the diet, respectively, for 15 months. In a second series of experiments employing the same two dose levels of AT, a low incidence of tumours (6-21%) could be detected in the treated fish at 12, 16 and 20 months (Halver, 1967). [There was no pattern of correlation in the incidence with either dose or duration. A rather high incidence (24% and 56%) was observed at 9 months, probably due

to the smaller numbers examined at this time (9 and 17) compared to the numbers examined at 12, 16 and 20 months (33 to 73). The hepatoma incidence in the controls did not exceed 1%. No details of the composition of the diet and its possible contamination were given.]

(b) Skin application

Mouse: No skin tumours were observed in 2 groups of 50 male and 50 female 2-4-month old C3H/Anf mice following weekly skin applications of either 0.1 or 10 mg AT (analytical grade) in 0.2 ml acetone:methanol mixture (65:35) for life. The median survival times in the treated groups ranged from 44-57 weeks, and histological examination of the skin was carried out in 33-40 males and females from each group (Hodge et al., 1966).

(c) Subcutaneous and/or intramuscular injection

Rat: Napalkov (1962) administered 125 mg/rat AT s.c. twice weekly for approximately 11 months and found 5 liver tumours and 5 thyroid tumours in 7 rats surviving at the appearance of the first tumour. [No information regarding controls was reported.]

In a further study in rats, Napalkov (1969) implanted s.c. at monthly intervals 3 pellets of 500 mg AT in paraffin and lanolin under the skin of the left flank. Control pellets of equal size consisting only of paraffin and lanolin were implanted under the skin of the right flank. From the 6th-12th months of the experiment the same rats were additionally injected s.c. with 0.2 ml sunflower oil containing 125 mg AT twice weekly under the skin of the left flank, and with 0.2 mg sunflower oil without AT under the skin of the right flank. In 7/14 animals that survived for 21 months or more, 1 polymorphous-cell and 6 spindle-cell sarcomas developed at the treatment sites, first the implantation and later the injection sites. No tumours were observed in the same rats at the sites of implantation of control pellets or of injection of oil without AT.

(d) Intraperitoneal injection

Rat: An inhibitory effect of AT on the liver tumourigenesis produced by 4-dimethylaminoazobenzene was demonstrated in 2-3-month old male albino rats given 0.06% dimethylaminoazobenzene in the diet, or this treatment

together with a 10% solution of AT (1000 mg/kg bw) by i.p. injection every second day. All surviving animals were killed 21 weeks after the start of treatment. The incidence of liver tumours was 12/16 in the positive controls compared with 4/19 in the group which received additional treatment with AT. The difference was significant ($P<0.01$). The liver carcinomas produced in the positive control rats were, for the most part, hepatocellular carcinomas, whereas those in the group given AT as well included both hepatocellular carcinomas and cholangiocarcinomas (Hoshino, 1960).

3.2 Other relevant biological data

In the rat, 5-^{14}C-labelled AT is rapidly and completely absorbed from the gastrointestinal tract. Highest levels of radioactivity were found in the liver, kidney and blood 1 hour after administration, and the levels decreased after 3-4 hours. During the first 24 hours, 70-95% of the radioactivity was excreted in the urine (Fang et al., 1964). Using randomly labelled ^{3}H-AT and ^{14}C-AT labelled at the 5 carbon atom, Fang et al. (1966) characterized two metabolites by determining the change in the $^{3}H/^{14}C$ ratio. One was derived from the substitution of the 5-H atom of the triazole ring and occurred in female and male rats. Another metabolite derived from substitution of one of the H-atoms in the amino group was found only in males.

No signs of intoxication were observed in a 39-year old woman after ingestion of a commercial preparation containing 30% AT (20 mg/kg bw) and 56% 3,4-dichlorophenyl N,N'-dimethylurea (diuron). Unchanged AT was found in the urine, but no metabolites were identified. Fifty per cent of the estimated intake was excreted in the urine within a few hours after ingestion (Geldmacher-v. Mallinckrodt & Schmidt, 1970).

3.3 Observations in man

Axelson et al. (1974) have reported a cohort study of Swedish railway workers exposed to a variety of herbicides. In the period of follow-up of 5 or more years after the initial exposure, 11 cancers at a wide variety of sites were observed, whereas only 4.8 were expected from the national experience. Within the subgroup who were exposed to AT (alone or in combination with other herbicides), the corresponding numbers were 7 observed

versus 1.9 expected. Two of the cancers were of the lung, whereas only 0.24 were expected; histologically, one tumour was of the oat-cell type and the other an adenocarcinoma. Both men concerned were smokers.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Amitrole induced thyroid and liver tumours in both mice and rats following oral and/or subcutaneous administration. An increased incidence of liver-cell tumours in the trout has also been reported following oral administration, but this cannot be considered as conclusive until additional studies using properly controlled diets are reported. Limited skin-painting studies in mice gave no evidence of skin carcinogenicity.

4.2 Human data

A single, small, cohort study raises the suspicion that amitrole may be carcinogenic to man, but the findings cannot be regarded as conclusive.

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[1] This section should be read in conjunction with the section, "General Remarks on Anti-thyroid Substances" (p. 23) and the section "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

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ETHYLENETHIOUREA*

1. Chemical and Physical Data

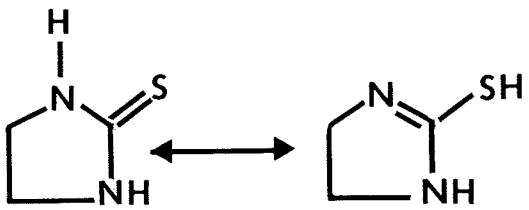
1.1 Synonyms and trade names

Chem. Abstr. No.: 96-45-7

4,5-Dihydroimidazole-2(3H)-thione; ethylene thiourea; N,N'-ethylene-thiourea; 1,3-ethylene-2-thiourea; ETU; 2-imidazolidinethione; 2-imidazoline-2-thiol; 2-mercaptoimidazoline; 2-thiol-dihydroglyoxaline

NA-22; NA-22-D; Pennac CRA; Sodium-22 neoprene accelerator; Warecure C

1.2 Chemical formula and molecular weight



$C_3H_6N_2S$ Mol. wt: 102.2

1.3 Chemical and physical properties of the pure substance

(a) Description: White crystals

(b) Melting-point: 203-204°C

(c) Solubility: Solubility in 100 ml of water: 2 g at 30°C, 9 g at 60°C, and 44 g at 90°C; moderately soluble in methanol, ethanol, ethylene glycol and pyridine; insoluble in acetone, ether, chloroform and benzene

1.4 Technical products and impurities

Ethylenethiourea is available in the US as white crystals or a finely-ground powder with a melting-point of 192°C. It is also available in the US and Japan as a white powder with a melting-point above 195°C. It is available in the US as a white powder consisting of an 80% dispersion of

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* Considered by the Working Group in Lyon, June 1974

ethylenethiourea in oil (E.I. Du Pont de Nemours & Co., Inc., 1972).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Although the first synthesis of ethylenethiourea was reported in 1872 (Hofman, 1872), commercial production was not reported in the US until 1951 (US Tariff Commission, 1952). It can be synthesized by the reaction of ethylenediamine with carbon disulphide, followed by refluxing with hydrochloric acid to obtain ring closure (Johnson & Edens, 1942). Whether this route is used for commercial production is not known.

Because only two US companies report commercial manufacture of ethylenethiourea to the US Tariff Commission, separate data on its production are not published. Data on US imports and exports are not available.

Ethylenethiourea is sold by several companies in the Federal Republic of Germany, France and the United Kingdom. No information is available on which of these companies manufacture the chemical or on the extent of the total Western European production (Chemical Information Services, Ltd, 1973; Econ Verlag GmbH, 1973-1975). In Japan, where this chemical is manufactured by four companies, 1972 production is estimated to have been over 200 thousand kg, and additional quantities were imported.

Ethylenethiourea has been widely used since 1948 as an accelerator for neoprene (polychloroprene) rubber. It is also recommended for use as part of a curing system for polyacrylate rubber. Although the curing of these rubbers converts almost all of the ethylenethiourea to other compounds, traces are still present in the cured products. The results of one test on a specific neoprene stock indicated that 0.01 mg unchanged chemical per square inch of surface could be extracted by water at 57°C over a period of seven days (E.I. Du Pont de Nemours & Co., Inc., 1972).

The various types of neoprene rubber are used almost exclusively in industrial applications. The following US consumption pattern was reported

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

for 1973: 25% in industrial and mechanical goods; 20% in automotive products; 20% exported; 10% in wire and cable production; 10% in construction; 10% in adhesives; and 5% in miscellaneous applications (Anon., 1973). Consumer products containing neoprene include shoes and closures for containers (e.g., aerosol dispensers).

Polyacrylate rubbers are also used for industrial purposes. Products such as seals, o-rings and gaskets for automotive and aircraft applications are believed to account for almost all the consumption.

Although ethylenethiourea has been proposed for a variety of uses and although numerous patents have been issued, no evidence was found that it is being used commercially for other than rubber-curing purposes.

In late 1973, the US Food and Drug Administration (FDA) revoked its approval of the use of mercaptoimidazoline (the isomeric form of ethylenethiourea) in the production of vulcanized natural or synthetic gaskets used in closures for food containers and of rubber articles intended for repeated use in processing, packing or holding food (US Federal Register, 1973). In May 1972, the FDA proposed to take a variety of regulatory actions against those human and animal drugs, cosmetics and medical devices in which 2-mercaptoimidazoline is used in the manufacture of components which may come into contact with a user, a patient or a drug product in its intended use (US Federal Register, 1974). By June 1974, however, no final regulation had been published.

2.2 Occurrence

Ethylenethiourea has been found by many investigators to be among the principal degradation products of the metal salts of ethylenebisdithiocarbamic acid, which are widely used as agricultural fungicides. Although most of the studies have been on the manganese salt (maneb), the sodium salt (nabam) and the zinc salt (zineb), Bontoyan et al. (1972) found ethylenethiourea to be present in 28 different ethylenebisdithiocarbamate commercial products. Yip et al. (1971) reported that treatment of kale and lettuce with maneb (which contained some ethylenethiourea) at a rate of 1.09 kg active ingredient per acre resulted in initial residues of 0.6 mg/kg ethylenethiourea, which decreased to undetectable levels within seven

days after application. Levels of 0.018-0.044 mg/kg ethylenethiourea have been reported in commercial apples purchased in the area around Ottawa, Canada (Newsome, 1972). A paper presented at a 1973 symposium on the origin and fate of ethylenethiourea fungicides reported that no detectable amount of ethylenethiourea residue occurred in tomato foliage, soil surfaces or ditch water treated with a co-ordination product of zinc ion plus the manganese salt of ethylenebisdithiocarbamic acid (Blazquez, 1973).

2.3 Analysis

An analytical method for the determination of ethylenethiourea in fruits, vegetables and milk at levels as low as 0.02 mg/kg has been developed by Onley & Yip (1971) using thin-layer and gas chromatographic procedures. A method has also been described for its determination in water and on tomato foliage (detection limit, 1 mg/kg) (Blazquez, 1973).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: In a screening study reported as a preliminary note, 2 groups of 18 male and 18 female mice of the (C57BL/6 x C3H/Anf)F_1 or (C57BL/6 x AKR)F_1 strains were given single doses of 215 mg/kg bw ethylenethiourea (ETU) in gelatin by stomach tube at 7 days of age, and the same absolute amounts were then administered daily until the animals were 28 days of age. Subsequently, ETU was administered in the diet at a concentration of 646 ppm; the experiment was terminated at 82-83 weeks after the start of the treatment. The incidence of hepatomas was 14/16 (male) and 18/18 (female) in (C57BL/6 x C3H/Anf)F_1 mice and 18/18 (male) and 9/16 (female) in (C57BL/6 x AKR)F_1 mice, compared with 8/79 male and 0/87 female and 5/90 male and 1/82 female negative controls of each strain, respectively. Lymphomas were also observed in 3/18 male and 4/16 female (C57BL/6 x AKR)F_1 mice, compared with 1/90 in male and 4/82 in female controls (Innes et al., 1969). [Full details of the study were not available to the Working Group.]

Rat: Administration of 175 or 350 ppm technical grade ETU (97%) in the diet of groups of 26 male and 26 female Charles River CD rats for 18 months, followed by administration of the control diet for 6 months, produced hyperplastic goitre in 17 males and 13 females at the high dose level and in 9 males and 6 females at the low dose level. In addition, thyroid carcinomas occurred in 17 males (2 with pulmonary metastases) and 8 females at the high dose level and in 3 males and 3 females at the low dose level, compared with none in 32 male and 32 female controls. The first carcinoma occurred after 68 weeks in a female given 350 ppm in the diet. One female at the high dose level and 2 females at the low dose level had solid-cell adenomas of the thyroid, and a total of 3 males and 1 female had hyperplastic liver nodules (Ulland et al., 1972).

3.2 Other relevant biological data

ETU fed to rats at levels of 50-750 ppm in the diet for periods of 30-120 days produced decreases in body weight, increases in thyroid:body-weight ratio and decreases in uptake of ^{131}I, the extent of the effects being related to dosage and time. At levels of 500 and 750 ppm, moderate to marked hyperplasia occurred in the thyroids, but no effect was observed at 50 ppm (Graham & Hansen, 1972).

ETU was teratogenic in rats at doses that produced no apparent maternal toxicity or foetal deaths, suggesting that transplacental transfer of the substance may take place (Khera, 1973).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Ethylenethiourea (ETU) has been tested only by the oral route in rats,

[1] This section should be read in conjunction with the section "General Remarks on Anti-thyroid Substances" (p.23) and the section "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

producing thyroid carcinomas. The reported increased incidence of liver-cell tumours in 2 strains of mice following oral administration awaits confirmation.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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METHYLTHIOURACIL*

1. Chemical and Physical Data

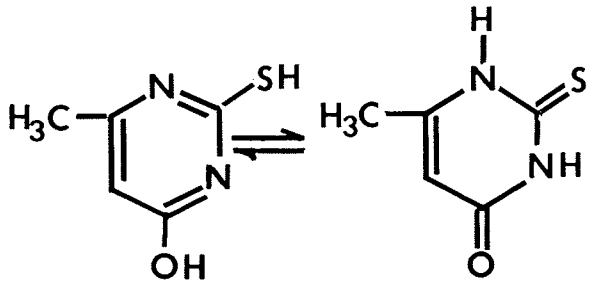
1.1 Synonyms and trade names

Chem. Abstr. No.: 56-04-2

2,3-Dihydro-6-methyl-2-thioxo-4(1H)-pyrimidinone; 2-mercapto-4-hydroxy-6-methylpyrimidine; 2-mercapto-6-methyl-4-pyrimidone; 2-mercapto-6-methyl-pyrimid-4-one; 6-methyl-2-thio-2,4(1H,3H)-pyrimidinedione; methyl thiouracil; methyl-thiouracil; 6-methyl-thiouracil; 6-methyl-2-thiouracil; 4-methyl-2-thiouracil; 4-methyluracil; MTU; 2-thio-6-methyl-1,3-pyrimidin-4-one; 6-thio-4-methyluracil; 2-thio-4-oxo-6-methyl-1,3-pyrimidine

Alkiron; Antibason; Basecil; Basethyrin; Metacil; Methacil; Methiacil; Methicil; Methiocil; Muracil; Orcanon; Prostrumyl; Strumacil; Thimecil; Thiomecil; Thiomidil; Thioryl; Thiothyron; Thiuryl; Thyreostat; Thyreostat I; Thyril; Tiotiron

1.2 Chemical formula and molecular weight



$C_5H_6N_2OS$ Mol. wt: 142.2

1.3 Chemical and physical properties of the pure substance

(a) Description: White, odourless, crystalline powder with bitter taste

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* Considered by the Working Group in Lyon, February 1974

(b) Melting-point: 326-331°C with decomposition

(c) UV absorption spectroscopy: λ_{max} 214 and 277 nm (in methanol)

(d) Identity test: The US Pharmacopeia (1955) describes identification tests based on (1) the blue colour produced after acidification of an alkaline mixture of the compound with sodium nitroprusside and (2) the formation of a stable white precipitate after boiling the substance with bromine and treating the bromine-free solution with barium hydroxide.

(e) Solubility: Very slightly soluble in cold water (0.1%) and ether; slightly soluble in alcohol, acetone and boiling water (0.7%); practically insoluble in benzene and chloroform; freely soluble in aqueous solutions of ammonia and in alkali hydroxides

(f) Volatility: Sublimes readily

(g) Reactivity: Forms complexes with metals and is oxidized by iodine and other sulphhydryl oxidizing agents

1.4 Technical products and impurities

Methylthiouracil, N.F. grade, with a specification of 97.0-101.5% $C_5H_6N_2OS$ (on a dry basis), 0.5% maximum loss on drying, 30 ppm maximum selenium, 20 ppm maximum heavy metals and 22.2-22.8% sulphur, was commercially available in the United States in the past (National Formulary Board, 1970).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Synthesis of methylthiouracil was first reported in 1886 by List (Merck & Co., 1968). It can be made by the condensation of ethyl acetoacetate with thiourea.

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

Although methylthiouracil has been listed in the US National Formulary, no evidence was found that it has ever been produced commercially in the US.

Methylthiouracil has been used in human medicine for the treatment of hyperthyroidism because of its ability to inhibit the synthesis of thyroid hormones. It is reported to be somewhat more effective than propylthiouracil (Goodman & Gilman, 1970). As of 1970, it was more widely used abroad than in the US, and no indication was found that methylthiouracil is presently being used in human medicine in the US.

In veterinary medicine methylthiouracil is reported to have been used as an anti-thyroid substance and was said to be more effective and less toxic than thiouracil. It was reportedly used for promoting growth and for fattening of swine and sheep (Merck & Co., 1968); however, no evidence was found that methylthiouracil is presently being used for this purpose.

2.2 Occurrence

Methylthiouracil has not been reported to occur in nature.

2.3 Analysis

Methylthiouracil may be estimated in tablets using 2,6-dichloroquinone chloroimide reagent (McAllister & Howells, 1952). For its determination in pharmaceutical preparations, the mercurimetric method of Abbott (1953) or its more recent potentiometric adaptation (Pinzauti et al., 1973) may be used. It may also be determined by potentiometric titration using 0.1 N chloramine T (Avakyanto & Murtazaev, 1969). For its detection in feed, Begliomini & Fravolini (1970) described a thin-layer chromatographic method sensitive to 1 µg. [See also the section, "General Remarks on Anti-thyroid Substances", p. 23.]

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

3.1 Carcinogenicity and related studies in animals

Experimental carcinogenesis with anti-thyroid chemicals including

methylthiouracil (MTU) has been reviewed by Christov & Raichev (1972a) and by Doniach (1970).

(a) Oral administration

Mouse: Of 15 NZO/Bl female mice given 0.05% MTU in the drinking-water for 42 weeks, diffuse hyperplasia of the thyroid with invasion of the surrounding tissue, and especially of the veins, was observed in 10 animals killed at that time. In another series, 14 mice, after a similar treatment for 42 weeks, were given thyroid powder for an additional 20 weeks; 12 mice developed involuted adenomas of the thyroid, and 7 had ectopic thyroid tissue in the lungs (Bielschowsky & Goodall, 1963).

In 25 male and 25 female C57 mice no evidence of neoplasia was observed after 69 weeks of administration of 0.05% MTU in the drinking-water. However, local papillary adenomas in 11/25 mice and a thyroid adenocarcinoma in 1/25 mice were observed in a group of mice treated similarly but kept on a low-iodine diet. [The levels of iodine in control and low-iodine diet were not specified.] No tumours occurred in 25 controls (Israel & Ellis, 1960).

No malignant changes were observed in the thyroid glands of 94 male and 82 female C3H/FIB inbred mice after administration of 0.1% MTU in the drinking-water for 22 months; the mice were maintained on a commercial diet. The higher number of hepatomas and of mammary tumours seen in treated animals was not statistically significant (Jemec, 1971).

Rat: Several workers have reported a high incidence of thyroid adenomas in rats after daily administration of 0.01-0.1% MTU in the drinking-water for over 42 weeks.

When MTU was administered continuously as a 0.01% solution in the drinking-water to 16 female 6-week old Wistar rats for up to 42 weeks, hyperplasia of the thyroid developed in 4/4 rats after 8-19 weeks of treatment. When the duration of treatment was extended to 42 weeks, 4 adenomas (1 multiple single adenoma at 21 weeks; 2 single adenomas and 1 large single cystic adenoma at 42 weeks) were detected in the remaining 12 animals (Hall, 1948). When administration of MTU was continued for 52-94

weeks, 2 malignant lesions of the thyroid (involving penetration of the capsule in one case and metastases in the lungs in the other) were found in 2/7 females killed between 78-94 weeks (Hall & Bielschowsky, 1949).

The production of adenomas of the thyroid in rats has also been reported by Napalkov (1959a), Ird (1968) and Tzelkov (1970).

In 9-month old Long-Evans female rats treated for 24-33 months with a daily dose level of 2.5 mg MTU given in a low-iodine diet, malignant thyroid tumours were observed in 8/24 animals and marked nodular changes in the thyroid in 15/24 rats. The 33% incidence of malignancy was reduced to 4% (1/28) when 0.3 mg/day thyroxine was administered simultaneously to a similar group of rats. Normal thyroids were observed in 25/31 rats given a low-iodine diet alone (Field et al., 1959).

Male and female Lister hooded rats of 10 weeks of age were given a saturated solution of MTU (approximately 0.1%) continuously in the drinking-water starting 24 hours after they had received 5, 30 or 100 μCi ^{131}I by i.p. injection. The animals were sacrificed at 15 months. Of the 20 survivors that had been treated with MTU only, adenomas of the thyroid gland were seen in 19 rats. The incidences of adenomas in groups administered 5 and 30 μCi ^{131}I only were 3/6 and 7/14, respectively. All of the 17 survivors that received 5 μCi ^{131}I + MTU had extremely numerous and large adenomas; some changes in the pituitary similar to those observed in the MTU groups, but no pituitary adenomas, were found. With an increase in the dose of radioactive iodine (30 μCi ^{131}I + MTU), all 20 survivors had adenomas, 5 of which were classified as malignant. Further increase in the dose of ^{131}I to 100 μCi did not result in increased malignancy. Micro-adenomas were seen in the thyroids of 7/9 control rats (Doniach, 1953).

Significant shortening of the latent period for the induction of thyroid adenomas was demonstrated in experiments with stock albino rats treated with 2-acetylaminofluorene (AAF) + MTU. However, the eventual yield of malignant thyroid tumours by the end of the experiment, which lasted for more than 18 months, proved to be equal in the group of animals treated with AAF in combination with MTU to that in the group receiving MTU alone (Napalkov, 1959b).

Data from several studies showed that the latent period for the induction of thyroid adenomas was shortened when injection of ^{131}I (latent period, 9 months) was followed by MTU treatment (latent period, 5-6 months) (Christov & Raichev, 1972a).

The effect of low-iodine diet and AAF in conjunction with chronic MTU treatment was studied by Lapis & Vekerdi (1962) in 2.5-4-month old Debrecen and CB albino rats. AAF was given at a dose level of 2.5 mg/rat thrice weekly by the intragastric route for the first 6 weeks, and 0.01% MTU was administered in the drinking-water for the total period (71 weeks) of the experiment. While treatment with AAF alone did not produce thyroid tumours in any of 30 animals, combined exposure to AAF + MTU and low-iodine diet resulted in adenoma formation in all animals surviving for 5 months or longer (number not stated). MTU alone produced a single case of micro-adenoma in 1/25 surviving animals maintained on a low-iodine diet.

Intact and castrated 4-5-week old albino female rats were administered 15 mg/day MTU on 5 days per week in the diet. All the rats were sacrificed after 9 months of treatment. In the intact and castrated rats treated with MTU, 10/27 and 10/16 rats developed kidney adenomas and adenocarcinomas, compared with 1/29 and 0/6 of controls (Akimova, 1962).

In a series of experiments summarized by Cherry & Glucksmann (1970), the incidence of cervico-vaginal sarcomas was studied in groups of 36-43 female hooded rats of the Lister strain given weekly intravaginal paintings of a 1% solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) in combination with 0.1% MTU in the drinking-water for 70 days prior to the DMBA treatment. Treatment with MTU increased the incidence from 72% and 25% in intact and ovariectomized controls given DMBA treatment alone to 77% and 90% in the MTU + DMBA treated animals. The latent period of tumour induction was 14-43 weeks in the combined treatment groups, compared with 29-57 weeks in the rats treated with DMBA alone.

Hamster: Groups of 3-month old female hamsters were administered 0.2% MTU in the drinking-water, alone or in combination with a single i.p. injection of 10 μCi ^{131}I given 24 hours before starting MTU administration,

which was then continued over a period of 52 weeks. Two further groups received ^{131}I only or served as untreated controls. Animals were killed at 2, 3, 4, 5, 6, 8, 10 and 12 months after the beginning of treatment. No adenomas were found in controls (4-6 animals killed at each time). Adenomas were first observed in the MTU group at 5 months (1/10), and the incidences increased to 3/10, 4/10, 5/12 and 7/12 animals at 6, 8, 10 and 12 months, respectively. In the ^{131}I group, adenomas were first detected at 10 months (2/8), and the incidence increased to 4/8 at 12 months. In the animals which received ^{131}I + MTU, adenomas appeared earlier, at 4 months (2/8), and the incidences progressively increased to 3/10, 4/8, 6/10, 7/8 and 11/12 at 5, 6, 8, 10 and 12 months. In addition, carcinomas were seen in 1, 3, 3 and 4 animals sacrificed from this group at 6, 8, 10 and 12 months. In all three groups multiplicity of adenomas increased with the duration of the experiment, the highest occurring in the ^{131}I + MTU group. The MTU-induced adenomas were chiefly of a papilliferous type and were localized in the peripheral zone of the gland. Thyroid carcinomas in the ^{131}I + MTU group showed marked infiltrative growth into the capsule of the gland or into the nearby soft tissue. Some tumour cells were found to have invaded medium and large blood vessels, and lung metastases were also observed (Christov & Raichev, 1972b).

(b) Subcutaneous implantation

Rat: Spindle-cell and polymorphous-cell sarcomas were detected in 3/31 rats, at the implantation site, after s.c. implantation of a pellet of 100 mg MTU in 200 mg lanolin and 100 mg paraffin each month for 393-618 days. In addition, adenomas of the thyroid and pituitary were found in 11/31 and 2/31 of the animals. No tumours were found near similarly implanted control pellets (Napalkov & Salyamon, 1968).

(c) Other experimental systems

Pre- and postnatal exposure: Napalkov & Alexandrov (1968) and Napalkov (1969) studied the incidence of thyroid tumours in several generations of rats exposed to MTU in utero as well as during postnatal life. Random-bred white rats of both sexes were given 5-6 mg MTU orally during their entire lifespan; treatment was continued in females during pregnancy

and lactation. Newly-born rats were also treated with MTU for their lifespan, and after maturation were mated and the same treatment was given to their offspring. In total, 130 rats surviving more than 500 days and belonging to 9 subsequent generations were studied under the conditions of the above permanent treatment. In a second experiment, the incidence of thyroid tumours was studied in rats of 17 successive generations also subjected to the continuous action of MTU, but bred by brother-to-sister mating in each litter. Three hundred and nineteen rats in this group survived more than 500 days. Thus, in both series, each animal was subjected to the MTU influence during all prenatal and postnatal oncogenesis, having been born of parents fed with the same compound. Rats which received MTU in postnatal life only and which were born of animals not exposed to MTU in utero were used as controls. When the tumour incidence in each generation was plotted against generation number, a wave-like distribution was observed in both experimental groups. Thus, in the random-mated rats, the tumour incidence among the first four generations was significantly higher than that in the controls (81.0 ± 4.6% versus 51.3 ± 8.1%). In the subsequent 5th-7th generations, the tumour incidence decreased sharply to 47.1 ± 8.7%, but again increased to 80.0 ± 13.3% in rats of the 8th and the 9th generations. A similar two-peak curve of distribution of tumour incidence was also shown by brother-to-sister mated rats observed for 17 generations, but the peak tumour incidence and its fall occurred 2-3 generations later than in the first group. The incidence of malignant tumours in both groups of experimental rats which had received MTU for successive generations was lower, or at least not higher (33.5 ± 3.6% and 41.2 ± 2.9%) than in controls treated during postnatal life only (53.1 ± 6.3%). However, low-differentiated and squamous-cell carcinomas of the thyroid were found in these rats, in addition to commonly observed benign and malignant adenomas. In the progeny of tumour-free animals and in those from parents bearing benign or malignant tumours, the average incidence of thyroid tumours was approximately the same (61.8 ± 5.9%, compared with 58.0 ± 7.1% and 69.9 ± 4.3%). However, malignant neoplasms were less frequent (27.6%) in rats born of parents with only benign thyroid tumours.

Prenatal exposure: A transplacental carcinogenic effect of MTU was demonstrated by Savel'eva (1971) who treated pregnant rats from the 13th-14th day after conception until the end of gestation with 10 mg/100 g bw MTU and subsequently exposed the offspring to 2 mg/100 g bw acetylaminofluorene (AAF) daily for 150 days beginning from the 45th day of life. Two additional groups of mothers were subjected either to thyroidectomy or to sham operation, and their offspring were treated with AAF as above. Liver tumours were observed in 62-72% of survivors from all three groups; while, in addition, 29% of the offspring of mothers treated with MTU during pregnancy and AAF postnatally developed thyroid tumours. Transplacental exposure to MTU alone without subsequent treatment with AAF did not result in thyroid tumour development in any of the 26 rats surviving until the end of the experiment. No tumours were observed in 25 intact control animals.

3.2 Other relevant biological data

Following the i.v. injection of 5 mg/rat MTU, 84-90% of the dose could be recovered from the carcasses of animals killed after 1 minute and 55-60% from the carcasses of animals killed after 3 hours. After 3 hours the concentration of MTU in the thyroid was approximately 1 mg/g of tissue (Williams & Kay, 1947).

MTU crosses the placental barrier and is excreted in the milk of lactating rats (Napalkov & Alexandrov, 1968).

3.3 Observations in man

In an extensive review of 1116 cases treated with methylthiouracil, Vanderlaan & Storrie (1955) described the adverse effects of this treatment. No specific mention of cancer is made in this series.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Methylthiouracil (MTU) administered to mice, rats and hamsters by the oral route produced thyroid tumours in all 3 species. It was similarly effective in rats following s.c. implantation. Kidney tumours were induced in female rats following oral administration. It enhanced the tumourigenic response of local application of DMBA, producing cervico-vaginal tumours in ovariectomized female rats. In rats and hamsters, combined treatment with ^{131}I and MTU, but not treatment with ^{131}I alone, increased the incidence of malignant thyroid tumours. There exists an inverse relationship between iodine content of the diet and MTU thyroid tumourigenicity.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] This section should be read in conjunction with the section, "General Remarks on Anti-thyroid Substances" (p. 23) and the section "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

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PROPYLTHIOURACIL*

1. Chemical and Physical Data

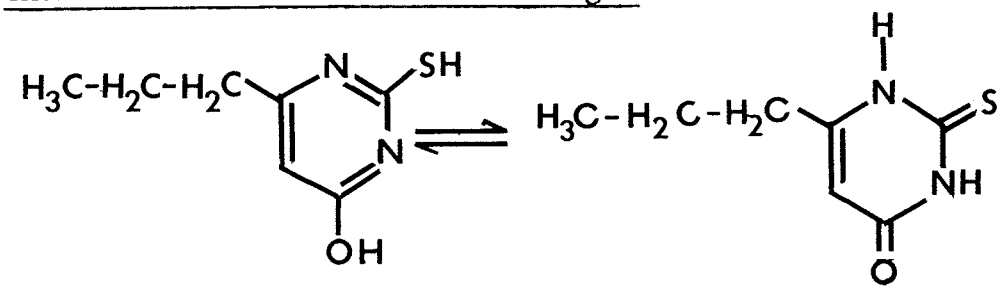
1.1 Synonyms and trade names

Chem. Abstr. No.: 51-52-5

2,3-Dihydro-6-propyl-2-thioxo-4(1H)-pyrimidinone; 2-mercapto-4-hydroxy-6-n-propylpyrimidine; 2-mercapto-6-methyl-4-pyrimidone; 2-mercapto-6-methylpyrimid-4-one; 6-propyl-2-thio-2,4(1H,3H)-pyrimidinedione; propyl thiouracil; propyl-thiouracil; 6-propylthiouracil; 6-n-propylthiouracil; 4-propyl-2-thiouracil; 6-propyl-2-thiouracil; 6-n-propyl-2-thiouracil; propythiouracil; PTU; 2-thio-4-oxo-6-propyl-1,3-pyrimidine; 2-thio-6-propyl-1,3-pyrimidin-4-one; 6-thio-4-propyluracil

Procasil; Propacil; Propycil; Propyl-thiorit; Propyl-Thyracil; Prothycil; Prothyran; Thyreostat II

1.2 Chemical formula and molecular weight



$C_7H_{10}N_2OS$ Mol. wt: 170.2

1.3 Chemical and physical properties of the pure substance

(a) Description: White, crystalline powder

(b) Melting-point: 219-221°C

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* Considered by the Working Group in Lyon, February 1974

(c) UV absorption spectroscopy: λ_{max} 214 nm; log ε 4.193} (in methanol)
λ_{max} 275 nm; log ε 4.199}

λ_{max} 207.5 nm; log ε 4.188}
λ_{max} 260 nm; log ε 4.029} (in methanol-KOH)
λ_{max} 315.5 nm; log ε 4.037}

(d) Identity test: The US Pharmacopeia (1970) gives two tests for identification of propylthiouracil: the formation of a complete solution of 25 mg of the substance in 1 ml strong ammonia, and the formation of a stable white precipitate with barium hydrochloride after treatment with bromine. The 2,6-dichloroquinone chloroimide test (McAllister & Howells, 1952) and the microscopic test (Ashley, 1953) may also be employed.

(e) Solubility: Slightly soluble in water at 20°C (about 1 part in 900); soluble in boiling water (1 part in 100), ethanol (1 part in 60) and acetone (1 part in 60); practically insoluble in ether, chloroform and benzene; freely soluble in aqueous solutions of ammonia and alkali hydroxides

(f) Reactivity: Forms complexes with metals and reacts with sulphhydryl-oxidizing agents

1.4 Technical products and impurities

Propylthiouracil is available in the United States as a USP grade containing 98-100.5% active ingredient on a dried basis (US Pharmacopeia, 1970). Small amounts of thiourea may be present as an impurity.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Synthesis of propylthiouracil was first reported in 1945 (Anderson

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

et al., 1945), and commercial production in the US was first reported in 1947 (US Tariff Commission, 1949). Propylthiouracil is believed to be made by the condensation of ethyl butyrylacetate with thiourea.

Two US companies reported commercial production of propylthiouracil to the US Tariff Commission in 1970, and another company is also believed to have been producing it. One of the reporting companies has since stopped production. No separate data are available on the quantity of propylthiouracil produced by the remaining reporting producer, but combined production of a group of ten chemicals classified as "other hormones and synthetic substitutes" and including propylthiouracil and two other anti-thyroid agents was reported to have been 88,000 kg in 1971 (US Tariff Commission, 1973).

Propylthiouracil is widely used as an anti-thyroid agent for the treatment of hyperthyroidism, the usual dose for this purpose being 100 mg every 8 hours. In a few cases (5%) this dosage may be raised to as much as 600 mg daily. Greer et al. (1965) found, from data collected over a 4-year period, that a single daily dose of 300 mg may be as effective as the divided dose.

Veterinary applications of propylthiouracil are reported to have included its use as a metabolic depressant to promote fattening in animals (Merck & Co., 1968); however, no evidence was found that propylthiouracil is presently being used for this purpose.

2.2 Occurrence

Propylthiouracil has not been reported to occur in nature.

2.3 Analysis

For its determination in pharmaceutical preparations the mercurimetric method of Abbott (1953) has been used; a modification of this method has been presented by Pinzauti et al. (1973). It may also be determined in such preparations by potentiometric titration employing 0.1 N chloramine T (Avakyants & Murtazaev, 1969) and can be estimated in feed material by chromatography over silica gel G (sensitivity, 1 μg) (Begliomini & Fravolini, 1970).

The colorimetric method of McAllister & Howells (1952) employing 2,6-dichloroquinone chloroimide reagent at pH 8.0 has been modified by Ratliff et al. (1972) to measure propylthiouracil concentrations of 0.5-10 µg/ml in serum. Marchant et al. (1971) separated propylthiouracil, thiourea and sulphate by thin-layer chromatography on cellulose and silica gel plates using the solvent systems ethanol:1 M ammonium acetate (7.5:3.0 v/v, freshly prepared) and chloroform:methanol:water (160:40:25), respectively. [See also the section, "General Remarks on Anti-thyroid Substances", p.23].

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

3.1 Carcinogenicity and related studies in animals

Experimental carcinogenesis with anti-thyroid chemicals, including propylthiouracil (PTU), has been reviewed by Christov & Raichev (1972) and by Doniach (1970).

(a) Oral administration

Mouse: In male A strain mice administered 0.8% PTU in a commercial diet with meat meal for a period of 77 weeks, beginning when the animals were 4-6 weeks old, chromophobe adenomas of the anterior lobe of the pituitary gland were observed in 3/4 mice, and all 4 mice had carcinomas of the thyroid. The pituitary glands of a similar group of surgically thyroidectomized mice were normal (Moore et al., 1953).

Administration of PTU at concentrations of 10 and 12 g/kg in a commercial diet to 4-5-week old C57BL mice for 17 months induced pituitary adenomas in 15/24 (62%) and 21/29 (72%) of animals surviving more than 17 months. The average weights of pituitary glands in these two groups (10 and 18 mg) were considerably higher than those of the 28 controls, in which no tumours were observed (King et al., 1963).

Rat: In 48 female Wistar rats administered 0.2% PTU in the diet and killed periodically from 2-14 months, 24 adenomas of the thyroid (4 solid and 20 cystic type) were observed (Van Dyke, 1953). With a lower dose (0.02%), no tumours were detected in 54 males and 54 females of the same

strain killed at intervals up to 15 months after the start of treatment (Sellers & You, 1951).

In a subsequent study, simultaneous administration of 0.02% sodium iodide or 0.02% dried thyroid powder together with 0.02% PTU or administration of 0.02% PTU alone in the diet for 15 months or more produced enlargement of the thyroid and some thyroid adenomas in 26, 24 and 37 male and female Wistar rats in the 3 groups, respectively. In 3 or 4 animals (1 with PTU alone and 2 or 3 with PTU + thyroid powder), metastatic thyroid tissue was found in the lungs. The pituitary glands of rats receiving PTU + thyroid powder were significantly heavier than those in the other groups, and single or multiple chromophobe adenomas occurred frequently in this group (Sellers et al., 1953).

A high incidence of thyroid tumours was reported in white rats maintained alternately on 0.1% PTU in the drinking-water (to produce hyperplasia, which occurred usually within 2 weeks of PTU treatment) and on 0.1% potassium iodide (KI) in the drinking-water (to involute the gland). Administration was continued for a period of about one year. A further group received 0.1% PTU alone. Thyroid tumours (19 adenomas and 1 carcinoma) developed in 17/29 survivors in the PTU + KI group; whereas among the 15 survivors in the PTU group, only 4 had thyroid tumours, all single and benign (Zimmerman et al., 1954).

Wistar albino rats of both sexes (6-8 weeks of age at the beginning of treatment) received PTU in the drinking-water for up to 18 months. The initial dose (0.2% PTU) was reduced to 0.1% at 13 weeks, to 0.05% at 26 weeks and to 0.025% at 52 weeks. In surviving male rats, thyroid adenomas were found in 11/18 and thyroid carcinomas in 3/18; while 20 adenomas and 4 carcinomas were observed in 30 surviving females. Additional treatment with ^{131}I (30 μCi, i.p., at the start of the experiment) did not significantly increase the tumour incidence. In the untreated controls killed at approximately 20 months of age, only thyroid adenomas were detected, the incidence being 2/20 in males and 1/20 in females. When PTU was administered in the drinking-water at concentrations adjusted to give an intake of 7 mg/kg bw/day intially, and rapidly reduced over a period of 3 months to 1 mg/kg

bw/day, which was then continued until termination of the experiment at 18 months (this intake being similar to the suggested human dose), adenomas or carcinomas of the thyroid were observed in 3/5 and 9/13 male and female survivors (Willis, 1961).

In male Long-Evans rats, 0.1% PTU alone or in combination with 250 ppm dried thyroid powder (DTP) was administered in the diet for 1 year after an initial single i.p. injection of 25 μCi ^{131}I. Of 65 animals examined that had received the combined treatment (PTU + ^{131}I + DTP), 64 developed tumours of the thyroid (51 adenomas and 13 carcinomas), while only adenomas (23/35) were found in the group that received ^{131}I + PTU. PTU alone also produced only thyroid adenomas in 16/33 rats (Lindsay et al., 1966).

Hamster: When 0.2% PTU was administered in the drinking-water to 3-month old Syrian (golden) hamsters of both sexes for 100 weeks, 13/58 males and 9/44 females developed malignant lesions of the thyroid with no metastases. In addition, 4 males and 6 females developed thyroid cancers with metastases. There were no controls, but the incidence of thyroid cancers in untreated hamsters was reported by Fortner et al. (1960) to be 8/523 (1.5%). No adenomas were seen (Sichuk et al., 1968).

Guinea-pig: Administration of 0.03% PTU in the drinking-water for a period of 104 weeks to 20 male guinea-pigs induced thyroid adenomas in 3/20 animals. Repeated s.c. injections with a thyroid-lipid extract in conjunction with 0.03% PTU in the drinking-water for 104 weeks substantially increased the tumour incidence (12/20), and the thyroid tumours appeared earlier (9 months compared with 14 months). No thyroid adenomas occurred in 20 untreated controls (Hellwig & Welch, 1963).

Dog: In a short-term study, Mayer (1947) found only hyperplasia of the thyroid gland in 12 beagle dogs treated from 5-6 weeks of age with 21-33 mg/kg bw/day PTU daily for a period of 6.5-8 months.

3.2 Other relevant biological data

Orally administered ^{35}S-labelled PTU is absorbed from the gastrointestinal tract in the rat and man. Plasma half-lives of 2.5 hrs (man) and 4.8 hrs (rat) have been reported (Marchant et al., 1971). In the rat, 75-90% of the ^{14}C-labelled PTU administered by the oral, i.p. and i.v. routes

was excreted in the urine within 24 hours; and 14% of the PTU was excreted in the bile as a glucuronide, demonstrating an enterohepatic circulation. The major urinary metabolite was also a glucuronide conjugate of PTU and accounted for approximately 40-48% of the activity excreted in the urine within 24 hours (Sitar & Thornhill, 1972).

PTU was concentrated by the thyroid gland, and four ^{35}S compounds were demonstrated by thin-layer chromatography in both rat and man: unchanged PTU, ^{35}S sulphate, an unknown PTU metabolite and protein-bound ^{35}S (Marchant et al., 1971).

Placental transfer of PTU was demonstrated in guinea-pigs (D'Angelo, 1967).

3.3 Observations in man

In an extensive review of 2491 cases treated with propylthiouracil, Vanderlaan & Storrie (1955) described the adverse effects of this treatment. No specific mention of cancer is made in this series.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Propylthiouracil produced thyroid tumours in mice, rats, hamsters, and guinea-pigs following oral administration, the only route tested. In mice, pituitary adenomas were also observed.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] This section should be read in conjunction with the section "General Remarks on Anti-thyroid Substances" (p.23) and the section "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

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THIOACETAMIDE*

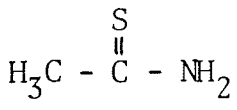
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 62-5-55

Acetothioamide; ethanethioamide; TAA

1.2 Chemical formula and molecular weight



C_2H_5NS Mol. wt: 75.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless leaflets; slight odour of mercaptans

(b) Melting-point: 113-114°C

(c) UV absorption spectroscopy: λ_{max} 210 nm; log ε 3.66
λ_{max} 261 nm; log ε 4.08 (in water)
λ_{max} 318 nm; log ε 1.80

(d) Solubility: Soluble at 25°C in water (16.3%) and in ethanol (26.4%); sparingly soluble in ether

(e) Reactivity: Forms addition compounds and sulphides with salts of heavy metals; hydrolyzed by acids or bases, forming acetic acid, ammonia, nitrogen and hydrogen sulphide

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* Considered by the Working Group in Lyon, February 1974

1.4 Technical products and impurities

Thioacetamide is available in the United States as a laboratory chemical containing 99.0% active ingredient.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Synthesis of thioacetamide by the reaction of ammonium acetate and aluminium sulphide was first reported in 1921 (Kindler & Dehn, 1921). Subsequently, methods based on the reaction of hydrogen sulphide with acetonitrile and on the reaction of tripotassium thiophosphate with acetamide were reported (Kindler, 1923; Schultz & Ranke, 1961). Whether any of these methods are used for commercial production of thioacetamide is not known.

Thioacetamide is produced in the US by four laboratory chemical manufacturers. Although the quantity of thioacetamide produced by these manufacturers is not known, it is believed to be quite small: All US companies are required to report production of a synthetic organic chemical to the US Tariff Commission when the annual output exceeds 454 kg in amount or $1,000 in value, but no companies have reported production of thioacetamide to the US Tariff Commission since 1967 when only one company did so (US Tariff Commission, 1969).

Thioacetamide appears to have only one significant use at present - as a substitute for hydrogen sulphide in the chemical laboratory. However, no information is available on the quantity used for this purpose. Hueper & Conway (1964) reported that thioacetamide has also been used as an organic solvent in the leather, textile and paper industries, as an accelerator in the vulcanization of buna rubber, as a stabilizer of motor fuel containing tetraethyllead, as a solubilizer for riboflavin and, in combination with mercury, as a mordant of seed grain. However, no evidence was found that thioacetamide presently finds use in these applications.

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

2.2 Occurrence

Thioacetamide has not been reported to occur in nature.

2.3 Analysis

A potentiometic method using a sulphide ion-selective membrane electrode following the titration of thioacetamide with silver nitrate has been described (Pápay et al., 1973). [See also the section "General Remarks on Anti-thyroid Substances", p. 23.]

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Eighty-nine male and female Swiss mice, 2 months old, were administered a stock diet containing 0.03% thioacetamide or the stock diet alone. About 6 males and 6 females were killed at the ages of 6, 9, 13 and 17 months in both treated and control groups. In the treated 17-month old mice, 6/6 males and 6/7 females developed liver tumours; although metastatic lesions were not seen, these tumours were diagnosed as carcinomas and were transplantable. In the mice killed at earlier times, hypertrophy of hepatic cells, hyperplasia of biliary cells and cirrhosis were observed. In controls, including those sacrificed at 17 months of age, no liver tumours were seen (Gothoskar et al., 1970).

Rat: Male albino rats (3 weeks of age) were divided into groups of ten animals and fed diets containing 0.005%, 0.01%, 0.025%, 0.05% or 0.1% thioacetamide for a period of 18 months. At the 0.005% and 0.01% dose levels, slight to moderate cirrhosis of the liver was observed, and 1/6 survivors developed a hepatic-cell adenoma. In the animals treated with 0.05% thioacetamide, one hepatocellular carcinoma was observed among an unspecified number of survivors. Liver tumours were not observed in the controls. The survival time of animals administered 0.1% thioacetamide was less than one month, and it was also reduced in the rats treated with 0.025% thioacetamide. In addition, cirrhosis was present among the various groups (Fitzhugh & Nelson, 1948).

In a group of 150 stock albino Wistar rats of both sexes administered 0.032% thioacetamide in the diet and killed at various intervals, localized areas of cholangiofibrosis were seen after 11 weeks. Of 36 animals killed between 9 and 23 weeks, 22/36 had cholangiofibrosis, 18/36 had bile duct tumours (not specified) and 5/36 showed cystic dilatations of bile ducts. No such changes were observed in 50 controls (Gupta, 1955). In a later paper it was reported that metastases of the liver tumours were observed in the ovaries in 4/5 rats similarly treated with thioacetamide for 47 weeks or longer (Gupta, 1956).

In a group of 90 male albino rats administered 0.03% thioacetamide in the diet and killed serially over a 14-month period of treatment, an unspecified number of rats developed benign, cholangiocellular neoplasms of the adenomatous type (Martini & Caravaglios, 1956).

Hamster: A 1% solution of thioacetamide in distilled water was administered weekly by stomach tube at a dose of 2.5 mg/animal to 10 male and 10 female Syrian golden hamsters for 30 weeks, and the animals were observed until death. The few tumours observed in various organs did not differ from those observed in control hamsters in that colony (Terracini & Della Porta, 1961).

3.2 Other relevant biological data

After s.c. injection of 6 mg ^{35}S-thioacetamide in rats, more than 80% of the radioactivity was excreted in the urine within 24 hours. About 25% was excreted unchanged, and the remainder was excreted as free or esterified ^{35}S-sulphate. The liver did not concentrate ^{35}S to a greater extent than did other tissues (Nygaard et al., 1954).

When 5 mg ^{3}H-thioacetamide were administered orally in the diet to male albino rats, less than 1% was excreted unchanged in the urine. Almost all of the thioacetamide was metabolized to acetate within 24 hours. Radioactivity was found in all organs examined (liver, kidney and adrenal gland) and was highest in the liver. Liver tissue slices were found to be 3 times more active than kidney slices in converting thioacetamide to acetamide. It was suggested that thioacetamide is metabolized *in vivo* to acetamide which is itself carcinogenic (Jackson & Dessau, 1961), and that

the acetamide is then hydrolyzed to acetate (Rees et al., 1966).

After i.v. administration of 40 mg/100 g bw ^{35}S-thioacetamide to rats, 80% of the radioactivity was excreted in urine as thioacetamide or the sulphoxide within 24 hours (Schlicht, 1971).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Thioacetamide is carcinogenic to mice and rats following oral administration, the only route tested. It induced liver-cell tumours in mice and liver-cell and bile duct tumours in rats. No carcinogenic effects were observed in hamsters following its oral administration.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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THIOURACIL*

1. Chemical and Physical Data

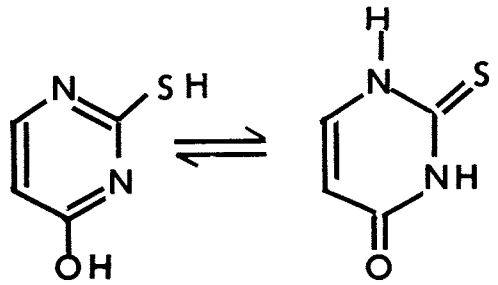
1.1 Synonyms and trade names

Chem. Abstr. No.: 141-90-2

2,3-Dihydro-2-thioxo-4(1H)-pyrimidinone; 6-hydroxy-2-mercapto-pyrimidine; 2-mercapto-4-hydroxypyrimidine; 2-mercapto-4-pyrimidinol; 2-mercapto-4-pyrimidone; 2-mercapto-pyrimid-4-one; 2-thio-4-oxo-1,3-pyrimidine; 2-thio-2,4-(1H,3H)-pyrimidinedione; 2-thio-1,3-pyrimidin-4-one; 2-thiouracil; 6-thiouracil; TU; 2-TU

Antagothyroid; Deracil

1.2 Chemical formula and molecular weight



$C_4H_4N_2OS$ Mol. wt: 128.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Minute crystals, prisms, or white or pale cream-coloured odourless powder with bitter taste

(b) Melting-point: No definite m.p.; about 340°C with decomposition

(c) UV absorption spectroscopy: λ_{max} 212 and 271 nm (in methanol)

(d) Identity test: The British Pharmacopoeia (1948) gives an identification test based on the melting-point (60-61°C) of the

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* Considered by the Working Group in Lyon, February 1974

purified product formed after refluxing thiouracil with phosphorus pentachloride. It may be identified by use of 2,6-dichloroquinone chloroimide reagent (McAllister, 1951), by a negative iodobismuthous acid test (McAllister, 1952) or by microscopic tests (Ashley, 1953; Kofler & Kolšek, 1970).

(e) Solubility: Very slightly soluble in water (0.05%); practically insoluble in alcohol, ether and acids; readily soluble in aqueous solutions of alkali hydroxide

(f) Reactivity: Forms precipitates and complexes with heavy metals and other compounds; reacts with various sulphhydryl reagents

1.4 Technical products and impurities

No information was available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Synthesis of thiouracil by the condensation of ethyl formylacetate with thiourea was first reported by Wheeler & Liddle (1908). Whether this method is used for commercial production of thiouracil is not known.

Thiouracil is produced in the US by one company. No separate data are available on the quantity of thiouracil manufactured by this company, but combined production of a group of ten chemicals classified as "other hormones and synthetic substitutes", including thiouracil and two other anti-thyroid agents, was reported to have been 88,000 kg in 1971 (US Tariff Commission, 1973).

Thiouracil has reportedly been used in human medicine as an anti-thyroid agent and in the treatment of angina pectoris and congestive heart failure (Merck & Co., 1968). However, no evidence was found that thiouracil presently finds use in the US in these applications.

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

The present major use for thiouracil in the US is believed to be as a chemical intermediate in the synthesis of iothiouracil sodium (sodium salt of 5-iodo-2-thiouracil), an anti-thyroid agent used in the treatment of hyperthyroidism. Total US sales of iothiouracil sodium for use in human medicine are estimated to be less than 100 kg annually.

2.2 Occurrence

Thiouracil has not been reported to occur in nature.

2.3 Analysis

The mercurimetric method of Abbott (1953) has been the basis for the determination of thiouracil in pharmaceutical preparations; a modification of this method has been presented recently by Pinzauti et al. (1973). Thin-layer chromatography using chloroform:methanol (90:10 v/v), isobutyric acid:ammonium hydroxide:water (33:1:16 v/v), butanol:water (86:14) and distilled water as solvent systems has been employed for verifying the purity of ^{14}C-thiouracil (Quinones et al., 1972).

Thiouracil and its derivatives are determined colorimetrically in serum, blood and urine by the use of Grote's reagent (prepared by treating sodium nitroprusside in sodium bicarbonate with hydroxylamine hydrochloride and then with bromine; excess bromine is removed either by aeration or, preferably, by the addition of phenol). Buffering at pH 8-9 is recommended for optimum colour intensity (Anderson, 1944; Christensen, 1945, 1946; Williams et al., 1944). However, since these colorimetric methods are non-specific, more recent determinations of thiouracil and its metabolites have employed ^{35}S-labelled thiouracil and separation by chromatographic procedures (Lees et al., 1973; Maloof & Soodak, 1965). [See also the section "General Remarks on Anti-thyroid Substances", p. 23.]

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Eighty-one mice of 3 inbred strains (A, C57 and I) were

administered 0.1% thiouracil (TU) in a commercial diet for periods of up to 80 weeks. In 69 mice examined, thyroidal follicular cysts were found and interpreted by the authors as non-malignant lesions, since they regressed following withdrawal of TU (Gorbman, 1947).

Of 52 C strain mice (40 males and 12 females) administered 0.375% TU in the diet for 6 months followed by 0.5% for life, the last animal died at 116 weeks, and nodular hyperplasis of the thyroid occurred in 28/39 mice surviving longer than 10 months (Dalton et al., 1950).

In (C57xCBA)F_1 female mice administered 0.2% TU in the diet for periods ranging from 11-29 months, hepatomas were observed in 6/21 of the treated animals. The hepatoma incidence in controls was not reported (Miller & Gardner, 1954).

After administration of 0.3% TU in the diet of C3H and TM male and female mice for 17 months, hepatomas developed in C3H mice (12/13 in males and 14/16 in females) but not in the TM strain mice (0/22 males and 0/22 females). In the C3H controls, hepatomas were found in 2/32 males and 0/24 females kept for the same period (Casas, 1963).

Rat: Nodular hyperplasia of the thyroid was observed in Stanford albino rats of both sexes after administration of 0.1% TU in the diet for up to 45 weeks. The incidence in males increased from 3/10 to 11/20 after treatment for 29 and 45 weeks, respectively. No increase was observed in female rats (3/10, compared with 5/15) examined after treatment for 29 and 38 weeks (Laqueur, 1949).

After administration of 0.05 or 0.1% TU in the drinking-water to Sherman rats for periods of 35-126 weeks, 11/20 rats developed adenomas and 1/20 a carcinoma of the thyroid. Simultaneous administration of 0.03% 2-acetylaminofluorene (AAF) in the diet increased the incidence of adenomas and carcinomas to 28/28 and 5/28, respectively, in rats autopsied after a treatment period of only 22-45 weeks (Paschkis et al., 1948).

Administration of 0.1% TU in the drinking-water of 81 male Sprague-Dawley rats resulted in the development of thyroid tumours (not specified) in 100% of the animals after 14 weeks of treatment (Money et al., 1953).

Similar results (65% incidence) were obtained in male Sprague-Dawley rats administered 0.2% TU in the drinking-water for 24 months (Clausen, 1954).

A low incidence of hepatomas (2/22) was observed in albino rats treated with 0.05% TU and 2% cholesterol in the diet for a period of 140 weeks. No hepatomas were observed in 17 rats fed TU alone for 126 weeks (Nelson et al., 1954).

The combined effect of low iodine and 0.25% TU in the diet was studied by Wollman (1961) in 5-9-week old Fischer 344, AxC 9935 and Marshall 520 rats. In 37 Fischer rats, 11 columnar papillary nodules, 5 small-cell papillary nodules and 1 cellular nodule of the thyroid were observed after 6-13 months of treatment. In 35 Marshall rats which were administered the diet for 1-8 months, 1 possible columnar papillary nodule of the thyroid was observed. In studies with 21 AxC rats, the TU level was increased to 0.5% after 9 months and continued for 24-30 months. After 24 months or more of treatment, 3 columnar, 1 small-cell papillary and 1 cellular nodule of the thyroid were found. Implants of pieces of hyperplastic thyroid tissue from each strain grew in isogeneic recipients. In the Fischer strain these transplants grew for several generations in rats without TU in the diet, as well as in those which underwent hypophysectomy.

Fish: The development of cutaneous melanomas was studied by Stolk (1959) in the female hybrids of Xiphophorus helleri Heckel x Xiphophorus maculatus Günther after administration of 2 g/kg TU in the food for a period of 27 weeks, starting when the fish were 54 weeks of age. Tumours were found in 30/30 TU-fed fish and in 21/30 controls, with an average of 5.2 and 3.1 tumours/tumour-bearing fish, respectively.

Other species: [The Working Group was aware of TU feeding studies in cats (McClosky et al., 1947), dogs (Steiner et al., 1949) and monkeys (Aranow et al., 1946), but the durations of treatment and numbers of animals used were too limited to allow any conclusions to be made concerning carcinogenicity.]

3.2 Other relevant biological data

TU is absorbed from the gastrointestinal tract in rats and man. In rats administered 5 mg by i.v. injection, 30% of the TU was recovered from the carcasses after 3 hours and only traces after 24 hours. In man, a single oral dose of 100 mg was almost completely eliminated from the blood within 24 hours. Fifteen per cent was broken down in the intestine and 30-50% in other tissues and body fluids, the remainder (approximately 30%) being excreted as TU in the urine (Williams & Kay, 1944).

In homogenized rat liver preparations from female Holtzman rats, 28-35% of the TU was metabolized within 3 hours. The pathway for the breakdown of TU was suggested to be as follows: uracil; β-ureidopropionic acid, which was further metabolized to β-alanine; ammonia and carbon dioxide (Spector & Shideman, 1959).

In Sprague-Dawley rats given a single i.p. injection of 5 mg ^{35}S-TU, thyroid accumulation of the ^{35}S-label began at 4 hours and reached a peak at 10 hours. The concentration gradient between thyroid tissue and plasma was 7.5 at 10 hours and 156 after 48 hours. Five ^{35}S compounds were detected in the thyroid by thin-layer chromatography: ^{35}S-sulphate, protein-bound ^{35}S, unmetabolized TU and two unidentified metabolites (Lees et al., 1973).

TU is transferred across the placenta in rabbits and dogs (Quinones et al., 1972).

3.3 Observations in man

Crane & Payne (1946) reported the case of a white female, 56 years of age, with hyperthyroidism, who was treated with thiouracil for 6 weeks before subtotal thyroidectomy. They observed in each lobe one small area with irregular glandular and papillary growth, which was interpreted as carcinoma. [The case is of doubtful significance.]

In an extensive review of 2490 cases treated by thiouracil Vanderlaan & Storrie (1955) described the adverse effects of this treatment. No specific mention of cancer is made in this series.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Thiouracil increased the incidence of liver-cell tumours in mice and produced thyroid tumours in several strains of rats following oral administration, the only route tested. Studies in cats, dogs and monkeys were inadequate in group size and duration to allow evaluation of carcinogenicity. The study in fish reported did not demonstrate a significant carcinogenic effect.

4,2 Human data

No adequate case reports or epidemiological studies were available to the Working Group.

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[1] This section should be read in conjunction with the section, "General Remarks on Anti-thyroid Substances" (p. 23) and the section, "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

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THIOUREA*

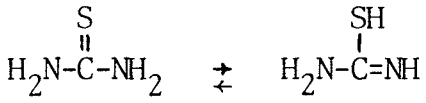
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 62-56-6

Thiocarbamide; 2-thiourea; THU

1.2 Chemical formula and molecular weight



CH_4N_2S Mol. wt: 76.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Almost colourless, rhombohedral crystals or needles (from ethanol)

(b) Melting-point: 180°C

(c) UV absorption spectroscopy: λ_{max} 241 nm (in methanol)

(d) Identity test: A 50 mg sample of thiourea warmed until it has melted and dissolved in 10 ml water gives a blood-red colour when treated with two drops of ferric chloride solution. An orange-coloured solution is formed by warming a mixture of 100 mg of the substance with dilute nitric acid. Thiourea may also be identified by Grote's reagent (see section 2.3).

(e) Solubility: Soluble in water at 25°C (1 part in 11); soluble in ethanol; sparingly soluble in ether

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* Considered by the Working Group in Lyon, February 1974

(f) Reactivity: Reacts with various sulphhydryl-oxidizing agents and forms complexes and adducts with metallic salts and many organic compounds including proteins and certain hydrocarbons

1.4 Technical products and impurities

Thiourea is available in the United States as a laboratory chemical containing 99% active ingredient. The specifications for a West German commercial product were as follows: 99% minimum active ingredient, 0.15% maximum water, 0.1% maximum ash, 0.4% maximum dicyanodiamide and traces of sulphate ion (SKW, 1971).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

A method for the synthesis of thiourea by the treatment of cyanamide with hydrogen sulphide was patented in the US in 1940 (US 2,173,067 granted to Robin Jr). In subsequent patents, synthesis of thiourea by the fusion of ammonium thiocyanate was reported (US 2,552,584 granted to Powers & Powers; US 2,560,596 granted to Mitchell). More recently, a method utilizing arsenic sulphide ore and cyanamide has been described (Rijavec & Živanović, 1956). The methods used for commercial production are believed to have been: (1) an improved version of the cyanamide-hydrogen sulphide method and (2) the reaction of carbon disulphide with ammonia.

Commercial production of thiourea was started in the US by two companies in 1938; however, one of these companies transferred production to Canada in 1952, and the other stopped production in 1954. In 1959, production of thiourea at the US-supplying plant in Canada was also stopped (Anon., 1964a). US requirements in 1964 were estimated to be 3.6 million kg and were reported to be supplied exclusively from one producing company in each of the following countries: the Federal Republic of Germany (where the product was made from cyanamide), France (by the reaction of

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

carbon disulphide with ammonia) and Japan (from cyanamide) (Anon., 1964b). At least two US companies announced plans to start production of thiourea in the early 1960's, but no evidence was found that commercial quantities have been produced to date.

From an analysis of import records at principal US ports of entry it is estimated that 1.2 million kg thiourea were imported in 1972 and 1.7 million kg in 1973 (with approximately 60% of the total in each year coming from Japan and lesser quantities from the Federal Republic of Germany and France).

The French company has been reported to have stopped production of thiourea (Anon., 1971) but in 1973 another French company was reported to be producing it (Anon., 1973). In 1969 a second company in the Federal Republic of Germany was reported to be building a thiourea plant with an annual capacity of 4 million kg (Anon., 1969a), and a third company was reported to be expanding its 3.5 million kg per year plant (Anon., 1969b).

Japan was reported to have 3 producers in 1966 with a combined production of over 2 million kg per year about 80% of which was reported to be exported to the US (Japan Chemical Week, 1966). One of these Japanese producers reportedly expanded its production capacity in 1968 (Japan Chemical Week, 1968).

The available literature on the uses of thiourea is characterized by frequent mention of the lack of information on which applications are the most important and which are only recommended uses that have not found commercial acceptance. The consumption pattern appears to have changed considerably in the last fifteen years, but these changes may be more apparent than real, since they are probably the result of corrections to past information rather than of changes in the relative importance of the various uses. Because of these uncertainties, the following discussion of uses of thiourea presents the available information in chronological order based on the date when the information was reported, and it undoubtedly includes potential uses as well as known commercial uses.

In 1961, one US company was reported to be consuming approximately 180 thousand kg thiourea per year as an intermediate in the manufacture of

fire-retardant resins for lacy fabrics. US consumption of thiourea in the production of diazo-type coatings for copy paper as an anti-yellowing agent was estimated in 1960 to have been approximately 570 thousand kg. In 1961, its use in boiler-water treatment to remove copper scale was reported to be a growing market; use in the photographic industry was said to be continuing, use in silver cleaning was said to be decreasing, and use in the synthesis of thioglycolic acid had essentially stopped; thiourea was reportedly being used in Canada to prevent the appearance of brown stain on hemlock wood (Anon., 1961).

A review article in 1963 on amino resins stated that use of thiourea in resins was very limited at that time (Wohnsiedler, 1963).

In 1964, use of thiourea in photo-sensitive papers was estimated to account for over 50% of the US market for thiourea, which was estimated to be about 1.8 million kg. The second largest US application was in the production of flame-retardant textile sizes, and use in boiler-water was believed to be the third largest. Other applications listed at that time were in photographic chemicals (as a silver toning agent), in hair preparations, in chelating agents, in dye intermediates, in dry-cleaning chemicals, in the synthesis of pharmaceuticals and insecticides and as a catalyst in the isomerization of maleic acid to fumaric acid (Anon., 1964b).

In 1964, one source reported that thiourea had been used as an antithyroid agent, as a fungicide, as an accelerator of sprouting in dormant tubers, as a flame-proofing agent for nylon, as a weighting agent for silk, as a dye-bath adjuvant of textiles, as a substitute for urea in urea-formaldehyde resins, as a pickling inhibitor and ingredient in cleaning and plating baths for metals, in the preparation of non-glare mirrors and in the synthesis of sulphathiazole (Hueper & Conway, 1964).

In 1966, approximately 1.7 million kg thiourea were reportedly being used in the US for the treatment of nylon to prevent running and to improve the handling properties of the final nylon-based products (Japan Chemical Week, 1966).

Thiourea has been reported to be useful as a peptizing agent for liquefying animal glues (Young, 1966).

One source has reported that thiourea was formerly used as an anti-thyroid agent and is used as a photographic fixing agent to remove stains from photographic negatives, as a vulcanization accelerator, as a reagent for determination of bismuth and selenite ions and in the manufacture of resins (Merck & Co., 1968).

Thiourea is used in the US as an intermediate in the production of thiouracil (see separate monograph) and of the anti-thyroid drugs, propyl-thiouracil (see separate monograph) and 5-iodo-2-thiouracil (Jones, 1969).

Thiourea may be used in the US as a chemical intermediate in the synthesis of thiourea dioxide, but this could not be verified. The quantity consumed for this purpose, if any, is believed to be quite small. In 1970, a Japanese company started construction of a thiourea dioxide plant with an annual capacity of 545 thousand kg. The main market for the chemical was said to be as a fibre bleaching agent (Japan Chemical Week, 1970).

In June 1970, two US companies were reported to be marketing thiourea-formaldehyde resins for use as flame-retardant treatments on nylon netting used for wedding gowns and window decorations (Anon., 1970).

Some product bulletins from manufacturers of thiourea have indicated that it finds use in galvanic products, agrochemicals, in the mineral oil and rubber industries, in photographic paper to prevent yellowing from the breakdown of diazo compounds, in paper making and in paper whiteners.

2.2 Occurrence

Thiourea has been found to occur naturally in laburnum shrubs and as a metabolite of Verticillium albo-atrum and Bortrylio cinerea.

The use of thiourea as a food additive has been prohibited in Italy and other countries (Duro, 1961). A conference held in Luxembourg in September 1955 on pesticides and public health pointed out the hazardous effect of use of thiourea in countries producing citrus fruits and the necessity of forbidding its use as a fungicide in agriculture (Plant Protection Ltd, 1957).

2.3 Analysis

Thiourea may be determined colorimetrically using Grote's reagent (prepared by treating sodium nitroferricyanide in sodium bicarbonate with hydroxylamine hydrochloride and then with bromine; excess bromine is removed either by aeration or preferably by addition of phenol (AOAC, 1960; Danowski, 1944)). The method is accurate for quantities in the range of 25-200 µg thiourea (Lashen & Starkey, 1970). An argentimetric method was described by Diwivedi & Yadav (1965). Holland et al. (1969) described a simple method for the detection (limit, 10^{-8} M) and determination (limit, 10^{-7} M) of thiourea by hydrogen overvoltage measurement.

For estimation in citrus fruits, Sperlich (1963) used a procedure involving extraction of thiourea by ethyl acetate:acetone, paper chromatography and spraying of the chromatogram with palladium chloride (limit, 1 µg thiourea; 0.5 mg thiourea in 100 g fruit material) or with 2,6-dibromoquinone chlorimide (limit, 1 µg thiourea; 1 mg thiourea in 100 g fruit material).

Duro (1961) described a paper chromatographic method for the identification or differentiation of thiourea and thiouracil when added as antioxidants in fruit juices. The two compounds could be separated using either n-butanol:acetic acid:water (4:1:5) or n-butanol saturated with water as solvent systems and could be detected by spraying with a 5% solution of sodium pentacyanoaminoferrate. The blue-violet spot of thiourea persisted, while the green-blue spot of thiouracil faded after 12 hours. Quantitative estimates of these compounds can be made by this method, when the concentration range is 2-20 µg.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Following administration of 2% thiourea in the diet to 31 A, 43 C57 and 17 I mice or 9 AxC3H and 4 AxCBA hybrids for periods extending

up to 81 weeks, follicular cystic changes in the thyroid, which were interpreted by the authors as non-malignant, were observed (Gorbman, 1947).

No tumours of the thyroid were reported in 21 female C3H mice administered 0.25-0.375% thiourea in the diet for 63 weeks (Dalton et al., 1948). Administration of 0.2-0.5% thiourea in the drinking-water to female R3 mice or 0.1% to female C3H mice for life did not result in a higher thyroid tumour incidence in treated as compared with control mice. In C3H virgin females, the incidence of mammary cancer was reduced from 40/96 (average age at death, 79 weeks) to 5/85 (average age at death, 81 weeks) in treated animals (Vazquez-Lopez, 1949).

Administration of 0.2-0.3% thiourea in the diet to 49 male and female C3H mice (castrated or intact) for 7 months resulted in hyperplastic thyroids in both castrated and intact treated animals. One adenoma of the thyroid was observed in a castrated animal receiving thiourea (Casas & Koppisch, 1952).

Rat: Administration of 0.25% thiourea in the drinking-water to Wistar rats for 23 months resulted in the development of thyroid tumours in an unspecified number of rats. In 2/30 rats, metastases of thyroid origin were found in the lungs, and invasion of the thyroid tumour into the thyroid veins was observed (Purves & Griesbach, 1946).

In groups of 10 male and 10 female local (Norwegian) strain rats and 10 male Wistar rats administered 0.25% thiourea in the drinking-water, thyroid tumours occurred in rats receiving the treatment for 12-24 months. Of the 9 Norwegian male rats treated for 16-23.5 months, adenomas were found in 4, a carcinoma in 1, adenomas and carcinomas in 2 and an adenoma, a carcinoma and a foetal adenoma in 1. All of the 8 females of the same strain examined after 12-23.5 months of treatment had tumours: adenomas in 5; an adenoma and a carcinoma in 1; adenomas, carcinomas and foetal adenomas in 2. In the 8 Wistar males treated for 12-21.5 months, 5 had adenomas and 1 had an adenoma as well as a foetal adenoma (Purves & Griesbach, 1947).

In groups of 18 Osborne-Mendel rats administered 0, 0.01, 0.025, 0.05, 0.1 or 0.25% thiourea in the diet for 104 weeks, the 0.25% dose level was incompatible with survival for more than 17 weeks. Of 29 treated rats sur-

viving the 104 weeks of treatment, 14 had hepatic-cell adenomas, compared with 0/18 in untreated controls (Fitzhugh & Nelson, 1948). In a subsequent personal communication (Deichmann et al., 1967) the liver tumour incidences in the above-treated rats were reported as: 3/5 (100 ppm), 4/8 (250 ppm), 2/8 (500 ppm) and 5/8 (1000 ppm), with a 1% spontaneous incidence of such liver tumours in untreated rats surviving for 2 years.

Of 19 male albino rats administered 0.2% thiourea in the drinking-water for up to 26 months, 1 developed a myxomatous tumour of the nose and 17 developed malignant tumours involving the area of the ear duct and the orbit, which were diagnosed as epidermoid carcinomas. No such tumours occurred in 12 controls observed for 104 weeks (Rosin & Ungar, 1957). In a later communication, Ungar & Rosin (1960) reported that in 7/8 male Hebrew University strain rats administered 0.2% thiourea in the drinking-water for 14-23 months, squamous-cell carcinomas of the Zymbal gland and/or Meibomian gland were observed.

The possible synergistic effect on tumourigenesis by administration of thiourea, aramite, methoxychlor, DDT and aldrin, in combination, to groups of male and female Osborne-Mendel rats was studied in a series of experiments by Radomski et al. (1965). In one experiment, 50 ppm each of the above compounds was mixed in the diet for 104 weeks. At the end of the observation period, liver tumours were found in 10/50 treated males and 9/49 treated females compared with 4/50 male and 6/50 female controls. [The difference, however, is not statistically significant ($P>0.05$).] In a second 104-weeks feeding experiment, the total tumourigenic dose of those compounds was increased, and each compound was administered at a level of 80 ppm in the diet alone or as a combination of all. In six groups (including controls) of 30 males and 30 females, no liver tumours were found in males from all 6 groups, and only one liver tumour was detected in a female from the control group. The incidence of other tumours was about the same in the treated groups as in the controls.

In a third experiment (Deichmann et al., 1967), a combination (thiourea, 50 ppm; aramite, 200 ppm; methoxychlor, 1000 ppm; DDT, 200 ppm) was used. Each of the constituents was also fed alone in the diet to 30

male and 30 female rats per group. The total number of tumours per group (mainly mammary, lung, blood and skin tumours) was: control, 15, 1 of these was malignant; thiourea, 21, 4 of these were malignant; mixture, 10, 2 of these were malignant.

Fish: Histopathologically confirmed hepatomas were observed in rainbow trout (4/40 and 12/42) after feeding with 1200 ppm thiourea in the diet for 15 and 20 months, respectively. Increasing the dose to 4800 ppm did not increase the tumour incidence (3/32 and 7/38) in fish examined at 15 and 20 months, respectively. The incidence of hepatomas in the controls was 0/400 (Halver, 1967). [No information on composition or contamination of the control diet was available.]

(b) Subcutaneous and/or intramuscular injection

Newborn mouse: A single s.c. injection of 2500 mg/kg bw thiourea was given to 42 ICR Swiss mice when the animals were 24-72 hrs of age. No increase in the number of lung adenomas as compared with controls was noted in mice killed 6 months after administration of the compound. A single dose of 1000 mg/kg bw urethane produced a 100% incidence of lung adenomas with an average of 17.2 tumours/mouse (Gargus et al., 1969).

(c) Other experimental systems

Intraperitoneal and oral administration: Twelve albino rats of both sexes were given 3 doses of 3, 4 and 4 ml of a 10% solution of thiourea by the i.p. route on 3 consecutive days, weekly for 6 months, followed by administration of 0.2% thiourea in the drinking-water. Of 6 rats surviving 12-16 months, 5 developed tumours involving the area between the ear duct and the orbit, 3 of which were diagnosed as squamous-cell carcinomas, one a mixed sarcoma and squamous-cell carcinoma and one a mixed-cell sarcoma. There were no such tumours in untreated rats in the colony (Rosin & Rachmilewitz, 1954).

3.2 Other relevant biological data

Thiourea is rapidly absorbed from the gastrointestinal tract in rats and man (Williams & Kay, 1945). When ^{35}S-labelled compound was given by i.p. injection to rats, 98% of the administered radioactivity appeared in

urine within 48 hours after the injection. Chromatography of the urine showed that the major product was undegraded thiourea, with small quantities of inorganic sulphate (6.2%) and ethereal sulphate (5.9%) (Schulman & Keating, 1950).

Maloof & Soodak (1957) showed that only a small amount (1.95% of the i.p. administered dose) of ^{35}S-labelled thiourea was concentrated in the thyroid of rats. Most of the ^{35}S in the gland was present as sulphate (56%) or bound to protein (13%); whereas in the serum, 75% of the ^{35}S label occurred as thiourea and 15% as sulphate. A cytoplasmic particulate system from the thyroid gland, which was not present in liver or kidney, was shown to desulphurate thiourea in vitro producing a protein-bound sulphur molecule (Maloof & Soodak, 1961).

^{35}S-Thiourea readily crosses the rat placenta, and the foetal thyroid: foetal serum concentration ratio became elevated above 1 on the 17th day of gestation and continued to increase until the 20th day (Shepard, 1963).

3.3 Observations in man

In an extensive review of 525 cases treated with thiourea, Vanderlaan & Storrie (1955) described the adverse effects of this treatment. No specific mention of cancer is made in this series.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Thiourea produced liver, thyroid and Zymbal gland tumours in rats following oral administration. Intraperitoneal injection followed by oral administration also led to the formation of Zymbal gland tumours in rats.

Oral and s.c. administration to mice did not produce thyroid tumours; however, the experiment using the s.c. route was inadequate. An increased

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[1] This section should be read in conjunction with the section, "General Remarks on Anti-thyroid Substances" (p. 23) and the section, "Animal Data in Relation to the Evaluation of Risk to Man" (p. 15) in the introduction to this volume.

incidence of liver-cell tumours in trout was reported, but this result cannot be considered as conclusive until additional studies using properly controlled diets are reported.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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URETHANE*

The name urethane is sometimes applied to high molecular weight polyurethanes used as foams, elastomers and coatings. Such products are not made from the chemical urethane and do not generate it on decomposition.

1. Chemical and Physical Data

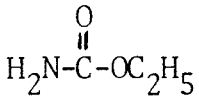
1.1 Synonyms and trade names

Chem. Abstr. No.: 51-79-6

Ethyl carbamate; ethyl ester of carbamic acid; ethylurethan; ethyl urethan; ethyl urethane; urethan

Leucothane; Pracarbamin

1.2 Chemical formula and molecular weight



$C_3H_7NO_2$ Mol. wt: 89.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless, almost odourless, columnar crystals or white, granular powder. Its solutions are neutral to litmus paper.

(b) Boiling-point: 182-184°C

(c) Melting-point: 48-50°C

(d) Solubility: At 25°C 1 g dissolves in 0.5 ml water, 0.8 ml ethanol, 0.9 ml chloroform, 1.5 ml ether, 2.5 ml glycerol or 32 ml olive oil.

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* Considered by the Working Group in Lyon, February 1974

(e) Volatility: Sublimes readily at 103°C at 54 mm Hg; volatile at room temperature

1.4 Technical products and impurities

Urethane is available in the United States as an NF grade in the form of a fused solid or crystals having a typical analysis of 99.43% active ingredient, 0.034% moisture and 1% maximum residue on ignition. It is also available in the form of a mixture with methyl carbamate.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Urethane has been produced commercially in the US for at least 30 years (US Tariff Commission, 1945). Although it reportedly can be made by the reaction of ethanol and urea under pressure and by warming urea nitrate with ethanol and sodium nitrite (Merck & Co., 1968), the method used for commercial production is probably the reaction of ethyl chlorocarbonate (made from ethanol and phosgene) with ammonia.

Separate data on US production of urethane are not available, since only one US company has reported the production of urethane to the US Tariff Commission in recent years. A second US company is also believed to be manufacturing urethane, although the amount produced annually is probably below the minimum of 454 kg needed before reporting production to the US Tariff Commission, as required by law.

One source reported in 1968 that urethane had found use in human medicine as an anti-neoplastic agent and formerly was used as a hypnotic, as an adjunct to sulfonamide therapy, as a component (with quinine) of a sclerosing solution for varicose veins, and as a topical bactericide (Merck & Co., 1968). A more recent source states that large doses of urethane produce bone-marrow depression, and that for a time it was used in the treatment of chronic leukaemia and multiple myeloma (Goodman & Gilman, 1970). No evidence was found that urethane presently finds use in the US

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

in human medicine.

Urethane has been reported to be used as a chemical intermediate in the preparation and modification of amino resins, and as a solubilizer and co-solvent for pesticides, fumigants and cosmetics (Merck & Co., 1968). The major present use of urethane is believed to be as a chemical intermediate, primarily for reaction with formaldehyde to produce N-hydroxymethyl derivatives, which are useful as cross-linking agents in textile treatments designed to impart wash-and-wear properties to fabrics. Some urethane may also be used to synthesize higher molecular weight carbamates, but this could not be verified.

Reported veterinary applications of urethane include its infrequent use as a hypnotic and its more frequent use as an anaesthetic for laboratory animals (Merck & Co., 1968).

2.2 Occurrence

Urethane has been reported to occur as a result of the reaction of ammonia and diethylpyrocarbonate added at levels of 10 µg/l in certain beverages at pH below 4.0. It was also reported to be present in diethylpyrocarbonate-treated wines at levels up to 50 µg/l; it was considered possible that some of this was of natural origin (WHO, 1972).

2.3 Analysis

A titration method for the estimation of urethane at concentrations greater than 3-5 mg/100 ml blood from patients treated for leukaemia with this compound was described by Archer et al. (1948).

Identification of urethane in mixtures of carbamates using gas chromatographic analysis on Carbowax 20 M, Versamid 900 or SE-30 columns has been described by Zielinski & Fishbein (1965a,b). Using thin-layer chromatography, McConnell Davis (1967) was able to distinguish 13 carbamates with 3 chromatographic systems and 3 spray reagents. Similar identification tests using paper chromatography were described by Fishbein & Cavanaugh (1965).

Microgram quantities of urethane may be analyzed as the trimethylsilyl derivative by gas chromatography on SE-30 columns, but interference

from biological materials was found when attempts were made to analyse urethane in tissues of experimental animals (Nery, 1969a).

Isotope dilution methods have been described for the determination of small quantities of urethane in beverages (wine, beer, orange juice and other soft drinks) resulting from the use of diethyl pyrocarbonate (Löfroth & Gejvall, 1971). These workers demonstrated urethane at concentrations ranging from 0.17 mg/l in orange juice to 2.6 mg/l in white wine. Levels of 0.014 mg/l in orange juice (pH 3.5) to 0.04 mg/l in Moselle wine (pH 3.5) were determined using an isotope dilution technique after the addition of known concentrations of ^{14}C-labelled diethyl pyrocarbonate (Fischer, 1972).

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

Since the observations by Nettleship et al. (1943) first indicated the carcinogenicity of urethane to mice, extensive carcinogenicity testing in several species of experimental animals has been carried out in a large number of laboratories. A general review on this subject has been published by Mirvish (1968). Some of the more classical and adequate studies are summarized below.

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: In a group of 100 male and 100 female Swiss albino mice administered 0.4% urethane in the drinking-water for two 10-day treatment periods commencing when the animals were 10 weeks of age, a nearly 100% incidence of multiple lung adenomas was observed. All animals had died or were killed after 42 weeks (i.e., when the animals were 1 year of age). Lung adenomas occurred in 9 male and in 23 female controls, mainly in animals dying after 66 weeks of age. Lymphomas (mainly lymphosarcomas) occurred in 28 females and in 15 males often before 40 weeks of age, compared with 16 and 4 in female and male controls, in which the tumours mostly occurred after 60 weeks of age. Multiple small haemangiomas of the liver occurred in several urethane-treated animals of both sexes which died

or were killed after the 40th week of age. In 3 treated males and 2 females a total of 9 papillomas and 1 sebaceous carcinoma of the skin were also observed (Toth et al., 1961a).

Groups of approximately 100 male and 100 female 5-week old outbred albino CTM mice were administered 0.4% urethane in the drinking-water for one or two 10-day periods or one, two or three 5-day periods with an interval of 10 days between each treatment period. All animals died or were killed within 60-80 weeks. The highest incidence of tumours occurred in animals given two 10-day treatments. In this group the incidence of lung adenomas was 80-84% in treated animals compared with 2-7% in controls. Lymphosarcomas occurred in 33% of males and in 27% of females, compared with 4.5 and 5.0% in controls. Of all the lymphosarcomas observed in treated animals 75% were of thymic origin. A slight increase in the incidence of liver angiomas and of Harderian gland tumours was also observed in treated animals (Della Porta et al., 1963a).

Further results in similarly treated mice of the same strain have been reported (Della Porta et al., 1963b). In addition to the tumours reported earlier in the female mice given one or two 10-day treatments of 0.4% urethane in the drinking-water, 34/83 and 21/70 mice, respectively, surviving after 15 weeks of age developed mammary tumours, compared with 15/119 virgin control females. The average latent period for the appearance of the first mammary tumour was 41.3 weeks in treated animals, compared with 52.5 weeks in the controls.

In two groups of 36 5-week old C3H female mice administered 0 or 0.1% urethane in the drinking-water for 13 weeks and observed up to 76 weeks, the incidence of pulmonary adenomas increased from 2/36 in controls to 15/32 in treated animals. In groups of 54-62 male or female 53-week old DBA mice administered 0.1% urethane in the drinking-water for 31 weeks and observed up to 45 weeks, pulmonary adenomatosis was observed in 20/54 males and 20/52 females, compared with 1/59 and 2/56 controls. Pulmonary adenomas also occurred in 10 of the treated males and in 11 treated females, compared with zero in the controls. Squamous-cell tumours occurred in 11 treated males and in 6 treated females; again, this tumour was not found in controls. The incidence of leukaemia was increased only

in C3H females, from 0/36 in controls to 8/36 in treated animals, as was the incidence of mammary carcinoma in DBA females, from 13/57 in controls to 34/54 in treated animals; the high spontaneous incidence in C3H controls was not increased, possibly due to the earlier death of treated females. Malignant mesenchymal tumours of the fat pad were also observed in 3 treated DBA males and in 3 treated C3H females. Tumours of this type were not found in controls (Tannenbaum & Maltoni, 1962).

In male (C57BL/6 x A/J)F1 mice administered 0.05 ml of a 10% solution of urethane in dioctyl ester of sodium sulphosuccinic acid (DSS) by stomach tube three times weekly for 5 weeks commencing when the animals were 7-8 days of age, 32/42 (76%) developed lymphocytic leukaemia, the mean age at death being 146 days. Pulmonary adenomas occurred in 100% of animals, hepatomas in 10% and stomach papillomas in 2% of the animals. Controls developed pulmonary adenomas only, with an incidence of 4/39 and 10/38 in the untreated or vehicle controls, respectively; the median ages at death were 441 and 444 days. In 41 males and 40 females treated similarly with a 5% solution of urethane, leukaemia occurred in 46% and 40% of animals, respectively. The incidence of hepatomas was 57% in males and 13% in females, and lung adenomas occurred in 98% of animals in both sexes. The average survival time was 270 days (Klein, 1962).

In groups of 20-25 male plus 20-26 female suckling (C57BL/6 x A/J)F1 mice administered a single dose of 1 mg/g bw urethane in DSS by stomach tube on days 1, 7, 14, 21 or 28 of age and observed up to 70 weeks of age, the incidence of pulmonary adenomas was 84-100% in all groups, compared with 24-36% in controls. The incidence of hepatomas was, however, related to the day of administration, being highest (91% in males and 77% in females) in mice treated at 7 days of age. The incidence in mice treated at 28 days of age was 17% in males and 5% in females. Controls receiving the solvent on day 7 had an incidence of 4% in males and 0% in females. In some treated groups a few animals developed Harderian gland tumours (4-9%) and stomach papillomas (4-17%). In controls, only 1/25 females developed a stomach papilloma, and no Harderian gland tumours occurred (Klein, 1966).

A single oral dose of 1, 4, 16 or 64 mg urethane given as a 5% solution in water by stomach tube to groups of male and female Swiss mice, followed by twice weekly skin applications of croton oil for 26 weeks produced a dosage-related increase in skin papillomas and lung adenomas. Of the controls, 2/20 mice had skin papillomas and 1/20 lung adenomas; whereas at the highest dose level 24/24 mice had an average of 5 and 7 tumours/mouse, respectively (Berenblum & Haran-Ghera, 1957).

Rat: In a group of 50 female Sprague-Dawley rats administered urethane (1 part in 1000) in the drinking-water for life, the average survival time was 12.5 (8-19) months. Of 40 animals on which autopsies were performed 33 had tumours, including 7 malignant lymphomas, 11 haemangiomas or haemangiosarcomas of the liver, spleen or uterus, 7 hepatomas, 10 adrenal cortex adenomas and 4 fibrosarcomas of the mesentery or uterus. No concomitant controls were used (Adenis et al., 1968).

Hamster: A group of 31 male and 30 female Syrian golden hamsters was given 0.2% urethane in the drinking-water for 20 weeks. The concentration was then increased to 0.4% for a further 20 weeks, at which time treatment was discontinued for 8 weeks due to the occurrence of diarrhoea among the animals. Treatment was resumed for a further 2 weeks, when the animals were left untreated and observed up to 80 weeks. Tumours were found in 22/27 male and in 21/25 female survivors, compared with 9/54 in male and 3/47 in female controls. Tumours in treated animals included: melanotic tumours of the skin (23 animals), papillomas and squamous-cell carcinomas of the forestomach (40 animals), malignant lymphomas (5 animals), mammary tumours (3 animals), hepatomas (3 males), haemangiomas (6 animals), haemangiosarcomas (2 animals), pulmonary adenomatosis (8 animals) and adenomatous polyps of the caecum (6 animals). Very few of these tumours occurred in the 12 tumour-bearing controls (Toth et al., 1961b). Similar results were obtained in a group of 52 male and 48 female 5-week old Syrian golden hamsters administered 0.1% urethane in the drinking-water for life (Toth & Boreisha, 1969).

Administration of 0.2% urethane in the drinking-water of 10 male and 10 female Syrian golden hamsters for life produced melanotic tumours of the

skin in 8/20 animals, compared to 1/63 in controls. In treated animals the tumours occurred mainly in males, possibly due to the short lifespan of treated females. In contrast, twice-weekly applications of a 20% solution of urethane in acetone to the skin in a similar number of animals produced no melanotic tumours (Pietra & Shubik, 1960).

(b) Skin application

Mouse: Two weekly doses of 120 mg urethane followed after an interval of 3 weeks by 18 weekly applications of 0.3 ml of a 0.5% croton oil solution in acetone produced 115 skin tumours in 22 surviving 'S' mice. Alternate applications of 60 mg urethane and 0.5% croton oil at 3- to 4-day intervals during 18 weeks produced 138 skin tumours in 17 surviving mice. When urethane was applied alone, either as two weekly applications of 120 mg or as 18 weekly applications of 60 mg, no tumours were produced in 19 and 17 mice, respectively. A similar result was observed when urethane was administered in conjunction with DMBA. Urethane produced no recognizable histological changes in mouse skin, even after prolonged application; and the tumours produced when it was given in conjunction with croton oil had the appearance of benign papillomas. One squamous epithelioma occurred 8 weeks after the end of treatment in the group given alternate treatments of urethane and croton oil (Salaman & Roe, 1953). Such findings were later confirmed (Berenblum & Haran-Ghera, 1955).

Three strains of mice (the F_1 hybrid of C57BL ♀ and C3H ♂ (CxH); dba; and C3H) received urethane in acetone at a dose of 12 mg/mouse, twice weekly, in the interscapular region, for 60-78 weeks. On microscopic examination, pulmonary tumours were observed in 30-52% of the 25-30 animals per group and were generally benign. Hepatic lesions occurred relatively early in 40-76% of the treated animals; and the lesions were observed grossly as elevated, blood-filled cysts, 1-8 mm in size, either localized or diffusely distributed. At times the peritoneal cavity was blood-stained or contained a haemorrhagic fluid. Microscopically, marked dilatation of the sinusoids, chronic, passive hyperaemia and blood cysts or lakes were observed. These were not interpreted as being haemangiomas. The incidence of mammary carcinomas was increased in treated females of all strains; and lesions of

the interscapular fat pad, which occurred as spongy, blood-filled tumours, were observed approximately 78 weeks after the beginning of application. Harderian gland adenomas were observed only in CxH mice, at approximately 104 weeks of age (Tannenbaum, 1961).

Male and female HR/De mice painted on the interscapular area with a 40% urethane solution in ethylene glycol developed significantly more pulmonary adenomas than did the corresponding controls painted with ethylene glycol only. Haired mice showed a higher tumour incidence (43/51) than did hairless ones (30/40); the average age at death was 14-15 months. A high incidence of epidermoid carcinoma (17/48) was observed only in hairless mice treated with urethane (Deringer, 1962).

Hamster: A 50% solution of urethane in acetone was applied to the skin of 40 male and 40 female hamsters 2 or 3 times per week; the total number of treatments varied from 50-105, and each application consisted of approximately 125 mg urethane. Animals were killed between 8-18 months from the beginning of treatment, and mammary tumours occurred in 4/40 females. In addition, a large number of melanotic tumours which grew upon transplantation occurred in both sexes; and in 2 animals bearing melanotic tumours metastases to the lungs were present. Tumours did not occur in control animals (Rivière et al., 1964a,b,c; 1965).

(c) Inhalation and/or intratracheal administration

Mouse: Three strains of mice (BLH, NMRI and C57BL) were exposed to 2 types of aerosol sprays (pressure and ultrasonic) containing urethane. The BLH and C57BL mice were 4-8 weeks of age at the start of the experiment, and the NMRI mice were less than 6 months old. Groups of mice were kept for 20-60 min/day in atmospheres saturated with the aerosols at urethane concentrations of 5, 10, 15 or 20%. The maximum period of treatment at a concentration of 20% varied from 14.5 weeks for BLH mice to 3.5 weeks for C57BL mice. Treatment of mice with pressure-spray yielded the first lung tumours after 10-22 weeks, and with ultrasonic spray after a period of 7-15 weeks. An increase in the survival time resulted in an increase in both tumour size and number, as well as in extent of dedifferentiation. Whereas BLH and NMRI mice showed only adenomas, C57BL mice exposed to 5%

urethane for 22 weeks mostly had solid tumours of a squamous type. In no cases were metastases observed (Otto & Plötz, 1966).

(d) Subcutaneous and/or intramuscular administration

Newborn or pre-weanling mouse: Malignant lymphomas developed in 3/14 (21.4%) Swiss albino mice which received a single dose of 1 mg urethane s.c. as a suspension in gelatin before 24 hours post-partum. The average age at which lymphomas developed was 14 weeks. Pulmonary adenomas were also found in 10/14 mice (Pietra et al., 1961). Similar results were reported by Fiore-Donati et al. (1961) who administered s.c. 0.05 ml of a 4% urethane solution in distilled water to Swiss mice and observed malignant lymphomas in 13/60 mice between 10-22 weeks of age.

Urethane (1 mg/g bw) administered 8 times at weekly intervals by the s.c. route to C57BL mice, first within 24 hours of birth, produced thymic lymphomas between the 12th and 27th weeks of life in 12/12 survivors (Doell & Carnes, 1962). The leukaemogenic activity of urethane administered s.c. in 1-, 5-, or 40-day old Swiss and AKR mice was studied by Fiore-Donati et al. (1962), who observed such activity in 1- (13/60) or 5-day old (7/39) Swiss mice but not in 40-day old treated animals (2/63). Neonatal treatment of AKR mice remarkably shortened the latent period of leukaemogenesis in this high leukaemic strain (14/37 mice developed leukaemia within 19 weeks, versus 1/60 within 23 weeks among the controls).

Continuation of the above-mentioned studies revealed the influence of age on susceptibility of the liver to urethane carcinogenesis. By the 60th week of age, 13/15 (87%) and 9/13 (70%) male Swiss mice treated on day 1 or day 5, respectively, developed liver tumours. Males treated with urethane at 20 or 40 days of age developed liver tumours in 8% and 0%, respectively. In females, no hepatomas occurred in the groups treated at 20 or 40 days, but 9 and 18% of females developed hepatomas in groups treated at 1 or 5 days of age (Chieco-Bianchi et al., 1963).

BALB/c, DBAf and C3Hf/Lw mice received s.c. 2 mg/animal urethane within 24 hours of birth. Hepatomas developed in 100% of 15 male and 14 female C3Hf/Lw mice and in 86% of 15 male DBAf mice but not in BALB/c mice. Lung adenomas were seen, regardless of sex, in 76% of 35 BALB/c mice, in

34% of 31 DBAf and in 17% of 29 C3Hf/Lw mice. In contrast to previous findings, none of these strains developed leukaemia, indicating significance of genetic background on urethane tumourigenesis (Trainin et al., 1964). However, BALB/c/Cb/Se mice developed lymphoid tumours when treated with 2 mg urethane (Ribacchi et al., 1964).

S.c. administration of 5 mg/mouse urethane in distilled water to dd mice at 8 days of age together with 3 additional weekly treatments of 10 mg/mouse produced thymic lymphomas in 22/59 (37%) males and in 27/43 (62%) females within 21 weeks (Ito et al., 1965).

In 7-day old dd/I mice injected s.c. with 1 mg/g bw urethane 4 times at weekly intervals, a broad spectrum of tumours was seen: thymic lymphomas in 33/47 (70%) at 11 weeks, lung adenomas in 45/47 (96%) at the same age, and Harderian gland tumours (44%) at 26 weeks. In addition, 6 liver tumours were observed (Matsuyama et al., 1969).

Newborn rat: Two strains of rats (August and Wistar) received 8 weekly s.c. injections of 1 mg/g bw urethane, the first injection being given within 24 hours of age. Melanotic lesions of the iris were observed only in the August strain (12/26 females and 8/25 males) after a period of 9 months (Roe et al., 1963).

Newborn or pre-weanling hamster: A single s.c. injection of 150 µg urethane administered to hamsters on the first day of life did not result in the development of tumours by the 60th week of age (Walters et al., 1967).

When 7-day old Syrian hamsters were injected s.c. with 1 mg/g bw urethane once a week for 6 weeks and observed for lifespan, 6 (30%) females and 2 (25%) males surviving at 52 weeks developed adrenal cortical tumours. In addition, a β-cell tumour of the pancreatic islet cells was found in one male dying at 112 weeks of age (Matsuyama & Suzuki, 1970).

The s.c. administration of a single dose of 1 mg/g bw urethane to 1-day or 8-week old Syrian golden hamsters resulted in the induction of higher numbers of forestomach papillomas in males and females receiving urethane at 8 weeks of age (33/78) than at birth (15/51). Squamous-cell

carcinomas were observed in 5 treated animals of both groups. The number of animals with dermal melanocytomas was 7/78 in those treated at 8 weeks of age and 13/51 in those treated at birth (Toth, 1970).

When similar groups were exposed to 10 weekly s.c. injections of 1 mg/g bw urethane, intestinal tumours were found with greater frequency in those hamsters receiving the treatment as newborns than as adults. Dermal melanocytomas and thyroid and lung tumours were induced, with similar incidences in the two age groups. Consistent with previous reports, significantly higher numbers of forestomach papillomas developed in animals in which treatment began at 8 weeks of age (Toth, 1971).

(e) Intraperitoneal administration

Mouse: Urethane was first tested for carcinogenicity in 1943, by i.p. injection in C3H female mice. Treatment consisted of 14 weekly injections of 1 mg/g bw with an interval of $7\frac{1}{2}$ months between the beginning of treatment and autopsy. The incidence of lung adenomas was increased from less than 5% known to occur in untreated mice to 70% (7/10) in treated mice. Tumours were shown to occur as early as 2-3 months after the start of treatment (Nettleship et al., 1943).

In groups of 29 BALB/c, 28 Zb and 25 Db female mice administered 11 i.p. injections of 1 mg/g bw urethane every 4 days, the incidences of lung adenomas were 100%, 71% and 44%, respectively, compared with known spontaneous incidences of 5%, 2% and 1% in these strains. In each case the latent period of tumour induction was reduced (Ida et al., 1962).

In 36 male C3H/HeA mice administered single i.p. injections of 25 mg urethane in distilled water at 2 months of age and killed after 13 months, the incidence of lung adenomas was 83%. In addition, hepatomas developed in 11/36 mice. Partial hepatectomy in similar groups of animals slightly increased the incidence of hepatomas when urethane was given before or after such treatment. The spontaneous incidence of hepatomas in male mice of this strain was 4% at the age of 15 months (Hollander & Bentvelzen, 1968). An increased incidence of hepatomas following 70% hepatectomy in conjunction with urethane was reported in BALB/c mice by Lane et al. (1970).

Newborn or pre-weanling mouse: In groups of 73-118 (C57BL x C3H)F_1 mice receiving a total dose of 2.1, 3.0 or 4.2 mg/g bw urethane administered as 6 i.p. injections at 3-day intervals, starting within 24 hours of birth (first series) or on the 7th day of age (second series), the incidences of leukaemia in the first series were 7%, 32% and 74%; and in the second series, 0%, 7% and 38%, respectively (Vesselinovitch & Mihailovich, 1966). When treatment with urethane was interrupted for 9 or 21 days between the third and fourth injections of urethane, the incidence of leukaemias was significantly decreased (Vesselinovitch & Mihailovich, (1967a).

In groups of 26-53 (C57BL x C3H)F_1 mice of both sexes administered 6 i.p. injections of 0.5 mg/g bw urethane at 3-day intervals, beginning on day 1, 4 or 7 of postnatal life, an increased incidence of malignant lymphomas, lung adenomas, hepatomas, Harderian gland tumours and stromal and epithelial ovarian tumours was observed. It was noted that only development of lung adenomas was not modulated by age or sex of animals, while other tumours appeared at different frequencies according to the age at which treatment was started (Vesselinovitch & Mihailovich, 1967a,b).

Seven-day old (C57BL x C3H)F_1 mice, administered a total of 6 i.p. injections of 0.5 mg/g bw urethane at 3-day intervals, were left intact or gonadectomized at 6 weeks of age. In intact animals hepatomas developed in 96% and 20% of males and females, respectively; whereas in the gonadectomized groups liver tumours developed in 62% and 67% of the animals, respectively (Vesselinovitch & Mihailovich, 1967c).

Rat: Groups of 15 male and 15 female Sprague-Dawley rats received 3 weekly i.p. injections of 0.5 mg/g bw urethane for 3.3 weeks starting when the animals were 1 or 2 weeks of age, or a similar dose 3 times weekly for 6.6 weeks starting when the animals were 2 weeks of age, or 100 mg/rat twice weekly for 14 weeks starting when the animals were 32 weeks of age. In females treated at 1 week of age, the incidence of mammary tumours was 100%, compared to 87% in controls; and the latent period of tumour induction was reduced from 85 weeks in controls to 49 weeks in treated animals. In males and females treated at 1 or 2 weeks of age, the incidence of Zymbal gland carcinomas reached 27% compared with 7% in male and female

controls; again, a significant reduction in the latent period was apparent. An increase in the number of angiomas and sarcomas at various sites, of malignant lymphomas, of kidney tumours and of epidermal cysts was generally apparent in treated animals (Tannenbaum et al., 1962).

Newborn and adult rats: In male and female MRC (Wistar) rats receiving a total dose of 3 or 5 mg/g bw urethane administered i.p. as 6-10 injections of 0.5 mg/g bw at 3-day intervals, starting within 24 hours of birth, 21% of 77 animals which received 3 mg urethane developed liver tumours by 110 weeks of age, while 53% of 83 treated with 5 mg urethane had liver tumours (Vesselinovitch & Mihailovich, 1968a). Neurogenic neoplasms, embryonal kidney tumours, Harderian gland adenomas and Anitschkow cell sarcomas of the heart were also observed in treated animals (Vesselinovitch & Mihailovich, 1968b).

When urethane was administered to the same strain of rats, beginning on day 1, 28 or 46 of life as 6 i.p. injections at 3-day intervals, a broad spectrum of tumours was produced within 146 weeks. Of 150 rats treated at 24 hours after birth, 18% had liver tumours, 2% Anitschkow sarcomas of the heart, 15.3% neurogenic tumours and 6% embryonal kidney tumours. In most cases none of the 118 controls developed such tumours. Adult rats were less sensitive to induction of these tumours; however, thyroid tumours occurred in up to 6.8% of the treated animals, compared with 0.8% in controls (Kommineni et al., 1970a,b).

Newborn hamster: Urethane was administered as an i.p. injection of 0.5 mg/g bw to groups of male and female white Syrian hamsters on the 1st day of life and was continued at 3-day intervals until the animals had received 2.5 mg/g bw. All surviving animals were killed after 120 weeks. Of 24 treated male and 30 treated females, 46% and 27%, respectively, died with melanotic tumours, a large number of which had metastases in the lymph nodes, liver, kidney and lung. The average age at death of tumour-bearing animals was 80 weeks, compared with 93 weeks in untreated controls (Vesselinovitch et al., 1970).

(f) Other experimental systems

Prenatal exposure: Female A mice were administered a single i.p. or

i.v. injection of 25 mg urethane 1, 2, 3, 4 or 5 days before parturition, and the offspring were observed for 6 months after birth. The incidence of lung tumours was 100% in offspring exposed in utero 1 day before birth, with an average of 8.9-10 lung tumours/mouse. Urethane administered on days 2-5 before parturition produced 60-80% incidences of lung tumours, with 1-2 lung tumours/mouse (Larsen, 1947a). Similar results were reported by Klein (1952) in AxC mice. When 25 mg urethane were given as an i.p. injection on day 17-19 of pregnancy, the incidence of lung tumours was 57% (0.9 tumours/mouse). When urethane was given 8-20 hours before delivery, the incidence was increased to 97%, and the number of tumours/mouse rose to 7.6.

Pregnant female A mice received a single i.v. administration of 25 mg urethane 24, 48 or 72 hours prior to delivery. During treatment the animals were kept in either a 10 or 100% oxygen environment or in the normal atmosphere. Increased oxygen concentration specifically enhanced multiplicity of lung tumours when urethane was administered 24 hours prior to delivery (DiPaolo, 1962).

A dose of 0.5 mg/g bw urethane was administered s.c. on 5 consecutive days to Swiss mice from the 7th-11th day of gestation and to C3H mice from the 11th-15th day of gestation. The above treatment resulted in an enhanced development of hepatomas in C3H mice, of ovarian tumours in Swiss and C3H mice and in a slight increase in the development of lung adenomas in both strains (Vesselinovitch et al., 1967).

The administration of 1 or 0.5 mg/g bw urethane to pregnant ICR/Jcl mice on days 7, 9, 11, 13, 15 or 17 of gestation resulted in incidences of lung tumours in the offspring ranging from 9.1% (7th day of gestation) to 69.4% (17th day of gestation). Additional groups of mothers received 1 mg/g bw urethane on days 2, 7, 12 and 17 of lactation in various combinations with prenatal exposure to urethane; and these experiments demonstrated an additive effect of combined treatment with regard to lung carcinogenesis (Nomura, 1973).

When MRC (Wistar) rats were administered 0.5 mg/g bw urethane once 4 days before parturition, a small number (4.5%) of hepatomas and sarcomas of

the heart were observed in the offspring (Kommineni et al., 1970a).

Pre-weanling exposure: Lactating Swiss mice were administered urethane on days 1, 3 and 5 following parturition. Each treatment consisted of 30 mg urethane. The suckling offspring were sacrificed at 20, 45, 90 and 210 days of life, and their lungs were examined for the presence of tumours. The incidences of lung adenomas in the above-mentioned age groups were 0%, 10%, 52% and 78%, respectively (de Benedictis et al., 1962).

Lactating Swiss mice were treated at 2-day intervals with a total of 12 i.p. injections of 6 mg urethane. Suckling offspring were killed at 8 months of age, and all animals were found to have lung tumours, with an average multiplicity of 9.3 tumours/mouse. Controls of the same age had lung tumours in only 25% of cases, with a multiplicity of 2 tumours/mouse (Adenis et al., 1971).

Combined treatment with X-irradiation: Kawamoto et al. (1958) were the first to demonstrate the augmenting effect of urethane plus concurrent X-irradiation on leukaemogenesis in mice. Berenblum & Trainin (1960) demonstrated that urethane enhanced X-ray leukaemogenesis in adult mice when it was given after irradiation, but that it did not do so when the sequence of the treatments was reversed; they concluded that urethane acts as a "promoting" agent in leukaemogenesis. Urethane alone, however, was shown to induce leukaemia either when administered once to newborn Swiss mice (Fiore-Donati et al., 1961; Pietra et al., 1961), C3Hf mice (Liebelt et al., 1961) and CTM mice (Della Porta et al., 1963a) or when administered repeatedly to newborn C57BL mice (Doell & Carnes, 1962) and (C57BL x C3H)F_1 mice (Vesselinovitch & Mihailovich, 1966). Kaplan (1964) indicated that leukaemogenesis occurs in mice only when there is a synchrony of 3 conditions: thymic immaturity, bone marrow damage and viral release, all of which are caused by urethane in newborn mice.

In a series of studies initiated to evaluate the effect of subliminal exposure of infant mice to urethane on the leukaemogenesis caused by X-irradiation administered to young adults, the exposure of (C57BL x C3H)F_1 mice on the 3rd, 6th and 12th days of life to low doses of urethane enhanced the leukaemogenic effect of X-irradiation given on the 42nd day of life

(Vesselinovitch et al., 1972). X-irradiation combined with the administration of urethane in the virus-particle-free 020 strain of mice led to the early production of mammary tumours containing B-particles; and cell-free extracts were effective in inducing mammary tumours (Timmermans et al., 1969).

3.2 Other relevant biological data

The metabolism of urethane in experimental animals has been reviewed by Haddow (1963) and by Mirvish (1968).

When urethane is administered to rats and pregnant mice, it is rapidly and evenly distributed throughout the body and is found in the body fluids of the rats and of the mouse foetuses (Boyland & Roden, 1949; Nomura et al., 1973). In mice, about 90% of the administered dose is excreted within 24 hours as CO_2 in the expired air, about 6% remains in the body and an approximately similar amount is excreted in the urine (Bryan et al., 1949; Skipper et al., 1951). Urethane injected i.p. in newborn mice is eliminated at only a tenth of the adult rate: in newborns 20% of the administered urethane is eliminated after 24 hours; in adults, however, 75% is eliminated within 6 hours. The rate of elimination of urethane increases slowly in newborn mice for the first 10 days after birth and sharply between the 15th-20th days. The longer retention time of urethane in newborn animal liver is attributed to the lack of an esterase which metabolizes urethane to CO_2, and which is active in adult liver microsomes (Kaye, 1960; Mirvish et al., 1964).

In rats, rabbits and humans (patients with multiple myeloma treated with urethane in conjunction with an alkylating agent), the urinary metabolites are: urethane (0.5-1.7% of the administered dose), N-hydroxy urethane (0.02-0.15%), acetyl-N-hydroxy urethane (0.1-0.6%), ethyl mercapturic acid (0.1-0.2%) and N-acetyl-S-ethoxy carbonylcysteine (0.9-2.1%). The urinary metabolites of N-hydroxy urethane are qualitatively similar but quantitatively different (Boyland & Nery, 1965). N-hydroxy urethane is also excreted as glucuronide, resistant to hydrolysis by mineral acids and by beta-glucuronidase (Mirvish, 1966).

N-oxidation of urethane to form N-hydroxy urethane, N-hydroxy esters

or free radicals, resulting from abstraction of a hydrogen atom attached to the amido nitrogen (Nery, 1968), leads to biologically active ethoxycarbonylating and/or, by the loss of CO_2, to ethylating species which produce derivatives of cytosine and sulphur-containing amino acids (Boyland & Nery, 1965; Boyland & Williams, 1969; Nery, 1968, 1969b; Williams et al., 1971). Such reactions occur with tissue sulphhydryl groups and S-ethyl, and S-ethoxycarbonyl derivatives of N-acetylcysteine are mainly excreted. Inorganic one-electron oxidation of alkyl-N-hydroxycarbamates leads to alkoxy carbonylating species and to the formation of the corresponding alkyl carbamates (Boyland & Nery, 1966a,b). The metabolic "reduction" of N-hydroxy urethane which is commonly observed in vivo (Boyland & Nery, 1965; Mirvish, 1966; Nery, 1968) may be mediated by such an oxidative mechanism. The conversion of N-hydroxy urethane to urethane is inhibited by SKF-525A (Kaye & Trainin, 1966).

In contrast to urethane and acetylurethane, N-hydroxy urethane and its acyl derivatives, under physiological conditions, react with nucleic acids or their constituent bases or with proteins, specifically acetylating the primary amino group of cytosine. Incubation of cytosine with the urethane metabolite, O-acetyl-N-hydroxy urethane, in the presence of mouse liver preparations, results in the formation of uracil, in addition to N-acetyl cytosine (Nery, 1969b). Also, although the carcinogenic activities of urethane and N-hydroxy urethane are approximately equal (Boiato et al., 1966), N-hydroxy urethane is much more active than urethane in assay systems where further metabolism of the compounds is suppressed, e.g., in the inactivation of DNA (Bendich et al., 1963), the inactivation of transforming DNA by Bacillus subtilis (Freese, 1965), in causing chromosomal aberrations in plants (Boyland et al., 1965) and animals (Borenfreund et al., 1964), as an antiviral agent (de Sousa et al., 1965), as a specific inhibitor of DNA synthesis (Young & Hodas, 1964), as a teratogen (Murphy & Chaube, 1964) and in the production of methaemoglobinaemia (Boyland, 1968).

Mullinix et al. (1973), using the degradation of bacterial DNA, which results in cellular death after exposure to N-hydroxy urethane, showed that 2 types of reaction occur between DNA and N-hydroxy urethane. One involves the degradation of DNA and is promoted by oxidizing agents and blocked under

reductive conditions by free-radical scavengers. The second reaction involves modification of deoxycytosine. This modification is blocked by oxidizing as well as by reducing agents and by free-radical scavengers. Evidence was presented that a common intermediate may be ethoxycarbonyl urethane. The protective effect of thymine or thymidine against chromosome damage by urethane (Boyland & Koller, 1954) and the inhibition of carcinogenesis by thymine, thymidine, cytosine, asparagine and aspartic acid (Elion et al., 1960) suggest that the carcinogenic action of urethane involves the inhibition of enzymic steps in nucleic acid metabolism, probably during pyrimidine biosynthesis. Aspartate carbamoyl transferase activity of mouse liver and lung is inhibited by urethane in vivo (Giri & Bhide, 1968) but not in vitro (Kaye, 1968). When (1-^{14}C)ethyl or ethyl (carboxy-^{14}C)carbamate is administered to mice, radioactive labelling of mouse tissues is found to result from the incorporation of ^{14}C-ethanol or $^{14}CO_2$ formed by hydrolysis of urethane. Most of the radioactivity in mouse liver RNA, however, is present in a single compound, ethyl cytosine-5-carboxylate, which cannot be detected in liver RNA of mice receiving (1-^{14}C) ethanol or sodium hydrogen-(^{14}C)carbonate (Boyland & Williams, 1969). The mechanism by which ethyl cytosine-5-carboxyl is formed remains unknown. Several unidentified radioactive labelled components are found in DNA and RNA in rats after the administration of (1-^{14}C)urethane or of (2-^{3}H)ethylcarbamate, whilst with ethyl(carboxy-^{14}C)carbamate, the amount of radioactivity associated with nucleic acids is negligible (Prodi et al., 1970). Actinomycin D, a potent inhibitor of RNA synthesis, inhibits skin tumour carcinogenesis by urethane and croton oil (Gelboin et al., 1965). In partially hepatectomized mice, the highest incidence of hepatomas is obtained when urethane is administered during a peak of RNA synthesis (Chernozemski & Warwick, 1970). Similarly, administration of (2-^{3}H)ethyl or ethyl(carboxy-^{14}C)carbamate results in preferential labelling of RNA fractions, when the highest RNA synthesis has occurred (Williams et al., 1971). When (2-^{3}H) ethyl carbamate is given in a single s.c. injection to newborn mice, mitochondrial DNA shows a higher radioactivity than do nuclear or microsomal DNA (Chavan & Bhide, 1973). In newborn animals, radioactivity remains bound to DNA and RNA in proteins for up to 2 weeks after treatment (Chavan

& Bhide, 1972). Although ethoxycarbonylation of nucleic acid cytosine may be an important initiating step in urethane carcinogenesis, Williams et al. (1971) observed higher levels of methoxycarbonylation of RNA cytosine after the administration to mice of the methyl carbamate (a compound which is not carcinogenic to rodents (Larsen, 1947b; Roe & Salaman, 1955)).

Thus, there appears to be an absolute structural requirement for the integration of the urethane molecule. Any chemical alteration of the molecular structure, except by N-hydroxylation, reduces the carcinogenic activity of urethane (Berenblum et al., 1959). The tumour-initiating properties of alkyl carbamates depend on the nature of the ester group, the presence of a free hydrogen in the amide position and the nature of the single substituent in the amide position (Pound, 1967). Evidence that urethane does not act *per se*, but needs metabolic action to exert its carcinogenic activity can be summarized as follows: irrespective of the route of administration, tumours are produced at many distinct sites throughout the body; mouse lung transplants become neoplastic only if they are previously exposed to urethane under *in vitro* metabolizing conditions (Rogers, 1955). Urethane is carcinogenic to hamsters but does not cause malignant transformation of hamster cells *in vitro* (Berwald & Sachs, 1963). Urethane causes chromosome damage in rats (Boyland & Koller, 1954) but not in cultured animal cells (Borenfreund et al., 1964) nor in plant cells (Boyland et al., 1965). Urethane and acetylurethane do not react with nucleic acids or proteins under physiological conditions (Nery, 1969b).

3.3 Observations in man

No case reports or epidemiological studies were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Urethane has been shown to be carcinogenic in mice, rats and hamsters

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

following administration by the oral, inhalation, subcutaneous or intraperitoneal routes, producing, among others, lung tumours, lymphomas, hepatomas, melanomas and vascular tumours. It is an initiator for skin carcinogenesis in mice both when given orally and topically. It was also shown to enhance the leukaemogenic effect of X-irradiation. It is carcinogenic in single dose experiments and following prenatal exposure. Neonatal and infant mice are more susceptible to cancer induction by urethane than are adult mice.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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NITROFURANS

2-AMINO-5-(5-NITRO-2-FURYL)-1,3,4-THIADIAZOLE*

1. Chemical and Physical Data

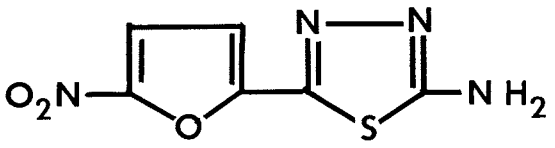
1.1 Synonyms and trade names

Chem. Abstr. No.: 712-68-5

5-Amino-2-(5-nitro-2-furyl)-1,3,4-thiadiazole; 2-(5-nitro-2-furyl)-5-amino-1,3,4-thiadiazole; 5-(5-nitro-2-furyl)-2-amino-1,3,4-thiadiazole

ASA-140; Furidazina; Furidiazina; Furidiazine; NF-475; Ph/778; Triafur

1.2 Chemical formula and molecular weight



$C_6H_4N_4O_3S$ Mol. wt: 212.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Yellow crystals

(b) Melting-point: 270-273°C (decomposition)

(c) UV absorption spectroscopy: λ_{max}379 nm: log ε 4.16 (in water)

λ_{max}287 and 373 nm (in ethanol

(d) Solubility: Slightly soluble in water and ethanol; soluble in dimethyl formamide

1.4 Technical products and impurities

No data were available to the Working Group.

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* Considered by the Working Group in Lyon, June 1974

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

The synthesis of 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole was first reported in 1960 (Skagius et al., 1960). It can be made by the dehydration of N-5-nitrofuroylthiosemicarbazide. The latter compound can be made by the reaction of 5-nitro-2-furoyl chloride with thiosemicarbazide or through the reaction of 5-nitro-2-furoylhydrazine with potassium thiocyanate in the presence of hydrochloric acid (Sherman, 1961). A British patent on this chemical was obtained by a Swedish pharmaceutical company (Aktiebolaget Pharmacia, 1960). Although it has been reported that there were two manufacturers of this compound in Western Europe in 1973 (Chemical Information Services, Ltd, 1973), there is no evidence that the product is presently manufactured in Europe. No evidence was found that it was ever produced commercially in the US or Japan.

Studies have been reported on the use of this chemical in the treatment of gastroenteritis (25 mg/kg bw/day) and as a topical agent for relief of the symptoms of haemorrhoids, anal fissures and proctitis (Miura & Reckendorf, 1967).

2.2 Occurrence

This chemical is not known to occur in nature.

2.3 Analysis

No data were 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

Rat: Weanling female Sprague-Dawley rats were fed a diet containing 2000 ppm 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole for 1 week, at which

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

time feeding of the chemical was stopped for 1 week because of failure of the rats to grow. Dietary administration was resumed at the start of the third experimental week at a dose of 200 ppm and was continued until the 46th experimental week when it was replaced by the control diet for an additional 20 weeks. The mean cumulative total dose per rat was 1.2 g (5.7 mmoles). Of 33 animals surviving 10 or more weeks, 32 developed a total of 38 tumours, including 4 benign and 28 malignant mammary tumours and 3 forestomach squamous-cell papillomas. The first mammary tumour was detected at 34 weeks. Hyperplasia of the epithelium of the renal pelvis was present in 5 rats, and 1 rat developed a transitional-cell carcinoma of the renal pelvis. Two solitary, benign mammary tumours were seen among 24 untreated controls (Cohen et al., 1974; Ertürk et al., 1970).

3.2 Other relevant biological data

When ^{35}S-labelled 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole was administered orally to mice, about half of the activity was excreted in the urine in 7 hours. Several radioactive metabolites were detected but not characterized (Campbell, 1964).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole is carcinogenic in rats following oral administration, the only species and route tested. It produced mammary carcinomas and forestomach papillomas.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

5. References

Aktiebolaget Pharmacia (1960) November 2, British Patent 852,795

Campbell, D.E.S. (1964) Studies of excretion of two new nitrofuran derivatives after oral application in mice. In: Proceedings of the 3rd International Congress of Chemotherapy, Stuttgart,1963, Stuttgart, Thieme, pp. 570-574

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Cohen, S.M., Ertürk, E., Von Esch, A.M., Crovetti, A.J. & Bryan, G.T. (1974) Carcinogenicity of 5-nitrofurans and related compounds with amino-heterocyclic substituents. J. nat. Cancer Inst. (in press)

Ertürk, E., Cohen, S.M. & Bryan, G.T. (1970) Comparative carcinogenicity of amino- and N-acetylamino-5-nitrofuran compounds in the rat. Fed. Proc., 29, 817

Miura, K. & Reckendorf, H.K. (1967) The Nitrofurans. In: Ellis, G.P. & West, G.B., eds, Progress in Medicinal Chemistry, Vol. 5, New York, Plenum, p. 367

Sherman, W.R. (1961) 5-Nitro-2-furyl-substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,3,5-triazines. J. org. Chem., 26, 88-95

Skagius, K., Rubinstein, K. & Ifversen, E. (1960) Potential chemotherapeutics. II. 2-(5-Nitro-2-furyl)-5-amino-1,3,4-thiadiazoles. Acta chem. scand., 14, 1054-1058

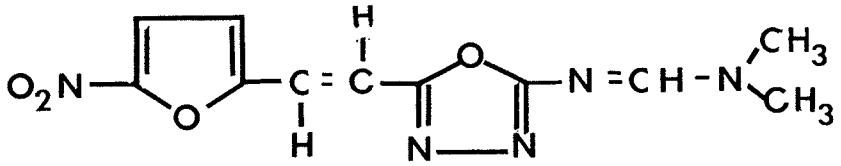
trans-2-[(DIMETHYLAMINO)METHYLIMINO]-5-[2-(5-NITRO-2-FURYL)VINYL]-1,3,4-OXADIAZOLE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 259-62-77-0

1.2 Chemical formula and molecular weight



$C_{11}H_{11}N_5O_4$ Mol. wt: 277.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Bright reddish-orange needles

(b) Melting-point: 204-207°C

(c) Solubility: Soluble in dimethyl sulphoxide, dimethyl formamide, ethylene glycol and diethyl ether

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Although there was some indication in the literature that this chemical

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* Considered by the Working Group in Lyon, June 1974

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

had found limited usage as a pharmaceutical, further investigation revealed that it has never been produced commercially in the US, Western Europe or Japan.

2.2 Occurrence

This chemical is not known to occur in nature.

2.3 Analysis

No data were 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

Rat: Weanling female Sprague-Dawley rats were fed a diet containing 2000 ppm trans-2-[(dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole during 46 weeks (total dose, 8.4 g, 30.3 mmoles) followed by the control diet for 20 weeks. Of 36 animals surviving 10 or more weeks, 23 developed a total of 30 tumours, including 22 mammary carcinomas, 3 forestomach squamous-cell papillomas, 2 benign and 2 malignant intestinal tumours and 1 pulmonary alveolar-cell carcinoma. Hyperplasia of the epithelium of the renal pelvis was present in 2 rats. Two solitary, benign mammary tumours were seen among 24 untreated controls (Cohen & Bryan, 1973; Cohen et al., 1974).

3.2 Other relevant biological data

No data were available to the Working Group.

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

trans-2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole is carcinogenic in rats following oral administration, the only species and route tested. It produced mammary and intestinal tract carcinomas.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

5. References

Cohen, S.M. & Bryan, G.T. (1973) Carcinogenesis caused by nitrofuran derivatives. In: Pharmacology and the Future of Man. Proceedings of the Fifth International Congress on Pharmacology, San Francisco, 1972, Vol. 2, Basel, Karger, pp. 164-170

Cohen, S.M., Ertürk, E., Von Esch, A.M., Crovetti, A.J. & Bryan, G.T. (1974) Carcinogenicity of 5-nitrofurans and related compounds with amino-heterocyclic substituents. J. nat. Cancer Inst. (in press)

2-(2-FORMYLHYDRAZINO)-4-(5-NITRO-2-FURYL)THIAZOLE*

1. Chemical and Physical Data

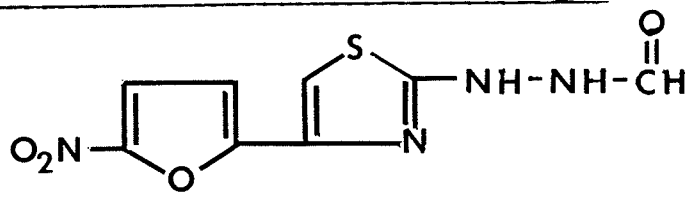
1.1 Synonyms and trade names

Chem. Abstr. No.: 3570-75-0

Formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide; 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)thiazol

AS-17665; FNT; Nefurthiazole; Nifurthiazol; Nifurthiazole

1.2 Chemical formula and molecular weight



$C_8H_6N_4O_4S$ Mol. wt: 254.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Bright yellow plates

(b) Melting-point: 215.5°C (decomposition) (Sherman & Dickson, 1962)

(c) UV absorption spectroscopy: λ_{max}385 nm (in 0.05 M sodium phosphate buffer, pH 7.4). In thin-layer chromatography 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)thiazole has the following Rf values: chloroform:methanol (9:1), 0.36; acetonitrile: ammonium hydroxide:water (10:3:0.5), 0.76; and chloroform:methanol:formic acid (6.5:3:0.5), 0.81 (Cohen et al., 1973a).

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* Considered by the Working Group in Lyon, June 1974

(d) Solubility: Soluble in n-butanol, dimethyl formamide, dimethyl sulphoxide, ethanol and polyethylene glycol

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Synthesis of 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)thiazole was first reported in 1962. It can be made by heating formic acid with 2-hydrazino-4-(5-nitro-2-furyl)thiazole (Dickson & Sherman, 1962).

No evidence was found that it has ever been produced commercially in the US, Western Europe or Japan.

2.2 Occurrence

This chemical is not known to occur in nature.

2.3 Analysis

Blood serum analysis was conducted by a 2-fold dilution method utilizing Bacillus subtilis 10707 as the test organism; sensitivity was 0.09 µg/ml (Holper et al., 1962). Cohen et al. (1970) and Ertürk et al. (1971) used infra-red spectroscopy, ultra-violet absorption and paper chromatography to analyze the compound. Urine samples were analyzed by paper chromatography, and hepatic and renal cytosol samples by Sephadex G-25 chromatography followed by ultra-violet absorption spectroscopy. Liquid scintillation counting procedures were employed to quantitate the pharmacologic distribution and metabolic transformation of ^{14}C-labelled 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)thiazole in mice and rats, with preliminary purification by paper or column chromatography (Cohen et al., 1973a).

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

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 group of 50 5-week old female Swiss mice was fed a diet containing 1000 ppm 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)thiazole (FNT) for 13 weeks. Due to failure to gain weight and to premature mortality, the control diet was then fed for a further 17 weeks, after which a diet containing 1000 ppm FNT was fed for an additional 16 weeks, followed again by the control diet for 7 weeks. The mean total consumption of FNT was 1.2 g per mouse. Because of the presence of a penicillin-sensitive pathogen in the lungs of some animals, bicillin LA was administered intramuscularly during the 13th and 28th experimental weeks to both treated and control mice. Of the 22 treated animals that survived for 18 weeks or more, all developed tumours, the total being 58 and including 14 squamous-cell carcinomas of the stomach, 4 mixed squamous-cell and adenocarcinomas of the stomach, 3 adenocarcinomas of the stomach, 9 pulmonary alveolar-cell carcinomas, 7 mammary adenocarcinomas, 19 generalized leukaemias and 2 tumours at other sites. Forestomach tumours frequently metastasized to the visceral peritoneum, liver and lungs. Of 44 control mice that survived for 40 or more weeks, 1 had a pulmonary alveolar-cell carcinoma and 15 had leukaemia; however, the incidences of these tumours in treated animals were significant ($P<0.01$ for the alveolar-cell carcinomas; $P<0.001$ for the leukaemias) (Cohen et al., 1970).

A diet containing 500 ppm FNT was fed to 30 5-week old female Swiss mice for 33 weeks (total dose, 710 mg per mouse) followed by the control diet for an additional 19 weeks. Of 20 treated mice that survived for 10 or more weeks, 19 developed a total of 24 tumours including 11 papillomas and 1 carcinoma of the forestomach, 2 pulmonary alveolar-cell carcinomas and 10 lymphocytic leukaemias ($P<0.001$, leukaemia being present in 2/29 control mice surviving for 10 or more weeks). Twenty-two of the control mice survived for more than 50 weeks (Cohen et al., 1973c).

Rat: Stein et al. (1966) first reported the production of tumours in

rats fed diets containing FNT, but no details were available.

A group of 20 60-day old female Holtzman rats was fed a diet containing 2000 ppm FNT for 36 weeks followed by the control diet for a further 19 weeks (total dose, 5.1 g, 20.1 mmoles). Three animals surviving for 24 or more weeks each developed a benign mammary tumour. No tumours were present in 5 control rats surviving for 36 or more weeks. In a second experiment, 36 22-day old female Holtzman rats were fed a diet containing 2000 ppm FNT for 44 weeks followed by the control diet for 17 weeks (total dose, 5.8 g, 23.0 mmoles); and 60 tumours developed in 26 rats surviving 17 or more weeks. These tumours included 19 benign and 6 malignant mammary tumours, 16 adenomas, 5 adenocarcinomas and 1 fibroma of the kidney, 5 small-bowel and 2 caecal or large-bowel adenocarcinomas, 4 carcinomas and 1 fibroma of the external auditory canal and 1 haemangioendothelial sarcoma of the liver. Three benign mammary tumours were present among 16 untreated controls surviving for 36 or more weeks. To attempt control of pulmonary infections, tetracyclin and penicillin were administered intermittently; and piperazine citrate was given to rats in both experiments to protect against pinworm infestation (Morris et al., 1969). A mammary adenocarcinoma arising in one of the FNT-treated rats was transplantable subcutaneously into unconditioned male and female newborn rats of the same strain (Ertürk et al., 1970b).

Thirty male and 30 female weanling Sprague-Dawley and 30 female weanling Buffalo rats were fed a diet containing 2000 ppm FNT for 46 weeks followed by the control diet for an additional 18 weeks (total doses, 8.4 g, 33.1 mmoles; 6.6 g, 26.0 mmoles; and 5.6 g, 22.1 mmoles per rat, respectively). The numbers of survivors at 14 weeks were 29, 26 and 29 rats, respectively. Of the 29 male Sprague-Dawley rats, all developed a total of 49 tumours including 8 benign mammary tumours (first detected at 41 weeks), 7 benign and 14 malignant renal tubular tumours, 5 transitional-cell carcinomas of the renal pelvis, 1 renal fibroma, 10 hepatic cystic adenomas and 4 hepatocellular carcinomas. The 26 female Sprague-Dawley rats all developed a total of 68 tumours including 9 benign and 16 malignant mammary tumours (the first detected at 31 weeks), 8 benign and 11 malignant renal tubular tumours, 9 hepatic cystic adenomas and 15 tumours at other sites. The 29 female Buffalo rats all developed a total of 68 tumours including 6 benign

and 22 malignant mammary tumours (the first detected at 31 weeks), 5 benign and 10 malignant renal tubular tumours, 4 transitional-cell carcinomas of the renal pelvis, 11 hepatic cystic adenomas, 4 lymphocytic leukaemias and 6 tumours at other sites. Two benign mammary tumours were present in 29 female Sprague-Dawley controls, while no tumours were found in the 29 male Sprague-Dawley controls nor in the 30 female Buffalo control rats. Bicillin LA was administered i.m. to all rats to control infection (Ertürk et al., 1971).

Both the benign and malignant renal tubular tumours arising in both strains and sexes were transplantable subcutaneously into unconditioned weanling Sprague-Dawley female rats, and some were maintained through three transplant generations (Ertürk et al., 1970a).

A group of 51 weanling female Sprague-Dawley rats was fed a diet containing 2000 ppm FNT for 2 weeks, followed by the control diet for 1 week, after which a diet containing 1000 ppm FNT was fed for 43 weeks, followed by the control diet until the 75th week (total dose, 5.09 g, 19.7 mmoles). Of 51 rats surviving 10 or more weeks, 49 developed a total of 78 tumours including 49 mammary adenocarcinomas, 8 forestomach papillomas, 8 renal tubular adenocarcinomas, 4 transitional-cell carcinomas of the renal pelvis, 3 lymphocytic leukaemias and 6 tumours at other sites. Of 71 control rats surviving 10 or more weeks, 18 developed 19 tumours including 12 benign and 6 malignant mammary tumours. FNT-treated rats received no other drugs (Cohen et al., 1973b).

Groups of 40 male and 40 female weanling Sprague-Dawley rats were fed a diet containing 2000 ppm FNT for 49 and 52 weeks, respectively, followed by the control diet for an additional 52 weeks (total dose, 9.9 g, 39.0 mmoles; 8.5 g, 33.5 mmoles, respectively). Among 40 males, 33 of which survived more than 12 months, a total of 63 benign and malignant tumours was present including 23 benign and 5 malignant renal tubular tumours, 8 benign and 16 malignant tumours of the gastrointestinal tract and 11 tumours at other sites. Of the 40 untreated control male rats, 39 survived for 12 months, 27 for 18 months and 4 for 2 years. Seventeen benign and malignant tumours were present in these rats, 14 of which arose in endocrine organs; no renal or gastrointestinal tract tumours were detected. Among 40 females

fed FNT, 31 of which survived for more than 12 months, a total of 71 benign and malignant tumours was present, including 23 benign and 4 malignant renal tubular tumours, 1 benign and 9 malignant tumours of the gastrointestinal tract, 18 benign and 4 malignant mammary tumours and 12 tumours at other sites. Of the 40 untreated control female rats, 39 survived for 12 months, 36 for 18 months and 18 for 2 years. Fifty-six benign or malignant tumours were present in these rats, 30 of which arose in endocrine organs and 18 in mammary tissue; no renal or gastrointestinal tract tumours were reported (Tekeli et al., 1973).

Hamster: A group of 24 weanling male Syrian golden hamsters was fed a diet containing 1000 ppm FNT for 48 weeks, followed by the control diet for an additional 22 weeks (total dose, 2.7 g, 10.8 mmoles). All of 24 hamsters surviving 6 or more weeks developed tumours, the total being 29 and including 13 forestomach papillomas, 9 urinary baldder transitional-cell carcinomas, 6 adrenal adenomas and 1 renal pelvis transitional-cell carcinoma. One adrenal adenoma was present among 24 untreated controls (Croft & Bryan, 1973).

3.2 Other relevant biological data

In mice and rats, the absorption, distribution, metabolism and excretion of ^{14}C-FNT (labelled in the thiazole ring) was studied. It was found that the urine was the major route of elimination and that more than 95% of ^{14}C was eliminated in 96 hours; however, less than 10% of ^{14}C in the urine was FNT. In addition, ^{14}C-FNT was covalently bound to renal and hepatic cytosol macromolecules (Cohen et al., 1973a).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole is carcinogenic in mice, rats and hamsters following oral administration, the only species and route tested. In mice it produced mainly stomach and pulmonary tumours and lymphocytic leukaemias. In rats it produced mainly mammary, renal and hepatic gastrointestinal tract tumours and lymphocytic leukaemias; in hamsters it produced mainly forestomach and urinary bladder tumours.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

5. References

Cohen, S.M., Ertürk, E. & Bryan, G.T. (1970) Carcinogenicity of formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide in Swiss mice. Cancer Res., 30, 906-912

Cohen, S.M., Alter, A. & Bryan, G.T. (1973a) Distribution of radioactivity and metabolism of formic acid 2-[4-(5-nitro-2-furyl-2-^{14}C-2-thiazolyl] hydrazide following oral administration to rats and mice. Cancer Res., 33, 2802-2809

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Cohen, S.M., Lower, G.M., Jr, Ertürk, E. & Bryan, G.T. (1973c) Comparative carcinogenicity in Swiss mice of N-[4-(5-nitro-2-furyl)-2-thiazolyl] acetamide and structurally related 5-nitrofurans and 4-nitrobenzenes. Cancer Res., 33, 1593-1597

Croft, W.A. & Bryan, G.T. (1973) Production of urinary bladder carcinomas in male hamsters by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide or formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide. J. nat. Cancer Inst., 51, 941-949

Dickson, D.E. & Sherman, W.R. (1962) June 29, Belgian Patent 612,121

Ertürk, E., Cohen, S.M. & Bryan, G.T. (1970a) Induction, histogenesis and isotransplantability of renal tumors induced by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide in rats. Cancer Res., 30, 2098-2106

Ertürk, E., Morris, J.E., Cohen, S.M., Price, J.M. & Bryan, G.T. (1970b) Transplantable rat mammary tumors induced by 5-nitro-2-furaldehyde semicarbazone and by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide. Cancer Res., 30, 1409-1412

Ertürk, E., Morris, J.E., Cohen, S.M., Von Esch, A.M., Crovetti, A.J., Price, J.M. & Bryan, G.T. (1971) Comparative carcinogenicity of formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide and related chemicals in the rat. J. nat. Cancer Inst., 47, 437-445

Holper, J.C., Otto, R.H., Kimura, E.T. & Bower, R.D. (1962) AS 17665, a new systemically active nitrofuran. In: Antimicrobial Agents and Chemotherapy, Ann Arbor, Michigan, Braun-Brumfield, pp. 268-274

Morris, J.E., Price, J.M., Lalich, J.J. & Stein, R.J. (1969) The carcinogenic activity of some 5-nitrofuran derivatives in the rat. Cancer Res., 29, 2145-2156

Sherman, W.R. & Dickson, D.E. (1962) 4-(5-Nitro-2-furyl)thiazoles. J. org. Chem., 27, 1351-1355

Stein, R.J., Yost, D., Petroliunas, F. & Von Esch, A. (1966) Carcinogenic activity of nitrofurans. A histologic evaluation. Fed. Proc., 25, 291

Tekeli, S., Biava, C.G. & Price, J.M. (1973) The carcinogenic activity of 3-hydroxymethyl-1-{[3-(5-nitro-2-furyl)allydidene]amino}hydantoin in rats. Cancer Res., 33, 2894-2897

5-(MORPHOLINOMETHYL)-3-[(5-NITROFURFURYLIDENE)AMINO]-2-OXAZOLIDINONE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

(a) dl-Form

Chem. Abstr. No.: 139-91-3

Furaltadone; furaltadone tartrate; (±)5-methyl morpholino-3-[amino (5-nitrofurfurylidene)]-2-oxazolidinone; 5-morpholinomethyl-3-(5-nitrofurfurylideneamino)-2-oxazolidinone; 5-(N-morpholinomethyl)-3-(nitrofurfurylideneamino)-2-oxazolidinone; (±)-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone; 5-(4-morpholinomethyl)-3-(5-nitrofurfurylideneamino)-2-oxazolidinone; 5-(4-morpholinomethyl)-3-(5-nitro-2-furfurylideneamino)-2-oxazolidinone; 5-(4-morpholinylmethyl)-3-{[(5-nitro-2-furanyl)methylene]-amino}-2-oxazolidinone; nitrofuraltadone; 3-(5-nitro-2-furfurylideneamino)-5-(4-morpholinomethyl)-2-oxazolidinone; N-(5-nitro-2-furfurylidene)-3-amino-5-(N'-morpholinylmethyl)-2-oxazolidinone

Altabactina; Altafur; Biofurin; Donafur; F-150; Furalton; Furamidone; Furasol; Furazol INE; Furazolin; Furazoline; Furfural Piper; Furitale; Furlate; Furlidon; Furmethonol; Furmetox; Fur-Novo; Germicina; Ibifur; Medifuran; Megafur; Neofuran; NF 260; Nifadone; Nitraldone; Nitrofur; Nitrofurmethone; Otifuril; Polival; Sepsinol; Sistogram; Spectrafur; Ultrafur; Unifur; Valsyn; Viofural

(b) l-Form

Chem. Abstr. No.: 3795-88-8

(-)-Furaltadone; levofuraltadone; (-)-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone; L-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone

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* Considered by the Working Group in Lyon, June 1974

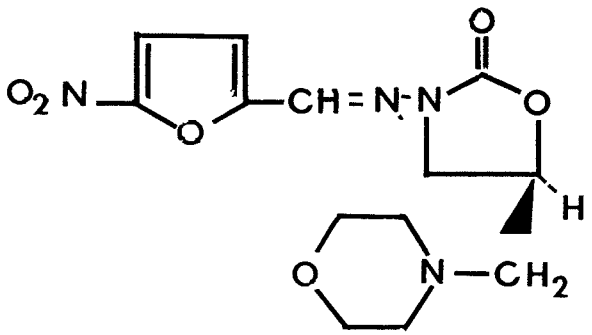
(c) dl-Form hydrochloride

Chem. Abstr. No.: 13146-28-6

(±)5-Methyl morpholino-3-[amino(5-nitrofurfuryliden)]-2-oxazolidinone hydrochloride; (±)-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene) amino]-2-oxazolidinone hydrochloride

1.2 Chemical formulae and molecular weights

Levo-form



$C_{13}H_{16}N_4O_6$ Mol. wt: 324.3

Hydrochloride

$C_{13}H_{16}N_4O_6.HCl$ Mol. wt: 360.8

Tartrate

$C_{17}H_{22}N_4O_{12}.3H_2O$ Mol. wt: 528.4

1.3 Chemical and physical properties of the pure substance

Levo-form

(a) Description: Crystalline yellow powder

(b) Melting-point: 206°C

(c) UV absorption spectrum: λ_{max} 258 nm } (in water)
λ_{max} 366 nm (log ε 4.5225) } (in water)
λ_{max} 302 nm } (in water)

(d) IR absorption spectrum: The principal peaks in KB_r pellets are at: 1755, 1532 and 1226 cm^{-1}.

(e) Stability: Stable at room temperature in absence of sunlight

(f) Solubility: Soluble in dilute acids, hot alcohol, dioxane, acetone, propylene glycol, polyethylene glycols and dimethyl formamide; slightly soluble in water; practically insoluble in ether, chloroform and benzene

Hydrochloride

(a) Description: Yellowish, odourless, sour powder

(b) UV absorption spectrum: λ_{max} 258 nm, λ_{max} 366 nm (log ε 4.4475), λ_{min} 302 nm (in water)

(c) Stability: Stable at room temperature in absence of sunlight

(d) Solubility: Soluble in water and methanol; slightly soluble in dimethyl formamide; insoluble in ethanol

Tartrate

(a) Description: Crystalline, yellow, odourless, bitter solid

(b) UV absorption spectrum: λ_{max} 258 nm, λ_{max} 365 nm (log ε 4.1858), λ_{min} 301 nm (in water)

(c) Stability: Stable at room temperature in absence of sunlight

(d) Solubility: About 5% in water at room temperature; practically insoluble in chloroform and ether

1.4 Technical products and impurities

This chemical is available in the US only as a component of veterinary medicines containing the chemical alone or in combination with procaine penicillin G in the form of a suspension in peanut oil. The hydrochloride and the tartrate are also available in Western Europe.

2. Production, Use, Occurrence and Analysis

Two reviews on nitrofurans have been published (Miura & Reckendorf, 1967; Paul & Paul, 1964).

2.1 Production and use[1]

Synthesis of 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone was first reported in 1957. It can be made by the reaction of 3-morpholinyl-1,2-epoxypropane with hydrazine hydrate, followed by condensation with diethyl carbonate and sodium in methyl alcohol to produce 3-amino-5-morpholinomethyl-2-oxazolidinone. Condensation of this intermediate with 5-nitro-2-furaldehyde produces 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone (Gever, 1957). Commercial production of this chemical was first reported to the US Tariff Commission by the only US producer in 1959 (US Tariff Commission, 1960). This company has not reported commercial production since 1960, although it is still believed to be producing the chemical for use in veterinary medicines. It has been produced in Japan in the past but is no longer made there.

In Europe, this compound, as well as the hydrochloride and tartrate salts, are known to be produced commercially by three companies, one each in Italy, The Netherlands and Spain. There may be additional producing companies in the Federal Republic of Germany, Hungary, Israel and Italy (Chemical Information Services,Ltd, 1973; Ragno, 1972). Total European production is estimated to be in excess of 10 thousand kg per year.

In 1964 it was reported that this chemical was being used for the treatment of salmonellosis and acrosacculitis in poultry, for bovine mastitis and for canine bacterial infections (Paul & Paul, 1964). Since April 1973, suspensions of 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone in peanut oil carrier, either alone or in combination with procaine penicillin G, have been approved for use against bovine mastitis

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

by the US Food and Drug Administration (FDA). However, a zero tolerance for residues of the chemical in the milk of dairy cows is applied (US Code of Federal Regulations, 1973).

In July 1971, the FDA announced its intention of withdrawing approval for the use of the compound in the treatment of bovine mastitis because it had been found to produce tumours in laboratory animals (US Code of Federal Regulations, 1971). Although an opportunity was given for interested parties to present facts to a hearing on the proposed withdrawal, by late 1973 the FDA apparently had reached no conclusion on the continued use of this substance (US Congress, 1973).

This chemical is also recommended in Europe for the treatment of bacterial enteritis in fowl, of colibacillosis in hogs and calves, of livestock mastitis and of bacterial disease in fish.

It was introduced in the US and in a few European countries for systemic treatment of staphylococcus infections, but its use in human medicine was stopped because of its neurotoxic properties (Miura & Reckendorf, 1967).

One European manufacturer recommends this compound in human medicine for the treatment of localized skin or mucous infections and of infections of the urinary tract.

2.2 Occurrence

5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone is not known to occur in nature.

Cox & Heotis (1963) reported that after 3 intramammary injections of 2 g of this chemical were given to Holstein cows following 3 consecutive milkings, the levels in the milk declined to 0-0.01 ppm 48 hours after the last medication.

2.3 Analysis

Biological samples can be extracted with organic solvents, and 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone can be determined by ultraviolet spectrophotometric, colorimetric or microbiological methods of analysis (Paul et al., 1960). Milk can be extracted using chloroform and 0.1 N hydrochloric acid prior to spectrophotometric analysis; the

sensitivity is 0.01 ppm with an accuracy of ±10% at the 95% confidence level (Cox & Heotis, 1963).

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 female Sprague-Dawley rats were administered 1000 ppm levo-5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone hydrochloride in the diet for 46 weeks (total dose, 5 g, 13.9 mmoles) followed by a control diet for 20 weeks. Of 32 rats that survived for 10 or more weeks, 31 developed a total of 41 tumours including 6 benign and 25 malignant mammary tumours, 7 lymphoblastic lymphomas and 2 transitional-cell carcinomas of the renal pelvis. The first mammary tumour was detected at 30 weeks. One benign mammary tumour was present among 25 untreated controls (Cohen et al., 1973).

3.2 Other relevant biological data

The LD_{50} in mice is 525 mg/kg bw (Paul & Paul, 1964).

In rats dosed with 100 mg/kg bw 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone, plasma levels of 3.2 mg/l were demonstrated after 4 hours, with about 10% bound to plasma proteins. When rats received 138 mg/kg bw, about 3.4% was recovered from urine within 48 hours. The compound was detectable by chemical and microbiological techniques in milk obtained from dogs or cows during a 4-hour period following the administration of 20 mg/kg bw 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone or its hydrochloride and in the bile of chickens and dogs administered the compound or its hydrochloride. The compound, its hydrochloride or its citrate, following oral or intravenous administration, were detectable in the cerebrospinal fluid of dogs within 0.5-4 hours (Paul et al., 1960).

5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone was enzymatically reduced by rat liver homogenate under anaerobic conditions

(Akao 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 were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone is carcinogenic in rats following oral administration of its hydrochloride. It produced mainly mammary carcinomas and lymphoblastic lymphomas. No other species or routes of administration were tested.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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5-NITRO-2-FURALDEHYDE SEMICARBAZONE*

1. Chemical and Physical Data

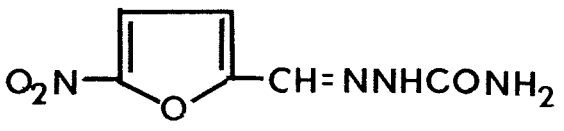
1.1 Synonyms and trade names

Chem. Abstr. No.: 59-87-0

Nitrofural; 5-nitrofuraldehyde semicarbazide; nitrofuraldehyde semicarbazone; 5-nitrofuran-2-aldehyde semicarbazone; 5-nitro-2-furancarboxaldehyde semicarbazone; 2-[(5-nitro-2-furanyl)methylene]-hydrazinecarboxamide; nitrofurazone; 5-nitro-2-furfuraldehyde semicarbazone; 5-nitrofurfural semicarbazone; 5-nitro-2-furfural semicarbazone; (5-nitro-2-furfurylideneamino)urea; 1-(5-nitro-2-furfurylidene)semicarbazide

Aldomycin; Alfucin; Amifur; Babrocid; Becafurazone; Biofuracina; Biofurea; Chemofuran; Chixin; Cocafurin; Coxistat; Dermofural; Dynazone; Eldezol F-6; Fedacin; Flavazone; Fracine; Furacilin; Furacillin; Furacin; Furacin-E; Furacine; Furacinetten; Furacin-HC; Furacoccid; Furacort; Furacycline; Furaldon; Furalone; Furametral; Furan-ofteno; Furaplast; Furaseptyl; Furaskin; Furaziline; Furazin; Furazina; Furazol W; Furazone; Furesol; Furfurin; Furosem; Fuvacillin; Hemofuran; Ibiofural; Mammex; Mastofuran; Monofuracin; Nefco; NF-7; NFS; NFZ; Nifucin; Nifurid; Nifuzon; Nitrofurazan; Nitrofurazone; NSC-2100; Otofural; Otofuran; Rivafurazon; Rivopon-S; Sanfuran; Spray-Dermis; Sprayforal; Vabrocid; Vadrocid; Veterinary nitrofurazone; Yatrocin

1.2 Chemical formula and molecular weight



$C_6H_6N_4O_4$ Mol. wt: 198

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* Considered by the Working Group in Lyon, June 1974

1.3 Chemical and physical properties of the pure substance

(a) Description: A microcrystalline, lemon-yellow, odourless solid, appearing also in polymorphous forms. Initially tasteless, then bitter

(b) Melting-point: 236-240°C (decomposition)

(c) UV absorption spectroscopy:

λ_{max} 365 nm (log ε 4.5707)	(in methanol)
λ_{max} 260 nm (log ε 4.5515)	
λ_{min} 302 nm	
λ_{max} 375 nm (log ε 4.5150)	(in ethanol-water 0.005%)
λ_{max} 260 nm (log ε 4.5150)	
λ_{min} 306 nm	

(d) Solubility: Soluble in dimethyl formamide (1 g in 15 ml); polyethylene glycol (1 in 86); propylene glycol (1 in 350); acetone (1 in 415); slightly soluble in water (1 in 4200); almost insoluble in chloroform (1 in 27000) and benzene (1 in 43500)

(e) Chemical reactivity: Stable in solid state when protected from light

1.4 Technical products and impurities

One sample of chemical grade 5-nitro-2-furaldehyde semicarbazone was reported to contain about 3% 5-nitro-2-furaldehyde azine as an impurity (Morris et al., 1969).

5-Nitro-2-furaldehyde semicarbazone is available in the US as creams, ointments, powders, solutions, sprays, suppositories and surgical dressings. In many of these products the concentration may be only 0.2%, and it may be used in combination with other pharmaceuticals (e.g., hydrocortisone acetate, phenylephrine hydrochloride) (American Society of Hospital Pharmacists, 1970).

5-Nitro-2-furaldehyde semicarbazone is available in Western Europe in the form of powders, creams, ointments and solutions; it is available in Japan as powders, tablets and ointments.

2. Production, Use, Occurrence and Analysis

Two reviews on the nitrofurans have been published (Miura & Reckendorf, 1967; Paul & Paul, 1964).

2.1 Production and use[1]

The action of this chemical as a topical antibacterial agent was first reported in 1944 (Dodd & Stillman, 1944), and the product was available for general use in 1945 (Miura & Reckendorf, 1967). Commercial production was first reported in the US in 1955 (US Tariff Commission, 1956). In 1947, a US patent was granted for a method of synthesis involving the reaction of 5-nitrofurfural with an aqueous solution of a mixture of semicarbazide hydrochloride and sodium acetate (Stillman & Scott, 1947). In 1960, a US patent was granted for a synthesis route based on the reaction of acetone semicarbazone or other semicarbazones with 5-nitrofurfuraldoxime (Gever & O'Keefe, 1960). Whether either of these routes is used for the commercial synthesis of 5-nitro-2-furaldehyde semicarbazone is not known.

This chemical is known to be produced by one company in the US and at least one company in Spain and one in Italy. There may also be additional producing companies in the Federal Republic of Germany, Italy, Israel, Hungary, The Netherlands and Spain, but this could not be verified (Chemical Information Services,Ltd., 1973; Ragno, 1972). 5-Nitro-2-furaldehyde semicarbazone was produced by 3 Japanese companies in the past, but now only one company does so. Annual production is estimated to be about 12,000 kg, but production is decreasing year by year; and in recent years this chemical has been neither imported nor exported by Japan.

5-Nitro-2-furaldehyde semicarbazone has been used in human medicine as an antibacterial agent for the treatment or prevention of infections in a variety of conditions involving the skin, eyes, ears, nose and the genito-urinary tract. It is used topically on burns, pyodermas, skin

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

grafts, ulcers and wounds (American Society of Hospital Pharmacists, 1970). It was used against wound infections in Europe during World War II and has been used in the USSR as a component of a protective paste for the hands of workers in industry (Miura & Reckendorf, 1967). It has been used for infections of eyelids and in conjunctivitis (Leopold, 1972). It was used as the antibacterial agent in solutions for treatment of bacterial otitis externa and bacterial otitis media, in nasal decongestant combinations, in urethral inserts for treatment of urethral inflammations and in vaginal suppositories for treatment of bacterial vaginitis and cervicitis (Kastrup, 1973). It reportedly has found limited use as a systemic treatment of infections in human medicine (Esplin, 1970). Some studies have indicated that it is useful in controlling metastases of malignant testicular tumours and in the control of the tropical illness, Chagas disease (Miura & Reckendorf, 1967). Total US sales of 5-nitro-2-furaldehyde semicarbazone for use in human medicine are estimated to be less than 100 kg annually.

In March 1973, the US Food and Drug Administration (FDA) proposed to withdraw approval for all pharmaceutical products containing this chemical except those used for topical applications (US Department of Health, Education and Welfare, 1973). No final action had been taken on this proposal as of April 30 1974.

5-Nitro-2-furaldehyde semicarbazone has found considerable use in veterinary medicine. It was used as an anticoccidial agent in poultry feeds but reportedly has been largely replaced by other agents (Hayes, 1967). It was used at one time for the treatment of bovine mastitis (Merck & Co., 1961). It is used topically against infections in surface lesions and is administered orally in drinking-water for the treatment of infectious enteritis in swine (Shor & Magee, 1970). Since April 1973, it has been approved by the FDA for the treatment of grey diarrhoea in minks and for use in combination with nifuroxime and diperodon hydrochloride for treatment of bacterial ear infections in dogs (US Code of Federal Regulations, 1973). 5-Nitro-2-furaldehyde semicarbazone reportedly has shown significant trypanocidal action in laboratory animals (Miura & Reckendorf, 1967) but whether it finds commercial use in this application is unknown.

In Western Europe this chemical is mainly used in veterinary applications. In Japan it is used for the prevention and treatment of suppurative diseases and as a preservative in foods: thus, during the period 1950-1966 it was approved by the Welfare Ministry of Japan as a food preservative for fish sausage, fish ham, meat sausage, meat ham and kamaboko (fish cake) at concentrations of 5 mg/kg. It was also permitted to be included in crushed ice at a level of 20 mg/kg for the preservation of fresh fish and shellfish (Matsuda, 1966).

2.2 Occurrence

5-Nitro-2-furaldehyde semicarbazone is not known to occur in nature.

2.3 Analysis

The separation and identification of 5-nitro-2-furaldehyde semicarbazone in medicated feeds has been reviewed by Fishbein (1972).

5-Nitro-2-furaldehyde semicarbazone was determined in feeds and premixes colorimetrically over a range of 0.0055-11% with a recovery of 97.7 ± 4.8 (S.D.)% following formation of 5-nitrofurfural phenylhydrazone and extraction with toluene (Buzard et al., 1956). A similar method was used for its determination in chicken tissues at 1-5 ppm (Herrett & Buzard, 1960) and for estimation of levels as low as 0.25 ppm in milk (Cox & Heotis, 1962). Using a photometric procedure, Paar (1962) detected levels down to 0.5 ppm in milk. Stone (1964) has further improved the method for milk by concentration on a chromatographic column, permitting quantitation to 0.01 ppm. A polarographic method for the estimation of the chemical in feed has been proposed (Hocquellet, 1972), and a method for identification using thin-layer chromatography has been developed (Bories, 1971).

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: In a group of 60-day old female Holtzman rats fed a diet containing 1000 ppm 5-nitro-2-furaldehyde semicarbazone (containing about 3%

5-nitro-2-furaldehyde azine) for 36 weeks followed by the control diet for 19 weeks (total dose, 3.5 g, 17.5 mmoles per rat), benign mammary tumours developed in 11 of the 18 rats which survived for 36 or more weeks. No tumours were present in 5 control rats surviving for 36 or more weeks. In a further experiment, 22-day old female Holtzman rats were fed a diet containing 1000 ppm 5-nitro-2-furaldehyde semicarbazone (containing the same azine impurity) for 44 weeks followed by the control diet for 17 weeks (total dose, 4.5 g, 22.8 mmoles per rat). All the 24 rats surviving 36 or more weeks developed benign mammary tumours. Three benign mammary tumours were present among 16 untreated controls surviving for 36 or more weeks. In an attempt to control pulmonary infections, tetracyclin and penicillin were administered intermittently; and piperazine citrate was given to rats in both experiments to protect against pinworm infestation (Morris et al., 1969).

Three of the benign mammary tumours were transplantable subcutaneously into unconditioned male and female newborn rats of the same strain (Ertürk et al., 1970).

In a group of 30 weanling female Sprague-Dawley rats fed a diet containing 1000 ppm 5-nitro-2-furaldehyde semicarbazone (free of any detectable impurity) for 46 weeks followed by the control diet for a further 20 weeks (total dose, 4.8 g, 24.2 mmoles per rat), 29 rats survived 22 or more weeks and 22 developed one or more benign mammary tumours. Two of 29 control rats developed benign mammary tumours. Bicillin LA was administered intramuscularly to all rats to control infection (Ertürk et al., 1970).

3.2 Other relevant biological data

The oral LD_{50} of 5-nitro-2-furaldehyde semicarbazone was about 600 mg/kg bw for rats and 650 mg/kg bw for mice; the animals were observed for 7 days following dosage (Miyaji, 1971).

Acute toxic effects of high doses of the chemical administered to animals include growth retardation, neurotoxicity, renal tubular, hepatic, adrenal and testicular cytotoxicity and inhibition of immunocompetence (Miyaji et al., 1964; Newberne & McEuen, 1957; Paul & Paul, 1964).

In rats dosed with 100 mg/kg bw, plasma levels of 4.5 mg/l 5-nitro-2-furaldehyde semicarbazone were found after 4 hours, 34% of which was bound to plasma proteins. Rats dosed with 200 mg/kg bw of the chemical excreted about 4.6% in urine and 0.5% in faeces within 48 hours. Orally administered 5-nitro-2-furaldehyde semicarbazone was detected in the cerebrospinal fluid of dogs within 2 hours (Paul et al., 1960). Rats dosed with 100 mg/kg bw 5-nitro-2-furaldehyde semicarbazone-(formyl-^{14}C) (0.12 µCi/mg) excreted about 66%, 35% and 1% of the activity in urine, faeces and in respired air as CO_2, respectively, within 96 hours, and the majority of ^{14}C activity was eliminated within 48 hours. Recovery of ^{14}C in the bile was about 27% after 48 hours. About 1% of ^{14}C was recovered from urine, faeces and bile as unchanged 5-nitro-2-furaldehyde semicarbazone, suggesting substantial metabolism of this substance in the rat (Tatsumi et al., 1971).

5-Nitro-2-furaldehyde semicarbazone was enzymatically reduced by rat liver homogenate under anaerobic conditions (Akao 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 were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

5-Nitro-2-furaldehyde semicarbazone (nitrofurazone) was tested only by the oral route in rats where it increased the incidence of benign mammary tumours. The available evidence is insufficient to evaluate the carcinogenicity of this compound.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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1-[(5-NITROFURFURYLIDENE)AMINO]-2-IMIDAZOLIDINONE*

1. Chemical and Physical Data

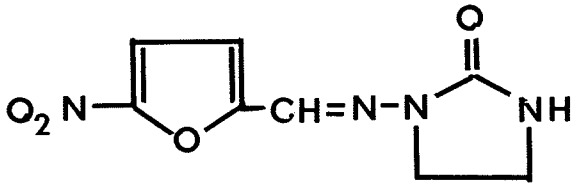
1.1 Synonyms and trade names

Chem. Abstr. No.: 555-84-0

Nifuradene; N-(5-nitro-2-furfurylidene)-1-amino-2-imidazolidinone; N-(5-nitro-2-furfurylideneamino)-2-imidazolidinone

NF-246; Nifuradine; Oxafuradene; Oxifuradene; Oxyfuradene; Renafur

1.2 Chemical formula and molecular weight



$C_8H_8N_4O_4$ Mol. wt: 224.2

1.3 Chemical and physical properties of the pure substance

(a) Description: Lemon-yellow crystals

(b) Melting-point: 261.5-263°C (decomposition)

(c) UV absorption spectroscopy: λ_{max} 387 nm; log ε 4.25 } (in water) } Michels & λ_{max} 273 nm; log ε 4.086} Gever, 1956

λ_{max} 257 nm and 365 nm (in ethanol)

(d) Solubility: Soluble in water, 88 mg/l (Paul et al., 1960); soluble in ethanol and dimethyl formamide

(e) Stability: Turns orange on exposure to air

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* Considered by the Working Group in Lyon, June 1974

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

The first laboratory synthesis of 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone, in which 5-nitro-2-furfural was condensed with 1-amino-2-imidazolidinone, was reported in 1956 (Michels & Gever, 1956). Although it was apparently marketed in the past as an antibacterial agent by a US manufacturer of nitrofurans, no evidence was found that it was ever produced commercially in the US.

There is one known producer of this chemical in Italy.

It has been reported to be used in the treatment of urinary tract infections (Miura & Reckendorf, 1967).

2.2 Occurrence

1-[5-nitrofurfurylidene)amino]-2-imidazolidinone is not know to occur in nature.

2.3 Analysis

Biological samples can be extracted with organic solvents and 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone determined by ultraviolet spectrophotometric, colorimetric or microbiological methods (Paul et al., 1960).

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 female Sprague-Dawley rats were fed a diet containing

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

1500 ppm 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone for 46 weeks followed by the control diet for 20 weeks (total dose, 6.8 g, 30.4 mmoles). Of 31 rats that survived for 10 or more weeks, all developed tumours, the total being 38 and including 2 benign mammary tumours and 29 mammary carcinomas, 5 lymphoblastic lymphomas and 2 tumours at other sites. The first mammary tumour was detected at 30 weeks. One benign mammary tumour was present among 25 untreated controls (Cohen et al., 1973).

3.2 Other relevant biological data

In rats dosed with 100 mg/kg bw, plasma levels of 4.7 mg/l 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone were demonstrated after 4 hours. When rats received 276 mg/kg bw, 10% was recovered from urine and 0.1% from faeces within 48 hours. Milk obtained from dogs and pigs during a 4-hour period following the administration of 20 mg/kg bw contained 18.6 mg/l 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone measurable by microbiological assay (Paul et al., 1960).

1-[(5-Nitrofurfurylidene)amino]hydantoin (nitrofurantoin) and 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone were identified as human urinary metabolites of 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone (Pugh et al., 1972).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone is carcinogenic in rats following oral administration, the only species and route tested. It produced mammary carcinomas and lymphoblastic lymphomas.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

5. References

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

This substance was previously evaluated in 1971 (IARC, 1972). Since that time a new crystalline form of this chemical has been tested, and further biological data concerning this compound have become available.

1. Chemical and Physical Data

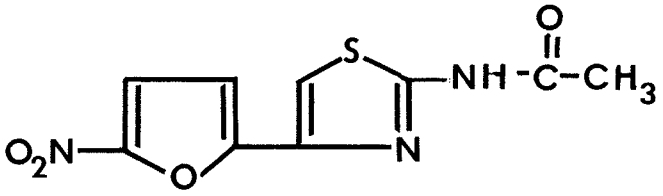
1.1 Synonyms and trade names

Chem. Abstr. No.: 531-82-8

2-Acetamido-4-(5-nitro-2-furyl)thiadiazole; 2-acetamido-4-(5-nitro-2-furyl)-thiazole; 2-acetylamino-4-(5-nitro-2-furyl)thiazole; furathiazole; N-[4-(5-nitro-2-furanyl)-2-thiazolyl]acetamide; N-[4-(5-nitro-2-furyl)thiazol-2-yl]acetamide

Furium; Furothiazole; NFTA

1.2 Chemical formula and molecular weight



$C_9H_7N_3O_4S$ Mol. wt: 253.2

1.3 Chemical and physical properties of the pure substance

(a) Description: A dark-yellow, crystalline powder

(b) Melting-point: 296°C (decomposition)

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* Considered by the Working Group in Lyon, June 1974

(c) Solubility: Insoluble in water; very slightly soluble in ethanol; moderately soluble in methanol; soluble in dimethyl formamide and dimethyl acetamide

(d) UV absorption spectroscopy: λ_{max}226, 250 and 273 nm (in methanol)
λ_{max}385 nm (in dimethyl formamide)

(e) Crystalline forms: N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide displays the unusual property of dependence on crystal form for its biological activity. Several polymorphs can be formed, each of which is distinctly different in its X-ray diffraction pattern and in its infra-red spectrum in Nujol mull (Sherman & Dickson, 1962).

1.4 Technical products and impurities

No impurities were detected by infra-red spectroscopy, ultraviolet absorption spectroscopy or paper chromatography in commercial N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide obtained from the manufacturer (Ertürk et al., 1970a).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

The first synthesis of this chemical was reported in 1962 (Sherman & Dickson, 1962). Reaction of thiourea with 2-bromoacetyl-5-nitrofuran produced the hydrobromide of 2-amino-4-(5-nitro-2-furyl)thiazole, which was then acetylated to N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide using acetic anhydride in pyridine (Hayes, 1967).

No evidence was found that this chemical has ever been produced or used commercially in the US or Japan. There is one producer in Italy.

It has been used medicinally in cases of cystitis and urinary calculi with secondary infection, following bile duct operations (400 mg per day) and in bacterial enteritis (250-400 mg per day for 3-9 days) (Miura & Reckendorf, 1967). It is believed, however, that the present production

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

is used almost exclusively in veterinary applications. [In 1967, it was reported that its chemotherapeutic activity was dependent on the crystal form (Hayes, 1967).]

2.2 Occurrence

N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide is not known to occur in nature.

2.3 Analysis[1]

This chemical in dimethyl formamide and methanol solution (1:9) can be determined by chromatography on silica gel using benzene:ethyl acetate (1:1) as eluent and a 0.5% solution of sodium fluorescein as developer under UV max 385 nm. Quantitation can be made spectrophotometrically (E_1^1 = 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 group of 50 female Swiss mice was fed a diet containing 1000 ppm commercial N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA) (see section 1.4) for 13 weeks followed by the control diet for a further 14 weeks. Of 16 mice that survived for more than 17 weeks, 15 had lymphatic leukaemia and 3 squamous-cell tumours of the stomach. No tumours occurred in 56 control animals observed over the 27-week period. Bicillin LA was administered intramuscularly to the mice to control infection (Cohen et al., 1970).

Groups of 30 female Swiss mice were fed diets containing 100, 250, 500 or 1000 ppm commercial NFTA (see section 1.4) for 14 weeks followed by the control diet for a further 14 weeks. Of animals that survived on these diets for more than 14 weeks, 7/14, 8/16, 9/13 and 8/9, respectively,

[1] Data from the manufacturer

developed leukaemia; and 3/14, 3/16, 0/13 and 6/9, respectively, developed squamous-cell stomach tumours. A single pulmonary adenoma occurred among 35 control animals (Cohen et al., 1970).

Groups of 20-36 female Swiss, RF, BALB/c and C3H mice were fed a diet containing 1000 ppm commercial NFTA (see section 1.4) for 14 weeks followed by the control diet for a further 14 weeks. In animals surviving for more than 14 weeks, leukaemia was found, respectively, in 22/22, 12/16, 21/29 and 12/24 mice of the Swiss, RF, BALB/c and C3H strains; while stomach tumours were found, respectively, in 8/22, 1/16, 2/29 and 2/24 animals. Among controls, only leukaemia was found in 3/16 RF mice (Cohen et al., 1970).

Groups of 30 female Swiss mice were fed diets containing 500 or 1000 ppm commercial NFTA (see section 1.4) for 14 weeks (total doses, 290 and 590 mg per mouse, respectively) followed by the control diet for 16 weeks. In mice surviving for more than 10 weeks, leukaemias occurred in 18/25 at the lower dose and in 26/27 at the higher dose level; all mice died by the 30th week. Leukaemia was present in 1/27 control mice surviving 10 or more weeks (Cohen et al., 1973b).

Groups of female Swiss mice which had undergone the following surgical procedures: thymectomy, partial thymectomy, sham thymectomy, splenectomy or sham splenectomy, were administered a diet containing 1000 ppm commercial NFTA (see section 1.4) for 14 weeks after which the control diet was fed. Appropriate control groups were included for each operative procedure. The incidences and sites of tumours observed in mice surviving 10 or more weeks and killed at 30 or 40 weeks are given in Table 1 (Cohen et al., 1973a).

Rat: Seventy female weanling Sprague-Dawley rats were fed a diet containing 1990 ppm commercial NFTA (see section 1.4) (100-200 mg/kg bw/day) for 46 weeks followed by the control diet for 20 weeks. Among 56 animals that survived for 16 or more weeks, 52 developed a total of 67 tumours, including 24 benign mammary tumours and 23 mammary carcinomas, 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.

TABLE 1*

INCIDENCES OF LEUKAEMIA AND FORESTOMACH TUMOURS
IN MICE TREATED WITH NFTA FOR 14 WEEKS

Operative procedure	Dose of NFTA (% by wt)	No. of mice alive at week 10	No. of lymphocytic leukaemias	No. of forestomach neoplasms	
				Papil-lomas	Carcin-omas
Thymectomy	0.0	18	0	0	0
Thymectomy	0.1	15	0	9	3
Partial thymectomy	0.0	4	1	0	0
Partial thymectomy	0.1	7	7	0	0
Sham thymectomy	0.0	27	1	0	0
Sham thymectomy	0.1	13	13	2	0
Splenectomy	0.0	23	0	0	0
Splenectomy	0.1	16	14	5	0
Sham splenectomy	0.0	20	2	0	0
Sham splenectomy	0.1	12	10	2	0
None	0.0	27	1	0	0
None	0.1	27	26	1	0

* From Cohen et al. (1973a)

Hyperplasia of the epithelium of the renal pelvis was seen in many animals, but only 2 of them developed transitional-cell carcinomas of the renal pelvis. No tumours were seen among 40 untreated controls. Bicillin LA was administered to all rats to control infection (Ertürk et al., 1970b).

Weanling female Sprague-Dawley rats (Madison, Wisconsin) were fed a diet containing 1990 ppm NFTA prepared by the method of Sherman & Dickson (1962) for 46 weeks followed by 20 weeks of the control diet. Of 69 rats that survived for 10 or more weeks, 39 developed a total of 40 tumours, including 37 benign mammary tumours, the first being noted at 42 weeks. Hyperplasia of the epithelium of the renal pelvis was seen in 12 rats, and 3 other rats developed transitional-cell carcinomas of the renal pelvis. Five benign mammary tumours were observed in 67 untreated controls (Cohen et al., 1974).

Hamster: A diet containing 1000 ppm commercial NFTA (see section 1.4) was fed to 24 weanling male Syrian golden hamsters for 48 weeks, followed by the control diet for an additional 22 weeks (total dose, 3.2 g, 12.6 mmoles per animal). Of the 24 hamsters that survived for 6 or more weeks, all developed tumours, including 16 urinary bladder and 1 renal pelvis transitional-cell carcinomas, 6 forestomach papillomas and 2 adrenal tumours. One adrenal adenoma was present among 24 untreated controls (Croft & Bryan, 1973).

Dog: Two female mongrel dogs were given commercial NFTA (see section 1.4) at a dosage of approximately 50 mg/kg bw/day for 30 months and observed for 3-5 additional months; both dogs developed 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 Other relevant biological data

Both humoral- and cell-mediated immunity of BALB/c mice were suppressed by NFTA fed in the diet for up to 70 days; and a dose-dependent, cell-mediated, immunosuppressive effect was demonstrated (Headley et al., 1974). Co-administration of NFTA and p-hydroxyacetanilide markedly inhibited the murine leukaemogenicity of NFTA; the inhibition was partially reversed by

supplementation with sodium sulphate (Cohen et al., 1972). NFTA was enzymatically reduced by rat liver xanthine oxidase or NADPH-cyt. c reductase (Wang et al., 1974a,b) prior to binding to SH-groups of protein (Wang et al., 1971). However, NFTA did not react with glutathione in the presence of rat liver homogenate (Boyland & Speyer, 1970).

3.3 Observations in man

No data were 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) is carcinogenic in mice, rats (independent of the crystalline form) and hamsters following oral administration, the only route tested. It produced generalized lymphosarcomas and forestomach tumours in mice; mainly mammary carcinomas, salivary gland carcinomas, lung carcinomas and transitional-cell carcinomas of the renal pelvis in rats; and, in hamsters, urinary bladder carcinomas and forestomach tumours. Two dogs given NFTA orally developed gall-bladder adenomas.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

5. References

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INDUSTRIAL CHEMICALS

ACETAMIDE*

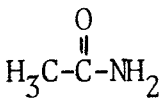
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 60-35-5

Acetic acid amide; ethanamide; methane carboxamide

1.2 Chemical formula and molecular weight



C_2H_5NO Mol. wt: 59.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Deliquescent crystals

(b) Boiling-point: $222^{o}C$

(c) Melting-point: $81^{o}C$

(d) Density: d_4^{20} 1.159

(e) Refractive index: n_D^{78} 1.4274

(f) Solubility: At $25^{o}C$, 1 g is soluble in 0.5 ml water, 2 ml ethanol or 6 ml pyridine; soluble in chloroform, glycerol and hot benzene

(g) Chemical reactivity: Neutral reaction, K_b at $25^{o}C = 3.1 \times 10^{-15}$

1.4 Technical products and impurities

In 1963 acetamide was reported to be available in the US as a technical grade (99% minimum acetamide and 0.3% maximum free acid) and as a chemically

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* Considered by the Working Group in Lyon, June 1974

pure, odourless grade (99.5-99.9% acetamide and a trace of free acid) (Lurie, 1963).

2. Production, Use, Occurrence and Analysis

A review article on acetamide has been published (Lurie, 1963).

2.1 Production and use[1]

A method for the synthesis of acetamide by fractional distillation of ammonium acetate was reported in 1923 (Coleman & Alvarado, 1923), and although many other synthesis routes are available, commercial production is believed to be based on this same distillation, the ammonium acetate being made by the reaction of ammonia with acetic acid at elevated temperature (Lurie, 1963). Acetamide has been produced commercially in the US for over 50 years: 5 producers reported a total production of 200 kg in 1921 (US Tariff Commission, 1922). In 1972, 2 US companies were believed to be making acetamide; but only 1 manufacturer was reporting commercial production to the US Tariff Commission, and separate production data were not given (US Tariff Commission, 1974).

Acetamide is produced in the following European countries (number of producing companies is shown in parentheses): the Federal Republic of Germany (3); France (1); Italy (2); and the United Kingdom (7) (Ben Brothers, Ltd., 1974; Chemical Information Services, Ltd., 1973; Econ Verlag GmbH, 1973-1975). The total annual European production is estimated to be less than 1 million kg.

It has been reported that acetamide has been used in cryoscopy, as a soldering flux ingredient, as a solvent, wetting agent and penetration accelerator for dyes, as a component of urea molding compounds, as an anti-acid in the lacquer, explosives and cosmetics industries, as a plasticizer in leather, cloth and coatings, as a humectant for paper, as an activator in bleach liquors, as a special food for molds, and as a chemical inter-

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

mediate in the synthesis of methylamine, thioacetamide, hypnotics, insecticides, medicinals and various plastics (Lurie, 1963). It was also reported that acetamide had found use as a stabilizer, in the manufacture of denatured alcohol and as an antidote in experimental fluoracetate poisoning (Merck & Co., 1968).

Whether acetamide still finds commercial use in any of the applications listed above could not be established.

2.2 Occurrence

Over-oxidized wine can contain acetamide (Datunashvili, 1963).

2.3 Analysis

Thin-layer chromatographic analysis of acid amides, including acetamide, has been described by Seeboth et al. (1966). Amounts down to 10 µg were detectable.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Rat: Dessau & Jackson (1955) first suspected a tumourigenic effect with acetamide when 1/5 Rockland albino rats developed a liver tumour described by the authors as a hepatocellular adenoma after receiving oral doses of 4 g/kg bw/day in distilled water on 5 days per week for 205 days.

In a later study, four groups of 25 1-month old male Wistar rats were fed a diet containing 0, 1.25, 2.5 or 5% acetamide for 1 year. One rat/group was killed at monthly intervals, and the remaining rats were killed after 1 year. Liver tumours (most of them described as trabecular carcinomas and some as adenocarcinomas with lung metastases) were seen in 4/24, 6/22 and 1/18 rats autopsied from the low, medium and high dose level groups, respectively. The first liver tumour was seen after 16 weeks. No liver tumours occurred in 25 controls. In a further group of 50 male Wistar rats fed 5% acetamide continuously in the diet, 1 rat was killed

weekly from 0-26 weeks, after which 1 rat was killed every other week. Liver tumours (described as being trabecular carcinomas and some as adenocarcinomas with lung metastases) were observed in 4/48 rats treated for 38-52 weeks, compared with 0/43 in controls. When acetamide was administered at a concentration of 5% in the diet to 99 male Wistar rats, with 2 rats returned to a control diet each week, liver tumours were found after treatment for 14-40 weeks in 22/81 rats autopsied (Jackson & Dessau, 1961).

Two groups of 40 male Wistar rats were fed diets containing 2.5% acetamide or 2.5% acetamide + 5.6% L-arginine L-glutamate, and 2 groups of 15 males were fed a diet containing 5.6% arginine glutamate or a control diet for 1 year. In 2/8 rats fed acetamide and killed after 1 year, hepatomas ranging "from highly differentiated to undifferentiated anaplastic growths" were observed; 7/16 rats fed acetamide for 1 year and maintained on a control diet for a further 3 months developed liver tumours. In contrast, 1/11 rats that received acetamide + arginine glutamate for 1 year developed a hepatoma, and 1/19 rats receiving such a diet for 1 year and the control diet for 3 months had hyperplastic liver nodules. No liver tumours occurred in the control group nor in rats fed 5.6% arginine glutamate alone (Weisburger et al., 1969).

3.2 Other relevant biological data

Maximum tolerated single doses for male rats and male mice are 7.5 and 8 g/kg bw, and the corresponding LD_{50} values are 10.3 and 10.1 g/kg bw (observed after 24 hours). Animals administered 1/20 the lethal single dose, 400 mg/kg bw, daily for 36 days showed decreased growth; but no other signs of toxicity or pathological lesions were observed (Caujolle et al., 1970).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Acetamide is carcinogenic in rats following oral administration, the only species and route tested, producing benign and malignant liver tumours.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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BENZENE*

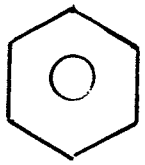
1. Chemical and Physical Data

1.1 Synonyms and trade names

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

Benzin**; benzine**; benzol; benzole; benzolene; bicarburet of hydrogen; carbon oil; coal naphtha; cyclohexatriene; motor benzol; phene; phenyl hydride; mineral naphtha; pyrobenzol; pyrobenzole

1.2 Chemical formula and molecular weight



C_6H_6 Mol. wt: 78.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Clear, colourless, highly flammable liquid

(b) Boiling-point: 80.1°C

(c) Melting-point: 5.5°C

(d) Density: d_4^{15} 0.8787

(e) Refractive index: n_D^{29} 1.5016

(f) Volatility: The vapour pressure is 74.6 mm Hg at 20°C.

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*Considered by the Working Group in Lyon, June 1974

** These two names are no longer used for benzene. They have been used for many years to describe a low-boiling petroleum fraction predominantly containing aliphatic hydrocarbons (Ayers & Muder, 1964).

(g) Solubility: Slightly soluble in water (0.8 part by weight in 1000 parts of water at 20°C); miscible with acetone, alcohol, carbon disulphide, carbon tetrachloride, chloroform, ether, glacial acetic acid and oils

1.4 Technical products and impurities

Commercial crystallizable benzene contains 99-100% benzene and has a boiling-point of 80-81°C. The typical specifications for commercial grades of benzene are: nitration grade - a distillation range not greater than 1°C and including the temperature 80.1°C; industrial grade - a distillation range not greater than 2°C and including the temperature 80.1°C; refined benzene - 0.0032 mg/l (1 ppm) maximum of thiophene, 0.15% maximum of non-aromatics and a distillation range not greater than 1°C and including the temperature 80.1°C. None of the grades must contain acidity (American Society for Testing Materials, 1968).

2. Production, Use Occurrence and Analysis

A review on benzene has been published (Ayers & Muder, 1964).

2.1 Production and use[1]

Benzene was first isolated by Faraday in 1825 from a liquid condensed by compressing oil gas. Synthesis, by the polymerization of acetylene, was first carried out in 1866 by Berthelot. Benzene has been produced commercially from coal since 1849 and from petroleum since 1941. Since 1959 the major US source of benzene has been petroleum (Ayers & Muder, 1964).

Various petroleum refinery techniques are used for the production of benzene: catalytic reforming (approximately 67% of the total US production is now made *via* this route), hydrodealkylation, pyrolysis of gasolene, toluene disproportionation and dealkylation of higher alkyl aromatics. High-temperature carbonization of coal yields coal-tar, which contains

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

benzene; however, due to increasing dependence on petrochemical derivatives, the amount of benzene derived from coal sources has become only a small part of total production and was estimated to be only 12% of US production in 1971.

In the US, Puerto Rico and the Virgin Islands there are 31 producers of petroleum-based benzene, and there are 9 in the US who manufacture coal-derived benzene.

US benzene production has shown steady growth from 459 million kg in 1940 (US Tariff Commission, 1941) to 3,928 million kg in 1972 (US Tariff Commission, 1974a). Preliminary information indicates that 1973 production was 4,649 million kg (US Tariff Commission, 1974b).

In 1972 the US exported 96.8 million kg benzene (US Department of Commerce, 1972a) and imported 317.2 million kg (US Department of Commerce, 1972b). Since 1967 there has been a decline in exports of benzene, whereas imports have grown steadily during this same period.

Benzene is produced for chemical purposes in the following Western European countries (the number of producers followed by the total production in 1972 in million kg are given in parentheses): Austria (1; 15), Belgium (3; 40), the Federal Republic of Germany (9; 827), France (7; 383), Italy (7; 503), The Netherlands (2; 554), Spain (4; 118), and the United Kingdom (8; 556) (EEC, 1973; Sindicato Industrias Químicas, 1972).

In Japan, benzene is produced by 23 companies whose primary methods of production are by extraction from reformed or cracked naphtha and by dealkylation of toluene. During the period 1970-1972, production grew at the rate of 14% per year, reaching 1,575 million kg in 1972. During the same period, imports grew from approximately 1.8 million kg in 1970 to nearly 18 million kg in 1972. Exports in 1972 were 129.8 million kg; 90% of this went to the US.

Although its major use in the US for many years was in blends with gasolene, this usage stopped almost entirely after World War II. An estimated 1971 US consumption pattern of benzene as a chemical intermediate and for other non-fuel uses follows: in ethylbenzene, 44.2%; in phenol, 19.2%;

in cyclohexane, 15.6%; in maleic anhydride, 3.9%; in detergent alkylate, 3.9%; in aniline, 3.5%; in dichlorobenzenes, 1.1%; in DDT, 0.5%; and in miscellaneous other non-fuel uses, 8.1%. The most significant increases in the use of benzene in the years since 1945 have been in the manufacture of cyclohexane (only 4.1% of total consumption in 1945) and ethylbenzene (only 22.8% in 1945).

Ethylbenzene is used in the production of styrene, which is used for the manufacture of a wide variety of elastomers and plastics. Phenol is used mainly as an intermediate for the synthesis of phenol-formaldehyde, phenol-furfural and other phenol-derived resins used primarily in the construction industry as adhesives and laminating resins. Cyclohexane is an important raw material for the manufacture of caprolactam for nylon 6 and of adipic acid and hexamethylenediamine, which are used chiefly in the synthesis of nylon 66.

It has been reported that benzene was formerly used in human medicine for the treatment of leukaemia, polycythaemia vera and malignant lymphoma; and it has been said to be useful in veterinary medicine to destroy screw-worm larvae in wounds (Merck & Co., 1968). Benzene has been used as a solvent in the manufacture of paints and, since it is also a solvent for rubber, in rubber cements (Ayers & Muder, 1964).

The following benzene consumption pattern in Western Europe in 1970 has been estimated: in styrene, 40%; in phenol (via cumene), 20%; in cyclohexane, 23%; and in miscellaneous uses, 17% (Waddams, 1973).

In Japan, the major uses for benzene are in the manufacture of: styrene monomer (via ethylbenzene), 48%; cyclohexane, 26%; phenol, 16%; alkylbenzene, 4%; and maleic anhydride, 2%; miscellaneous uses account for 2%.

2.2 Occurrence

Benzene occurs in straight-run petroleum distillates and in coal-tar distillates, e.g., light oil from coke-oven gas (Ayers & Muder, 1964). It has been reported that all motor gasolenes (petrols) contain small quantities of benzene, usually less than 5%; although special motor fuels can contain up to 30% benzene. Analysis of the ambient air in gasolene filling

stations and at bulk tanker-loading installations gave mean benzene concentrations of 0.001-0.008 mg/l (0.3-2.4 ppm) at 9 gasolene filling stations and 0.001-0.02 mg/l (0.3-6.7 ppm) during normal operations at bulk filling facilities handling normal motor gasolene. Some variation occurred depending on weather conditions; in damp misty weather with no wind, concentrations did not exceed 0.06 mg/l (19.5 ppm) (Parkinson, 1971).

Using a gas chromatographic method, Lonneman et al. (1968) detected an average concentration of benzene of 0.00005 mg/l (0.015 ppm) by volume in Los Angeles air. The highest measured concentration was 0.0002 mg/l (0.057 ppm).

The US Occupational Safety and Health Administration health standards for air contaminants require than an employee's exposure to benzene does not exceed an eight-hour time-weighted average of 0.032 mg/l (10 ppm) in the workplace air in any eight-hour workshift for a forty-hour work week. In addition, the exposure may not exceed a ceiling concentration of 0.08 mg/l (25 ppm) except for a time period of 10 minutes maximum in which the concentration may be as high as 0.16 mg/l (50 ppm) (US Code of Federal Regulations, 1974). It is reported that a level of approximately 0.08 mg/l (25 ppm) is generally considered to be the maximum admissible in other countries, but the maximum concentration permitted in the USSR is approximately 0.02 mg/l (6 ppm) (ILO, 1968).

2.3 Analysis

An indicator-tube method based on the colour reaction between benzene vapours and silica gel treated with a 5% solution of cerium sulphate in fuming sulphuric acid has been described. The method is sensitive to 0.005 mg/l (1.5 ppm), with an accuracy of ± 15% for low concentrations of benzene and of ± 5% for high concentrations (Koljkowsky, 1969).

Maffett et al. (1956) described a quick method for the determination of pure benzene in air (less than 0.1 mg/l (35 ppm)). The benzene is collected in a sampling tube containing silica gel (collection rate,1.2-1.5 l/min), and the amount retained is determined spectrophotometrically by comparing the absorbance in iso-octane at 254.5 nm with a standard calibration curve. Collection of benzene in air and on various gels and its

determination by gas chromatography have been described by Sherwood & Carter (1970). A similar procedure for breath samples is also described, together with a method for determination of the concentration of phenol in the urine of workers exposed to benzene. By such a method, a breath concentration of 0.14 ppm (0.0004 mg/l) benzene has been determined (Sherwood, 1972). Colorimetric and gas chromatographic methods for the determination of phenol in the urine of workers exposed to benzene have been described by Van Haaften & Sie (1965).

The qualitative detection of specific compounds in industrial atmospheres using activated charcoal columns, gas chromatography and mass spectrometry for the collection, separation, analysis and qualitative identification of compounds has been described. The limit of identification of benzene in airborne mixtures of organic vapours in these studies was 20 ppm (Cooper et al., 1971).

Gas chromatographic determination of traces of benzene in industrial solvents (Hoffelt & Bourdon, 1969) and in automobile exhaust gases (Ishii & Musha, 1971) have also been described.

Other analytical methods including one recommended by IUPAC are included in a review on benzene (ILO, 1968).

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

A recent review on the biological effects of benzene has been published (Deutsche Forschungsgemeinschaft, 1974).

3.1 Carcinogenicity and related studies in animals

(a) Subcutaneous and/or intramuscular injection

Mouse: Lignac (1932) reported the occurrence of leukaemias in 8/33 male and female albino mice injected s.c. with 0.001 ml benzene in 0.1 ml olive oil weekly for 17-21 weeks (total dose, about 1 mg/kg bw). In the 8 tumour-bearing mice the time between the first injection and death ranged from 4 to 11 months. No concurrent controls were used.

In 20 F mice (sex unspecified) given weekly s.c. injections of 0.001 ml benzene in sesame oil, 6/20 mice (30%) developed leukaemia between 200 and 300 days of age. Of 212 untreated mice, 29 mice (14%) developed leukaemia before 300 days of age (Kirschbaum & Strong, 1942). [The increase is not statistically significant.]

Groups of 30 male AKR, DBA_2, C3H or C57BL6 mice were given weekly s.c. injections of 0.001 ml benzene in 0.1 ml olive oil for life. No tumours were found in mice of the DBA_2, C3H or C57BL6 strains, the maximum lifespan being 730 days. Between the 7th and 16th month of treatment 16/30 treated AKR mice died with leukaemia, 8 having died before the age of 9 months without leukaemia. However, leukaemia was also observed in 30/35 male AKR untreated mice which lived, on average, longer than the test animals (Amiel, 1960).

Of 5 male and 5 female inbred Swiss mice injected s.c. each week with 0.1 ml of a 1% solution of reagent-grade benzene in olive oil, 2 died within 8 weeks. The remainder were treated for 10 weeks and of these 8 mice, 2 males and 3 females were found to have subcutaneous sarcomas at autopsy performed 162-253 days after the start of the treatment. Three of the 5 sarcomas were transplantable in syngeneic mice. No other tumours were observed, and no controls were used (Hiraki et al., 1963).

(b) Skin application

Mouse: Many experiments have been carried out in which a variety of chemicals have been applied to the skin of mice as solutions in benzene. In these experiments, a large number of control animals have been treated with benzene alone, but in none has there been any indication to suggest that benzene treatment has induced skin tumours. It should be noted, however, that all possible tumour sites have not been examined in all of the experiments. Some of the most pertinent studies were carried out by Baldwin et al. (1961), Burdette & Strong (1941), Coombs & Croft (1966), Kirschbaum & Strong (1942) and Laerum (1973).

(c) Inhalation and/or intratracheal administration

No long-term carcinogenicity tests using exposure to vapours were

available to the Working Group. This route is the most important mode of human exposure.

3.2 Other relevant biological data

(a) Animals

The oral LD_{50} of reagent grade benzene in non-fasted male Sprague-Dawley rats was reported to be 0.93 (0.71-1.23) g/kg bw (Cornish & Ryan, 1965). Kimura et al. (1971) reported oral LD_{50}'s for benzene in male Sprague-Dawley rats of 3.4 g/kg bw in young adults (80-160 g) and 4.9 g/kg bw in older animals (300-470 g). An oral LD_{50} of 5.6 g/kg bw in male Wistar rats was reported by Wolf et al. (1956). In acute inhalation experiments 3/8 male Long Evans rats died within 24 hours after exposure to 130 mg/l (40,000 ppm) benzene for five 20-35-minute periods. Death occurred in 2/10 rats exposed to 33 mg/l (10,000 ppm) benzene for 12.5-30 minutes daily from 1-17 days (Furnas & Hine, 1958). Wolf et al. (1956) reported that the no-effect level for blood changes in rats, guinea pigs and rabbits was below 0.28 mg/l (88 ppm) when the animals were exposed for 7 hrs/day for up to 269 days. At this level slight leucopaenia was observed in rats; leucopaenia was also seen in rats given 132 daily oral doses of 10 mg/kg bw during 187 days. Jenkins et al. (1970) found no effects on the blood picture in rats, guinea pigs and dogs exposed continuously to 0.056 mg/l (17.6 ppm) for up to 127 days. Slight leucopaenia has been reported to occur in rats exposed to 0.14 mg/l (44 ppm) benzene for 5 hrs/day, on 4 days/week for 5-7 weeks (Deichmann et al., 1963).

When benzene is administered orally or injected s.c. into rabbits in single doses of 0.25-1 g/kg bw, 30-75% is eliminated unchanged in the expired air, the amount being dose-related (Williams, 1959). Only small amounts are excreted unchanged in the urine unless a large dose, 3 ml, is administered. When given orally, 1-2% of the benzene is eliminated within 3 days as respiratory CO_2, 23.5% as urinary conjugated phenol, small percentages as urinary conjugated catechol (2-hydroxyphenol), quinol (4-hydroxyphenol) and hydroxyquinol (2,4-dihydroxyphenol), up to 1% as phenylmercapturic acid and 1.3% as trans-trans-muconic acid. The conjugated phenol appears mainly as phenylsulphuric acid and partly as phenylglucuronide.

The hydroxyphenols are excreted mainly as ethereal sulphates.

In non-fasted rats, the major metabolites of benzene are conjugated phenols other than glucuronides (Williams, 1959). In fasted rats, however, the major excretory products of benzene are free phenol and glucuronide conjugates with no ethereal sulphate conjugates (Cornish & Ryan, 1965).

Phenol, phenylsulphate and phenylglucuronide are also produced in vitro by rat and rabbit liver preparations to which ^{14}C-benzene is added. Pre-treatment of animals with benzene results in an in vitro increase in both the hydroxylation of benzene and in the subsequent conjugation of phenol. In the in vitro studies, when low benzene concentrations were used, most of the phenol was conjugated; at higher concentrations more free phenol remained (Snyder et al., 1967).

It has been suggested that benzene is metabolized by microsomal mixed function oxidase to benzene oxide which isomerizes spontaneously to phenol or is converted by microsomal enzymes to the trans-dihydrodiol or to S-(1, 2-dihydro-2-hydroxyphenyl)-glutathione (Daly et al., 1972). The binding of benzene to cytochrome P-450 appears to be a significant factor in determining the rate of metabolism of benzene (Gonasun et al., 1973).

(b) Man

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

Benzene is readily absorbed via the lungs, and about 40-50% is retained (Srbová et al., 1950). The rate of absorption of benzene through the skin has been reported to be 0.4 mg/cm^3/hr. It is taken up preferentially by fatty and nervous tissues, and about 30-50% of the absorbed benzene is excreted unchanged via the lungs; a three-phase excretion pattern is seen at about 0.7-1.7 hrs, 3-4 hrs and 20-30 hrs (Deutsche Forschungsgemeinschaft, 1974).

Following inhalation of benzene, 0.1-0.2% is excreted unchanged in the urine and the remainder as water-soluble metabolites (Srbová et al., 1950). Subjects inhaling concentrations of 0.34 mg/l (110 ppm) benzene in air for 5 hours excreted 29% as phenol, 3% as catechol and 1% as quinol, mostly as ethereal sulphates. Most of the phenol and catechol was excreted within 24 hours and the quinol within 48 hours (Teisinger et al., 1952). Rainsford & Lloyd Davies (1965) showed a correlation between the concentration of benzene in air during 8-hour exposures and the excretion of phenol in urine in a small number of workers.

Forni (1966) found chromosome aberrations in 2 patients with benzene-induced leukaemia; Forni & Moreo (1967, 1969) later described similar aberrations in one case of benzene anaemia which developed into an acute myeloblastic leukaemia and in a case of acute erythroleukaemia.

Forni et al. (1971) compared 34 workers in a rotogravure plant exposed to benzene and toluene or to toluene only to 34 controls and found the frequency of unstable chromosome aberrations in lymphocytes of the peripheral blood to be higher in the benzene-exposed group. Tough et al. (1970) found an unusual number of chromosome aberrations in people exposed to benzene, but the lack of adequate control groups makes this study difficult to interpret.

3.3 Observations in man

(a) Case reports

The association between long-term benzene exposure and the occurrence of leukaemia was suggested as early as 1928 by Delore & Borgomano (1928), who described acute lymphoblastic leukaemia in a worker who had been exposed to benzene for 5 years.

In a series of papers by Bowditch & Elkins (1939), Hunter (1939) and Mallory et al. (1939), the industrial exposure to commercial benzene (benzol) in 89 workers involved either in the manufacture of artificial leather or in the manufacture of shoes (which involves the use of rubber cements containing benzene) and their short- and long-term health records were investigated. One further case included was that of a receptionist who habitually removed ink and paints from a telephone exchange board using a

benzene-containing solvent. Average exposures were usually below 100 ppm, but some of the workers may have been exposed to 200 or more ppm benzol at various times during the manufacture of the artificial leather; exposures ranged from 5 months to 12 or more years. Among these workers there were 10 cases of fatal poisoning, and histological material was obtained at necropsy in 8/10 cases; one subject died from acute myeloblastic leukaemia (see below) (Hunter, 1939).

Mallory et al. (1939) reported on 19 cases (14 autopsies and 5 biopsies) with prolonged exposure to commercial benzene from which material for histological examination was available. These cases included the 8 fatal cases mentioned above by Hunter (1939). Most cases presented a blood picture characterized by anaemia, leucopaenia and thrombocytopaenia and often involved a purpuric syndrome. Two cases of leukaemia were reported: one (an acute myeloblastic type) occurred in a 28-year old male who had been exposed to commercial benzene for 10 years, and the other (a lymphoblastic type) occurred in a 12-year old boy who for 'several' years in his father's paint shop had used a paint remover known to contain commercial benzene to remove paint from toys.

DeGowin (1963) reported on an indoor painter who for 13 years before developing aplastic anaemia had thinned his paints with benzene. He was not exposed to benzene subsequently but developed acute myeloid leukaemia 15 years later.

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

Vigliani & Saita (1964), reporting on the possible association between exposure to benzene and leukaemia in Italy, found that of 47 cases of benzene blood dyscrasias seen at the Clinica de Lavoro, Milan, between 1942 and 1963, 6 were leukaemia. These cases included a spreader and calender operator at a leathercloth factory (9 years' exposure), an assistant operator in a rotogravure firm (5 years' exposure), a spray varnisher (8 years'

exposure), a rotogravure operator (11 years' exposure) and 2 workers using glues containing benzene (9 years' and 3 years' exposure). At the Institute of Occupational Health in Pavia, of 41 cases of benzene blood dyscrasias seen between 1961 and 1963, 5 were leukaemias. These cases refer to workers exposed to glues containing benzene in shoemaking. In addition, 13 further cases of 'benzene leukaemia' were reported in Italy between 1941 and 1963. Finally, examination of insurance data for the period 1960-1963 in the Milan and Pavia areas showed 11 cases of leukaemia among 68 blood dyscrasias resulting from benzene exposure; within this group there were 26 deaths (11 with leukaemia and 15 with aplastic anaemia).

During the period 1950-1965, 50 cases of leukaemia were observed in workers exposed to benzene in industries in the region of Paris. Goguel et al. (1967) described 44 cases (37 men and 7 women), including 13 cases of chronic myeloid leukaemia (aged 26-61 years), 8 cases of chronic lymphoid leukaemia (aged 33-63 years) and 23 cases of acute leukaemia among which were 2 erythroleukaemias (aged 26-68 years). Exposure to benzene was confirmed for all 50 cases by study of the work exposure. Furthermore, in 19 of the 44 cases, benzene was measured in the blood; and in 7 a high level of benzene was reported. In a control group of non-occupational leukaemias, no benzene was found in the blood. Six of the reported cases were found in rubber cement workers in the raincoat industry; at the 1962 census, 1088 people were reported to be occupied in that branch of industry.

Similarly, 4 cases of acute leukaemia were reported in shoemakers in Istanbul exposed to benzene for 6-14 years (Aksoy et al., 1972). According to a related paper (Aksoy et al., 1971), the concentration of benzene in the working environment, which ranged between 15-30 ppm outside working hours, rose to a maximum of 210 ppm when adhesives containing benzene were being used. Three of the 4 cases were of the myeloblastic type and the fourth a monocytic type associated with thrombocythaemia in the pre-myeloproliferative stage. Two of the 4 patients were reported to have had aplastic anaemia prior to the leukaemia. Ludwig & Werthemann (1962) described one case of myeloid leukaemia and one with a tumour-like reticulosis which occurred among 44 laboratory workers exposed to benzene and toluene over the years 1940-1961 in two chemical manufacturing plants.

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

In a study carried out during 1966-1969, 401 patients (246 men and 155 women) entering Lyon hospitals with blood disorders were examined and their exposure to benzene or toluene assessed. Average ages were 43 years for men and 68 years for women. A total of 124 patients (79 men and 45 women, average ages, 50 and 46 years) entering hospital for other illnesses were used as controls; but the way in which these were selected is not indicated. Of the patients showing blood disorders, 17/140 (12%) with acute leukaemia, 9/61 (15%) with chronic lymphoid leukaemia and 4/56 (7%) with myeloid leukaemia had been exposed to benzene or toluene. Five cases of acute leukaemia were found among the 124 controls (Girard & Revol, 1970).

(b) Epidemiological studies

A case-control study of leukaemia has been reported from Japan (Ishimaru et al., 1971). All cases diagnosed as definite or probable leukaemia between 1945 and 1967 and resident in Hiroshima or Nagasaki City at the time of the onset of the disease were included. One control per case was chosen from the Atomic Bomb Casualty Commission Leukaemia Registry sampling frame, matched (on 5 characteristics) for city, sex, date of birth ± 30 months, distance from the atomic bomb explosion and alive and resident in either Hiroshima or Nagasaki at the time of disease onset in the patient. Of the 492 leukaemia cases identified, information could be obtained only for 413 matched case-control pairs. Ten occupations were considered to involve exposure to benzene, and these occupations taken together were associated with an increased risk for leukaemia (30 cases, 14 controls, relative risk = 2.3, $P<0.01$). Twenty-four leukaemia cases were too far from the atomic bomb explosion for radiation to have influenced the increased risk. The increased risk, however, could be associated with exposures other than to benzene, as in none of the ten occupations considered would benzene be the only chemical encountered.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Benzene has been tested only in mice by subcutaneous injection and skin application. The data reported do not permit the conclusion that carcinogenic activity has been demonstrated.

4.2 Human data

It is established that exposure to commercial benzene or benzene-containing mixtures may result in damage to the haematopoietic system. A relationship between such exposure and the development of leukaemia is suggested by many case reports, and this suggestion is strengthened by a case-control study from Japan.

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DIAZOMETHANE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 334-88-3

Azimethylene

1.2 Chemical formula and molecular weight

Diazomethane is probably a resonance hybrid of the three forms:

$$H_2C^{\ominus}-N^{\oplus}\equiv N \rightleftharpoons H_2C = N^{\oplus} = N^{\ominus} \rightleftharpoons H_2^{\oplus}C-N = N^{\ominus}$$

CH_2N_2 Mol. wt: 42.0

1.3 Chemical and physical properties of the pure substance

(a) Description: Yellow gas

(b) Boiling-point: $-23^{\circ}C$

(c) Melting-point: $-145^{\circ}C$

(d) Solubility: Soluble in ether and dioxane

(e) Chemical reactivity: May explode when heated to $100^{\circ}C$ or in contact with ground-glass surfaces or alkali metals. In contact with copper, diazomethane forms insoluble polymethylene with evolution of nitrogen. Can methylate SH-groups, acidic (carboxylic or phenolic) hydroxyl groups and amino- and imino-groups

1.4 Technical products and impurities

Because of its toxicity and its explosive nature, diazomethane is not manufactured for distribution and sale. When used as a methylating agent

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* Considered by the Working Group in Lyon, June 1974

in the laboratory, it is produced and used in situ. Thus, there is no available information on technical products and impurities.

2. Production, Use, Occurrence and Analysis

In 1938, a review on the chemistry of aliphatic diazo compounds (including diazomethane) and related compounds was published (Smith, 1938).

2.1 Production and use[1]

Synthesis of diazomethane was first reported in 1894 (Peckmann, 1894). Although free gaseous diazomethane can be prepared, it is usually produced as a solution (most frequently in ether). Reaction of N-nitrosamides (e.g., nitrosomethylurea, N-nitroso-β-methylamino-isobutyl methyl ketone, nitroso methylurethane, N-methyl-N'-nitro-N-nitrosoguanidine or N-nitroso-methyl-p-toluenesulphonamide) with an alkali (e.g., potassium hydroxide, potassium carbonate) has been used to prepare diazomethane (Merck & Co., 1968).

During the late 1940's and the 1950's, most laboratory preparations were probably made from N-methyl-N'-nitro-N-nitrosoguanidine; however, in most countries in recent years it is believed to be made from N-nitroso-methyl-p-toluenesulphonamide. One US company offers a self-contained reaction kit for generating ether solutions of diazomethane as needed without exposing the user to the diazomethane or to the N-nitrosomethyl-p-toluenesulphonamide.

The only known use for diazomethane is as a laboratory methylating agent. It reacts with a wide variety of organic chemicals but is especially useful for reaction with phenols and carboxylic acids and with sensitive compounds requiring neutral conditions (Smith, 1938).

2.2 Occurrence

Diazomethane is not known to occur in nature.

The US Occupational Safety and Health Administration health standards

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

for air contaminants require that an employee's exposure to diazomethane does not exceed an eight-hour time-weighted average of 0.2 ppm (0.4 mg/m^3) in the workplace air during any eight-hour workshift for a forty-hour work week (US Code of Federal Regulations, 1974).

2.3 Analysis

The detection of diazomethane, using a modification of the colour reaction between alkylating agents and 4-(4-nitrobenzyl)-pyridine, has been described (Preussmann et al., 1969).

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

3.1 Carcinogenicity and related studies in animals

It should be noted that due to the volatility of the substance, inhalation exposure also involves skin exposure and vice versa.

(a) Inhalation and/or intratracheal administration

Mouse: Twelve male A/2G mice were exposed twice weekly to the vapour of 1 ml of an ethereal solution of diazomethane containing 0.1-3.3 mg/ml for 3 mins per exposure. Groups of 6 animals were placed in a 6.25 l chamber during each exposure. After 10 days, exposures were reduced to 1-2 mins, and treatment continued twice weekly for 6 months. Twelve male controls were exposed to ether for only 2 mins per exposure twice weekly for 6 months. Of the treated mice, 2 died before 10 days, and the remainder had died by 10 months. Multiple pulmonary adenomas were diagnosed in 7/10 mice, compared with 2/8 in controls. Four of the controls survived longer than 10 months (Schoental & Magee, 1962).

Of 8 male Swiss mice similarly exposed to diazomethane for 1.5 mins twice weekly for 5 months and 6 male controls exposed to ether for 1.5 mins twice weekly for 5 months, 2 treated mice died before 10 days and the remaining 6 before 8 months. No tumours were seen in control or treated mice (Schoental & Magee, 1962). In a later experiment, 5 male Swiss mice were treated with diazomethane and 6 male controls were treated with ether for 12 exposures during the first 6 weeks. Lung tumours developed in 5/5

treated mice and in 3/6 controls surviving after the age of 6 months (Schoental, 1963).

Rat: Seven male albino rats originally of the Porton strain and 6 male LAC 606p* rats were exposed in a 6.25 l chamber in groups of 2-3 to 1 ml of an ethereal solution of diazomethane containing 0.1-3.3 mg/ml, twice weekly for 6 months (2-3 min exposures) or 4.5 months (1.5 min exposures), respectively. Two male control rats of each strain were similarly exposed to ether. Of 7 treated rats surviving longer than 10 months, 3 rats had pulmonary adenomas. One of these 3 also had a squamous-cell carcinoma of the lung with a metastasis attached to the diaphragm and invading the skeletal muscle. No tumours were reported in the 4 controls, which were killed after 11 months (Schoental & Magee, 1962).

(b) Skin application

Mouse: Of 12 male A/2G mice painted on the clipped dorsal skin with 2-3 drops of an ethereal solution of diazomethane (0.1-3.3 mg/ml) 5 times weekly for 5 months, 8/8 animals dying between 5-12 months had lung adenomas. No controls were used (Schoental & Magee, 1962).

(c) Subcutaneous and/or intramuscular injection

Mouse: Ten male Swiss mice were given 8 monthly s.c. injections of 0.1 ml ethereal diazomethane (0.1-3.3 mg/ml) in an equal volume of arachis oil, plus 4 monthly injections of 0.1 ml undiluted ethereal diazomethane. Of these, 9 mice survived up to 26 months; 1 had a spindle-cell sarcoma invading the adjacent muscle, and 1 mouse developed multiple pulmonary adenomas. No controls were used (Schoental & Magee, 1962).

3.2 Other relevant biological data

(a) Animals

Exposure to a concentration of 175 ppm diazomethane for 10 minutes caused haemorrhagic emphysema and oedema of the lungs in cats dying within 3 days (Flury & Zernik, 1931). Exposed guinea-pigs showed symptoms of

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* Animals supplied by the Laboratory Animals Centre, Carshalton, from an inbred strain No. 606p

severe respiratory tract irritation and pulmonary oedema (Sunderman et al., 1938). Acute exposure of rabbits to an atmosphere containing 2-12 mg/l diazomethane for 5 to 20 mins, 1-4 times, resulted in bronchopneumonia, followed by death before 7 days (Vyskočil et al., 1966).

As a methylating agent, diazomethane reacts with compounds containing various forms of active hydrogen atoms, such as amino, imino, carboxylic, enolic or phenolic hydroxyl groups (Hanusch et al., 1966). Reaction of diazomethane with deoxyguanosine yielded deoxy-1-methylguanosine, deoxy-(O-6)-methylguanosine and deoxy-7-methylguanosine; reaction with deoxythymidine yielded deoxy-1-methylthymidine, while deoxyadenosine and deoxycytidine were recovered unchanged under the experimental conditions used (Friedman et al., 1965). Acid hydrolysis of diazomethane-treated DNA yielded 7-methylguanine and 3-methyladenine; and acid hydrolysis of diazomethane-treated RNA yielded 7-methylguanine, 1-methyladenine and 1-methylcytosine (Kriek & Emmelot, 1964).

A proposal that diazomethane was the reactive intermediate in dimethylnitrosamine or N-methyl-N-nitrosourea metabolism was not supported by the studies of Lijinsky & Greenblatt (1972) and Lawley & Shah (1973), respectively.

(b) Man

Inhalation of diazomethane by man, depending on the degree of exposure, caused chest pains, asthmatic symptoms, cough and fever, fulminating pneumonia, moderate cyanosis, shock and death (Sunderman, 1970).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Limited studies indicate that diazomethane is carcinogenic in mice and

[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

rats, the only species tested. In mice it increased the incidence of lung tumours following skin application; and exposure to the gas induced lung tumours in rats.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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ortho- AND para-DICHLOROBENZENE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

(a) ortho-Dichlorobenzene

Chem. Abstr. No.: 95-50-1

DCB; 1,2-dichlorobenzene; o-dichlorobenzene; o-dichlorobenzol; ODB; ODCB; orthodichlorobenzene; orthodichlorobenzol

Chloroben; Cloroben; Dizene; Dowtherm E

(b) para-Dichlorobenzene

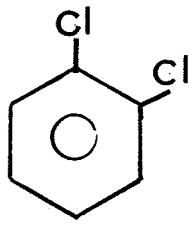
Chem. Abstr. No.: 106-46-7

1,4-Dichlorobenzene; p-dichlorobenzene; p-dichlorobenzol; para-dichlorobenzene; paradichlorobenzol; PDB; PDCB

Di-chloricide; Paracide; Paradi; Paradow; Paramoth; Santochlor

1.2 Chemical formulae and molecular weights

(a) ortho-Dichlorobenzene



$C_6H_4Cl_2$ Mol. wt: 147

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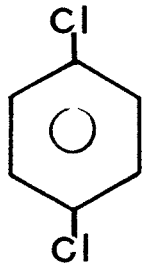
* Considered by the Working Group in Lyon, June 1974

(b) para-Dichlorobenzene



$C_6H_4Cl_2$ Mol. wt: 147

1.3 Chemical and physical properties of the pure substance

ortho-Dichlorobenzene

(a) Description: Colourless liquid

(b) Boiling-point: 180.5°C

(c) Melting-point: -17.5°C

(d) Density: d_4^{20} 1.3059; d_4^{25} 1.3003

(e) Refractive index: n_D^{25} 1.551

(f) Solubility: Practically insoluble in water; miscible with alcohol, ether and benzene

(g) Volatility: The vapour pressure is 1.15 mm Hg at 20°C.

para-Dichlorobenzene

(a) Description: Volatile, monoclinic crystals

(b) Boiling-point: 174°C

(c) Melting-point: 53-54°C

(d) Refractive index: n_D^{60} 1.5285

(e) Solubility: Practically insoluble in water; soluble in ethanol, ether, benzene, chloroform and carbon disulphide

1.4 Technical products and impurities

(a) ortho-Dichlorobenzene

In 1937, commercial ortho-dichlorobenzene was described as the liquor remaining after the separation of crystalline para-dichlorobenzene and containing: ortho-dichlorobenzene, 48.8%; para-dichlorobenzene, 28%; trichlorobenzene, 15%; monochlorobenzene, 6%; tetrachlorobenzene, 2%; and benzene, 0.2% (Cameron et al., 1937). This chemical is available in the US as a technical grade typically containing 98.7% by weight of the ortho-isomer and 1.3% of the meta- and para-isomers combined. It has a typical boiling range of 1.4°C and a moisture content of 80 ppm. ortho-Dichlorobenzene is also available in the US in a grade which typically contains 83% of the ortho-isomer, 17% of the meta- and para-isomers and has a boiling range of 3°C. An emulsifiable form of this latter product containing 93.5% dichlorobenzenes plus an emulsifier can also be obtained in the US.

(b) para-Dichlorobenzene

Commercial para-dichlorobenzene is available in the US as a technical grade liquid typically containing 0.08% by weight of a mixture of the meta- and ortho-isomers. This grade has a freezing-point of 52.93°C and a typical residue of 10 ppm. It is also available as crystals in several particle sizes containing no detectable impurities (GLC method). This grade has a freezing-point of 53.0°C and a typical residue of 8 ppm.

2. Production, Use, Occurrence and Analysis

A review on chlorinated benzenes, including the dichlorobenzenes, has been published (Hardie, 1964).

2.1 Production and use[1]

(a) ortho-Dichlorobenzene

ortho-Dichlorobenzene has been produced commercially in the US since

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

at least 1921 (US Tariff Commission, 1922). It is believed to be produced commercially by chlorinating benzene or by chlorinating monochlorobenzene at 150-190°C in the presence of a ferric chloride catalyst (Hardie, 1964). Either the resulting mixture of ortho- and para-dichlorobenzene (in which the para-isomer predominates), or the residue left after the recovery of monochlorobenzene by distillation, can be separated into their two principal components (only small amounts of the meta-isomer are present) by fractional distillation or by crystallization of the para-isomer (Hardie, 1964). The exact methods used by the 8 US producers of ortho-dichlorobenzene are not known; some use fractional distillation of purchased chlorobenzenes.

US production of ortho-dichlorobenzene in 1972 was 28 million kg (US Tariff Commission, 1974). In 1960, only 11 million kg were produced, but 8 million kg of a mixture of ortho- and para-dichlorobenzene were also manufactured (US Tariff Commission, 1961). In October 1973, US production for that year was predicted to be between 32-41 million kg (Anon., 1973).

Separate data on US exports of ortho-dichlorobenzene are not available, but US imports through the principal customs districts in 1972 were reported to have been only 6,300 kg (US Tariff Commission, 1973).

ortho-Dichlorobenzene is produced in the following Western European countries (the number of producers is given in parentheses): the Federal Republic of Germany (4); France (2); Italy (1); Spain (1); and the United Kingdom (2) (Chemical Information Services, Ltd., 1973). Combined production of ortho- and para-dichlorobenzene in Western Europe in 1972 is estimated to have been approximately 30 million kg, but how much was ortho-dichlorobenzene is not known.

Production of ortho-dichlorobenzene by the 6 Japanese producers is estimated to have been 11 million kg in 1972, approximately 15% below the level of the previous year. Separate data on Japanese imports and exports of ortho-dichlorobenzene are not available.

Hardie (1964) reported a variety of uses for ortho-dichlorobenzene: as a component of a solvent mixture used to remove lead and carbonaceous deposits from engine parts; as a component of a rust-proofing mixture; as a heat-exchange medium; as a magnetic coil coolant; as a dye intermediate;

as an insecticide; and, to a small extent, as a degreasing agent and a lacquer and resin solvent.

Merck & Co. (1968) stated that ortho-dichlorobenzene was used as a solvent for a variety of materials; as an insecticide for termites and locust borers; for the desulphurization of illuminating gas; for degreasing leather, metals and wool; as a component of metal polishes; as a heat transfer medium; and as a dye intermediate.

A manufacturer's product bulletin published in 1970 indicated that ortho-dichlorobenzene had a variety of uses as a solvent: in the production of isocyanates; in paint formulations; in compounds for removing a variety of polymeric materials, sulphur and oxides of metals; in engine-cleaning compounds; in various cleaning and polishing formulations; in degreasing agents; and in wood-preserving compounds. It was also reported to be used as a chemical intermediate, for making agricultural chemicals and dye intermediates - chiefly 3,4-dichloroaniline, and as a heat transfer medium and coolant for magnetic coils. The emulsifiable form was recommended for deodorizing garbage and sewage (PPG Industries, 1970).

The use of ortho-dichlorobenzene as an intermediate for agricultural chemicals has apparently grown rapidly in recent years. The following US consumption pattern for this chemical was estimated in late 1973: 53% for organic synthesis (chiefly pesticides); 20% as a process solvent in toluene diisocyanate production; 15% in miscellaneous solvent uses; 8% in the manufacture of dyes; and 4% for miscellaneous uses (Anon., 1973).

ortho-Dichlorobenzene is known to be used as a pesticide and as a solvent in Japan, but no consumption pattern is available.

(b) para-Dichlorobenzene

para-Dichlorobenzene has been produced commercially in the US since at least 1921, when 5 producers reported a total production of 183 thousand kg (US Tariff Commission, 1922). It is believed that para-dichlorobenzene is produced commercially by chlorinating benzene or by chlorinating monochlorobenzene at 150-190°C in the presence of a ferric chloride catalyst (Hardie, 1964). The resulting mixture of ortho- and para-dichlorobenzene

(in which the para-isomer predominates) can be separated by fractional distillation or by crystallization of the para-isomer. When high yields of the para-isomer are desired, an orienting catalyst such as an arylsulphonic acid can be used; or chlorination can be carried further so that the ortho-isomer is converted to 1,2,4-trichlorobenzene, from which the para-dichlorobenzene can be readily separated by distillation (Hardie, 1964). The exact methods of manufacture used by the 8 US producers of para-dichlorobenzene are not known; some use fractional distillation of purchased chlorobenzene.

US production of para-dichlorobenzene in 1972 was 35 million kg (US Tariff Commission, 1974). In 1960, only 29 million kg were produced, but 8 million kg of a mixture of ortho- and para-dichlorobenzene were also manufactured (US Tariff Commission, 1961).

US exports of para-dichlorobenzene in 1973 were estimated to have been 3-4.5 million kg; this figure was considered to be unusually high and was due to increased international usage, particularly in the Far East, for unknown reasons (Anon., 1973). Separate data on US imports are not available, but these are believed to be small (No imports through the principal US customs districts have been reported in recent years).

para-Dichlorobenzene is produced in the following Western European countries (the number of producers is given in parentheses): the Federal Republic of Germany (4); France (3); Italy (3); and the United Kingdom (2) (Chemical Information Services, Ltd., 1973). Combined production of ortho- and para-dichlorobenzene in Western Europe in 1972 is estimated to have been approximately 30 million kg, but how much was para-dichlorobenzene is not known.

Production of para-dichlorobenzene by the 6 Japanese manufacturers is estimated to have been 16 million kg in 1972, approximately 9% below the level of the previous year. Separate data on Japanese imports and exports of para-dichlorobenzene are not available.

Hardie (1964) reported that 65-70% of all para-dichlorobenzene was used in the production of moth repellants and space deodorants, and that it found minor application as an extreme pressure lubricant.

In 1968, para-dichlorobenzene was reported to be used for killing moths and their larvae, roaches, peach-tree borers and some other insects, and for preserving furs, woollens and rugs (Merck & Co., 1968).

A manufacturer's product bulletin published in 1972 indicated that over 90% of para-dichlorobenzene consumption in the US is of products in which use is made of the vapour produced by sublimation. Thus, it is used as a moth repellant, as a mildew control agent and as a space deodorant, e.g., in toilets and refuse containers. It was also recommended for use in the production of the dye intermediate 2,5-dichloroaniline, insecticides, pharmaceuticals and other organic chemicals (PPG Industries, 1972).

The following US consumption pattern for para-dichlorobenzene was estimated in early 1973: 50% as a space odorant, 40% for moth control and 10% for other uses (Anon., 1973).

Some amounts (probably less than 2 million kg) of para-dichlorobenzene are also believed to be used in the manufacture of polyphenylene sulphide resins (by reaction with sodium sulphide). These resins are used for surface coatings and molding resins.

In Japan, approximately 90% of para-dichlorobenzene is believed to be used as a moth control agent and 10% as a dyestuff intermediate.

2.2 Occurrence

(a) ortho-Dichlorobenzene

This chemical is not known to occur in nature.

The US Occupational Safety and Health Administration health standards for air contaminants require that an employee's exposure to ortho-dichlorobenzene does not exceed a ceiling value of 50 ppm in the workplace air (US Code of Federal Regulations, 1974). The maximum allowable concentration in the USSR is 20 mg/m^3 (ILO, 1970).

(b) para-Dichlorobenzene

This chemical is not known to occur in nature.

Small amounts may be present in polyphenylene sulphide resins. The

US Food and Drug Administration has approved the use of these resins in coatings of articles intended for repeated use in contact with food only if the level of residual para-dichlorobenzene does not exceed 0.8 ppm (US Code of Federal Regulations, 1973).

The US Occupational Safety and Health Administration health standards for air contaminants require that an employee's exposure to para-dichlorobenzene does not exceed an eight-hour time-weighted average of 75 ppm in the workplace air during any eight-hour work shift (US Code of Federal Regulations, 1974). The maximum allowable concentration in the USSR is 20 mg/m^3 (ILO, 1970).

2.3 Analysis

Several methods for the separation and detection of dichlorobenzene by gas chromatography have been described (Cowan & Hartwell, 1961; Habboush & Tameesh, 1970; Karasek & Fong, 1971; Nadeau & Oaks, 1961). Its determination in chemical plant effluent is described by Sprowl et al. (1962).

Estimation of para-dichlorobenzene and its metabolites in the urine using electron-capture gas chromatography has been employed to measure exposure (McKinney et al., 1970).

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

3.1 Carcinogenicity and related studies in animals

A number of studies in which ortho- and para-dichlorobenzene have been administered by the oral, subcutaneous and inhalation routes to rodents were available to the Working Group (Hollingsworth et al., 1956; Hollingsworth et al., 1958; Parsons, 1942). [These studies were too short in duration and involved too few animals to have any significance for the evaluation of the possible carcinogenicity of these compounds.]

3.2 Other relevant biological data

(a) Animals

The median lethal dose (LD_{50}) of para-dichlorobenzene in rats by the

i.p. route is about 2500 mg/kg bw. Administration of this substance by inhalation to rabbits produced signs of central nervous depression, irritation of mucous membranes, granulocytopaenia and degeneration of renal tubules (Zupko & Edwards, 1949); and in guinea-pigs, signs of liver damage occurred (Coppola et al., 1963; Frada & Cali, 1958; Totaro, 1961).

The maximum tolerated dose for rats of ortho-dichlorobenzene administered by gavage on 5 days a week for about 28 weeks lies between 19-190 mg/kg bw/day. Minimal liver and kidney damage occurred at higher dosage levels. Inhalation of ortho-dichlorobenzene vapour was also reported to cause liver and kidney damage (Hollingsworth et al., 1958).

The more pronounced toxicity to the liver of the ortho-isomer has been associated with a more pronounced binding of the compound or its intermediate metabolites to liver proteins (Reid & Krishna, 1973).

After its oral administration to rabbits, ortho-dichlorobenzene is metabolized mainly to 3,4-dichlorophenol; but 2,3-dichlorophenol, 3,4-dichlorophenylmercapturic acid and 3,4- and 4,5-dichlorocatechol are also formed. para-Dichlorobenzene is converted to 2,5-dichlorophenol and 2,5-dichloroquinol conjugated with glucuronic or sulphuric acid (Azouz et al., 1955).

The effect of inducers and inhibitors of microsomal mixed-function oxidases on the rate of metabolism and the extent of binding of ortho- and para-dichlorobenzene to cellular constituents suggests that arene oxides (epoxide) may be precursors of the excreted metabolites, and that these arene oxides may be responsible for the differing biological properties of the parent compounds (Reid & Krishna, 1973).

(b) Man

There is evidence that accidentally inhaled or ingested para-dichlorobenzene is toxic in man. One case of pulmonary granulomatosis (Weller & Crellin, 1953) and 2 cases of haemolytic anaemia (Campbell & Davidson, 1970; Hallowell, 1959) were reported. A case of allergic purpura after exposure to para-dichlorobenzene has also been described (Nalbandian & Pearce, 1965).

No evidence in workers of organic injury or of untoward haematological

effects has been found attributable to exposure to air containing ortho-dichlorobenzene at concentrations ranging from 1-44 ppm (average, 15 ppm) for many years (Hollingsworth et al., 1958).

para-Dichlorobenzene is converted to 2,5-dichlorophenol and 2,5-dichloroquinol by man (Hallowell, 1959; Pagnotto & Walkley, 1965); the amount of 2,5-dichlorophenol present in urine can serve as an indication of exposure. The phenolic metabolites are excreted as conjugates of glucuronic or sulphuric acids (Hallowell, 1959).

3.3 Observations in man

(a) Case reports

Girard et al. (1969) reported 5 cases of blood disorders occurring in subjects exposed to ortho- or para-dichlorobenzene as a solvent for other chemicals or in chlorinated benzene mixtures. No evidence of exposure to benzene was found. The haematological changes included 2 cases of chronic lymphoid leukaemia, 2 cases of acute myeloblastic leukaemia and 1 case of myeloproliferative syndrome. In the 2 subjects with chronic lymphoid leukaemia, one had been exposed to a glue containing 2% ortho-dichlorobenzene from 1945-1961, and the other had been exposed from 1940-1950 to a solvent containing ortho- (80%), meta- (2%) and para- (15%) dichlorobenzene, which was used for cleaning electrical parts. One of the 2 cases of acute myeloblastic leukaemia had been exposed to the same mixture of ortho-, meta- and para-dichlorobenzene taken from the same factory and used for the cleaning of clothes (2 litres per year for several years); and the other case was a 15-year old girl who had for 'some' time removed stains from her own clothes with a product containing 37% ortho-dichlorobenzene.

4. Comments on Data Reported and Evaluation

4.1 Animal data

No adequate studies on which to base an evaluation of carcinogenicity were available to the Working Group.

4.2 Human data

One report has suggested an association between leukaemia and exposure

to dichlorobenzenes, but this is insufficient evidence from which to assess the carcinogenic risk of this compound.

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ETHYL METHANESULPHONATE*

1. Chemical and Physical Data

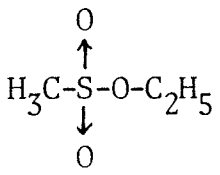
1.1 Synonyms and trade names

Chem. Abstr. No.: 62-50-0

EMS; ethyl ester of methanesulfonic acid; ethyl ester of methanesulphonic acid; ethyl ester of methylsulfonic acid; ethyl ester of methylsulphonic acid; ethylmethanesulfonate; ethylmethane sulfonate; ethyl methanesulfonate; ethyl methane sulfonate; ethylmethanesulphonate; ethylmethane sulphonate; ethyl methane sulphonate; ethyl methansulfonate; ethyl methansulphonate; methanesulfonic acid ethyl ester; methanesulphonic acid ethyl ester

"half-Myleran"; NSC 26805

1.2 Chemical formula and molecular weight



$C_3H_8O_3S$ Mol. wt: 124.0

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless liquid

(b) Boiling-point: 213-213.5°C (761 mm Hg); 104°C (14 mm Hg)

(c) Density: d_4^{22} 1.1452

1.4 Technical products and impurities

No data were available to the Working Group.

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* Considered by the Working Group in Lyon, June 1974

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Ethyl methanesulphonate has been prepared from the reaction of methanesulphonic anhydride and ethyl alcohol (Billeter, 1905).

No indications were found that ethyl methanesulphonate is produced commercially, although it has been produced for research purposes.

The monoesters of methanesulphonic acid have been considered for use as reversible male chemosterilants for insects and mammalian pests and as possible human male contraceptives (Jackson, 1964).

2.2 Occurrence

Ethyl methanesulphonate is not known to occur in nature.

2.3 Analysis

Preussmann et al. (1969) have described the use of 4-(4-nitrobenzyl)-pyridine to detect alkylating agents.

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

3.1 Carcinogenicity and related studies in animals

(a) Subcutaneous and/or intramuscular injection

Newborn mouse: Single s.c. injections of 100 µg ethyl methanesulphonate (EMS) in distilled water on the first day of life produced lung tumours (adenomas or adenocarcinomas) in 17/32 (53%) BALB/c mice (sex unspecified) surviving between 36-43 weeks, at which time the experiment was terminated. The average number of lung tumours/mouse was 1.34, compared with 0.09 in 2/21 (9.5%) controls given 0.02 ml aqueous gelatine (Roe et al., 1962, 1963).

Two groups of 50-60 BALB/c mice (sex unspecified) were injected s.c. with 200 µg EMS in 0.02 ml arachis oil on the first day of life, or with

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

200 µg daily for the first 5 days of life, and lung adenomas were observed in 9/45 (20%) and 8/51 (15.7%) survivors at 40 weeks, respectively, with mean numbers of lung tumours per mouse of 0.20 and 0.22. Lung tumours occurred in 5/40 (12.5%) controls injected with 0.02 ml arachis oil on the first day of life and in 4/34 (11.8%) controls given arachis oil daily for the first 5 days of life. When 200 µg EMS were given to a similar group of BALB/c mice by s.c. injection in 0.02 ml of a 3% aqueous gelatine solution daily for the first 5 days of life, 31/31 survivors at 40 weeks developed lung tumours (13.6 tumours/mouse), compared with 4/48 controls receiving aqueous gelatine only (0.12 tumours/mouse). In C57BL mice (sex unspecified) given 200 µg EMS in 0.02 ml arachis oil daily for the first 5 days of life, 5/39 (12.8%) survivors at 55-60 weeks developed lung adenomas, compared with 0/47 controls given 0.02 ml arachis oil alone (Walters et al., 1967).

(b) Intraperitoneal injection

Mouse: Of an unspecified number of male CBA mice given 3 i.p. injections of 200 mg/kg bw EMS in arachis oil at 3-week intervals, starting when the animals were 11-14 weeks old, 33% of the animals developed kidney tumours (unspecified) and 89% lung tumours (unspecified), compared with 3% and 20% in controls. The average survival times were 693 days and 762 days, respectively (Alexander & Connell, 1963).

Single i.p. injections of 3 mmoles/kg bw (372 mg/kg bw) undiluted EMS given to a group of 36 male and female CFW/D mice produced lung adenomas in 20/22 mice surviving 21-210 days, at which time the experiment was terminated. Lung tumours occurred in 4/29 controls killed after 210 days and in 3/103 controls killed after 365 days (Frei, 1971).

In male RF mice treated with single doses of 175 mg/kg bw EMS in saline, 18/31 (58%) mice developed lung tumours (unspecified), compared with 25/52 (48%) controls given 0.32 ml saline alone. The first tumours appeared at 350 and 367 days, respectively (Clapp, 1973).

Rat: Of 24 female Wistar rats of the Porton strain given 3 i.p. injections of 27.5 mg EMS in 1 ml of a 0.9% saline solution at days 0, 2 and 9, starting when the animals were about 5 weeks of age (100 g bw), 12 rats developed renal carcinomas, the first appearing after 7 months. No

such tumours occurred in an unspecified number of controls. After single doses of 350 mg/kg bw EMS, 1/22 rats developed an ependymoma of the brain (Swann & Magee, 1969).

In 66 male and female Sprague-Dawley rats given 3 i.p. doses of 33 mg EMS in saline at 7-day intervals, 12/35 males and 23/31 females killed at 12 months developed a variety of benign and malignant tumours, including carcinomas of the lung, compared with 0/20 in male and 0/20 in female controls (Hrushesky et al., 1972).

In 4 groups of 20 male and 20 female Wistar rats given single i.p. injections of 0, 100, 200 or 300 mg/kg bw EMS in 1 ml saline and observed for 110 weeks, 5/78 animals given 100 or 300 mg/kg bw doses developed malignant kidney tumours (3 epithelial and 2 mesenchymal) after 90-97 weeks. No kidney tumours occurred in rats receiving 200 mg/kg bw EMS, nor in the controls. In similar groups of rats receiving the same doses of EMS, following single i.p. injections of 30 mg/kg bw dimethylnitrosamine (DMN) 8 hours before, an additive effect in relation to the incidence of malignant kidney tumours was produced by the combination when compared with that produced by DMN alone (Montesano et al., 1974).

3.2 Other relevant biological data

A single injection of 100 mg/kg bw EMS in rats produced subfertility during the first 3 weeks, and a larger dose (300 mg/kg bw) caused complete sterility (Jackson et al., 1961). $^{14}C_2H_5$-EMS was shown to react with the thiol group of cystein *in vivo*; its reported half-life in rat blood serum was $6\frac{1}{2}$ hours (Roberts & Warwick, 1958).

Swann & Magee (1971) showed that administration of EMS also led to an ethylation in the 7-position of guanine in nucleic acids of several organs of the rat.

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Ethyl methanesulphonate (EMS) is carcinogenic in mice and rats following subcutaneous or intraperitoneal injection, the only species and routes tested. It produced mainly lung and kidney tumours in both species. It is carcinogenic following administration of a single dose.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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METHYL METHANESULPHONATE*

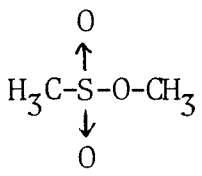
1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 66-27-3

as-Dimethyl sulphite; methanesulfonic acid methyl ester; methanesulphonic acid methyl ester; methyl ester of methanesulfonic acid; methyl ester of methanesulphonic acid; methyl ester of methylsulfonic acid; methyl ester of methylsulphonic acid; methyl mesylate; methylmethanesulfonate; methylmethane sulfonate; methyl methanesulfonate; methyl methane sulfonate; methylmethanesulphonate; methylmethane sulphonate; methyl methane sulphonate; methyl methansulfonate; methyl methansulphonate; MMS

1.2 Chemical formula and molecular weight



$C_2H_6O_3S$ Mol. wt: 110.0

1.3 Chemical and physical properties of the pure substance

(a) Description: Colourless liquid

(b) Boiling-point: 203°C (753 mm Hg); 59°C (0.6 mm Hg)

(c) Density: d_4^{20} 1.2943

(d) Refractive index: n_D^{20} 1.4140

(e) Solubility: Soluble in water at 25°C (1 part in 5); in dimethyl formamide and in propylene glycol (1 in 1); slightly soluble in non-polar solvents

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* Considered by the Working Group in Lyon, June 1974

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Methyl methanesulphonate has been prepared from the action of methyl iodide upon methyl sulphite (Arbusow & Pischtschimuka, 1909).

No indications were found that methyl methanesulphonate is produced commercially, although it has been produced for research purposes.

The monoesters of methanesulphonic acid have been considered for use as reversible male chemosterilants for insects and mammalian pests and as possible human male contraceptives (Jackson, 1964). The chemical has been tested clinically at doses between 2.8-800 mg/kg bw as a cancer chemotherapeutic agent (Bateman et al., 1966).

2.2 Occurrence

Methyl methanesulphonate is not known to occur in nature.

2.3 Analysis

Preussmann et al. (1969) have described the use of 4-(4-nitrobenzyl)-pyridine to detect alkylating agents.

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

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Of 63 male RF/Un mice administered methyl methanesulphonate (MMS) in the drinking-water for life at a level of 20 mg/100 ml (equivalent to a daily intake of about 30 mg/kg bw), 16 mice were sacrificed before 12

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

months. Of the remaining 47, 70% developed lung tumours (diagnosed macroscopically) at an average age of 20 months, compared with 39% of 162 controls developing tumours at an average age of 22 months (χ^2 14.4; $P<0.0002$). Thymic lymphomas developed in 15% of the treated mice, compared with 4% of controls (Clapp et al., 1968).

(b) Subcutaneous and/or intramuscular injection

Rat: Of 12 male BD rats given weekly s.c. injections of 8 mg/kg bw MMS in oil for 48 weeks (total dose, 368 mg/kg bw), 4 rats were lost through infection. Tumours at the injection site were observed in 3/8 remaining rats dying at 438, 470 and 555 days; these included 2 squamous-cell carcinomas and 1 polymorphic-cell sarcoma. Weekly s.c. injections of 4 mg/kg bw for 48 weeks resulted in sarcomas at the injection site in 3/12 rats and in a nephroblastoma in 1/12 rats dying between 572-776 days. No concurrent controls injected with oil were used (Druckrey et al., 1970).

(c) Intraperitoneal injection

Mouse: Single i.p. injections of 6 mmoles/kg bw MMS (660 mg/kg bw) in Sorensen's buffer to 35 male and female CFW/D mice when the animals were 6-8 weeks old did not significantly increase the incidence of lung adenomas or of lymphomas compared with the incidence found in the controls. The incidence of lung tumours at 365 days was 8/35 in treated animals, compared with 3/103 in controls; and that of lymphomas 2/35 in treated mice, compared with 3/103 in controls (Frei, 1971).

In RF mice treated with single i.p. injections of 150 mg/kg bw MMS in saline, 16/28 (57%) treated mice surviving at the time of the appearance of the first tumour (324 days) developed lung tumours, compared with 25/52 (48%) controls surviving after 367 days (Clapp, 1973).

Rat: Three groups of rats comprising 9-10 male and 8-10 female Wistar rats of the Porton strain (150-170 g bw) were given single i.p. injections of 120, 96 or 72 mg/kg bw MMS. Two rats given 96 mg/kg bw developed an oligodendroglioma and a malignant neurofibroma, and 2 rats given 72 mg/kg bw developed an astrocytoma and a meningioma of the spinal cord. The times of appearance of the tumours were not stated. An oligo-dendroglioma was also observed in 1/15 rats given 3 doses of 100 mg/kg bw

MMS. A group of 15 male and 15 female controls was used; no tumours were reported in these animals, and, furthermore, such tumours have not been reported in untreated rats of that colony (Swann & Magee, 1969).

(d) Intravenous injection

Rat: In 2 groups of 12 male BD rats given 8 mg/kg bw or 4 mg/kg bw MMS in saline i.v. weekly for 48 weeks (total doses, 368 and 184 mg/kg bw), 1 rat developed a myoma under the ventral skin after 515 days, and a further rat developed a round-cell sarcoma in the region of the neck after 675 days. No neurogenic tumours were observed (Druckrey et al., 1970).

(e) Other experimental systems

Prenatal exposure: Single i.v. injections of 20, 40 or 68 mg/kg bw MMS in saline given on day 15 or 21 of gestation to 12 female BD IX rats resulted in the development of 7 neurogenic tumours in 6/32 offspring surviving 140-507 days after birth. Tumours observed included 5 malignant neurinomas, 1 mixed glioma and 1 oligodendroglioma. No neurogenic tumours developed in 35 offspring of rats treated with doses of 10, 20 or 40 mg/kg bw MMS on day 9 of gestation. No controls were used (Kleihues et al., 1972).

3.2 Other relevant biological data

(a) Animals

Single i.p. doses of 50 mg/kg bw MMS given to male rats resulted in infertility during the second and third week after injection, and 100 mg/kg bw caused sterility for 28 days (Jackson et al., 1961).

In mice (Cumming & Walton, 1970) and rats (Kleihues et al., 1974) MMS is rapidly distributed throughout the body, including the central nervous system. In pregnant rats (21st day of gestation), transplacental passage into foetuses occurred within 2 minutes after i.v. injection (Kleihues et al., 1974).

Following i.v. injection of 100 mg/kg bw to rats, no detectable amounts of MMS were found in blood serum after 2 hours (Swann, 1968).

Various urinary metabolites (methylmercapturic acid sulphoxide, 2-

hydroxy-3-methylsulphinylpropionic acid, methylsulphinylacetic acid and a mixture of methylmercapturic acid and N-(methylthioacetyl)glycine) were identified in rats after i.v. administration of $^{14}CH_3$-MMS during the first 16 hours. About 80% of the excreted radioactivity was accounted for by these metabolites, resulting from an initial methylation of cysteine residues by MMS (Barnsley, 1968). Pillinger et al. (1965) found a conjugation of $^{14}CH_3$-MMS with glutathione in rat liver.

In rats, approximately 30% of the radioactivity injected as $^{14}CH_3$-MMS was exhaled as $^{14}CO_2$ within 30 hours, and an additional 20% was recovered from urine (Swann, 1968). In mice given a single i.p. dose of $^{14}CH_3$-MMS, approximately 34% of the radioactivity was recovered from the urine and 27% as $^{14}CO_2$ (Cumming & Walton, 1970).

Intravenous injection of $^{14}CH_3$-MMS led to a methylation of nucleic acids in various organs of the rat, including the liver and brain. Alkylation products in DNA included 7-methylguanine, 3-methyladenine and, to a much lesser extent, O-6-methylguanine (Kleihues & Magee, 1973; Margison et al., 1973; McElhone et al., 1971; O'Connor et al., 1972; Swann & Magee, 1968). Intravenous injection of $^{14}CH_3$-MMS to pregnant rats led to a similar degree of alkylation of nucleic acids in various maternal and foetal tissues (Kleihues et al., 1974).

(b) Man

Therapeutic application of total doses of between 2.8-800 mg/kg bw over a period of up to 350 days to 13 cancer patients led to significant gastrointestinal and hepatic toxic effects (Bateman et al., 1966).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

Methyl methanesulphonate (MMS) is carcinogenic in rats following subcutaneous and intraperitoneal injection, producing local tumours and tumours of the nervous system. Following oral administration in mice it increased the incidence of lung tumours and of lymphomas. In rats, it is carcinogenic on administration of a single dose as well as following prenatal exposure.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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POLYCHLORINATED BIPHENYLS*

1. Chemical and Physical Data

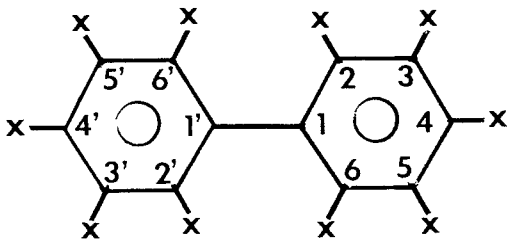
1.1 Synonyms and trade names

Chem. Abstr. No.: 13-36-36-3

Chlorinated biphenyl; chlorinated diphenyl; chlorinated diphenylene; chloro biphenyl; chloro 1,1-biphenyl; polychlorinated polyphenyls; polychlorobiphenyl

Aroclor; Clophen; Chlophen; Chlorextol; Dykanol; Fenclor; Inerteen; Kanechlor; Montar; Noflamol; Phenochlor; Phenoclor; Pyralene; Pyranol; Santotherm FR; Sovol; Therminol; Therminol FR-1

1.2 Chemical formula



In the above formula, X represents either a chlorine or a hydrogen atom.

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* Considered by the Working Group in Lyon, June 1974

1.3 Chemical and physical properties of the pure substance

(see also Tables 1 & 2)

(a) The melting- and boiling points of some chlorobiphenyls are given below*:

	MP °C	$BP_{(760)}$ °C	BP(mmHg)°C
2-chlorobiphenyl	54	267-268	154(12)
3-chlorobiphenyl	89	284-285	
2,2'-dichlorobiphenyl	59, 61-62		
4,4'-dichlorobiphenyl	148	315-319	
2,4,5-trichlorobiphenyl	78-79		
3,5,4'-trichlorobiphenyl	88		
3,4,3',4'-tetrachlorobiphenyl	172		230(50)
2,4,2',4'-tetrachlorobiphenyl	83		
2,4,5,3',4'-pentachlorobiphenyl	179		195-220/10)
3,4,5,3',4',5'-hexachlorobiphenyl	198		
2,3,4,5,2',4',5'-heptachlorobiphenyl			240-280(20)
2,3,4,5,6,2',3',4',5',6'-decachlorobiphenyl	310		

(b) Polychlorinated biphenyls are insoluble in water but soluble in most of the common organic solvents.

1.4 Technical products and impurities

Products vary from mobile oily liquids to white crystalline solids and hard non-crystalline resins. Technical products vary in composition, in the degree of chlorination and possibly according to batch. For example: Kanechlor 500 has an average content of 55.0% pentachlorobiphenyl, 26.5% tetrachlorobiphenyl, 12.8% hexachlorobiphenyl and 5.0% trichlorobiphenyl (lot 360). Kanechlor 400 has an average content of 43.8% tetrachlorbiphenyl, 32.8% trichlorobiphenyl, 15.8% pentachlorobiphenyl, 4.6% hexachlorobiphenyl

* Data from Hubbard (1964)

TABLE 1

CHEMICAL AND PHYSICAL DATA ON KANECHLORS

Kanechlor		300	400	500
Appearance		colourless, gummy oil	colourless, gummy oil	colourless, gummy oil
Specific gravity	(at 15°C) (at 100°C)	1.337-1.339 1.310-1.322	1.453-1.468 1.376-1.389	1.460-1.475
Viscosity	(at 75°C)	3.5-4.4	5.4-7.3	12-19
Centistokes	(at 98°C)	2.1-2.6	2.8-3.7	5.0-6.6
Refractive index	(at 25°C)	1.6230-1.6260	1.6295-1.6325	1.6370-1.6390
Pour-point	(°C)	(-19)-(-15)	(-8)-(-5)	8-12
Acid value KOH	(mg/g)	0.005>	0.005>	0.005>
Distillation temperature range	(°C of 760 mm HG)	325-360	340-375	365-390

TABLE 2

CHEMICAL AND PHYSICAL DATA ON AROCLORS

		Aroclor 1221	Aroclor 1242	Aroclor 1248	Aroclor 1254
Appearance		colourless, mobile oil*	almost colourless, mobile oil*	yellow-green-tinted, mobile oil*	light-yellow, viscous oil*
Density	(at 20°C)	1.18-1.19*	1.38-1.39**	1.45-1.47**	-
	(at 90°C)	-	-	-	1.47-1.49**
Refractive index	(at 20°C)	1.617-1.618*	1.625-1.627**	1.6305-1.6325**	1.638-1.640**
Pour-point	(°C)	+1*	-18 max**	-6 max**	+12 max**

* From Hubbard (1964)

** From Monsanto Co. (1973)

and 3.0% dichlorobiphenyl (lot 471). Kanechlor 300 has an average content of 59.8% trichlorobiphenyl, 23.0% tetrachlorobiphenyl, 16.6% dichlorobiphenyl and 0.6% pentachlorobiphenyl (lot 348). Monsanto, the only US manufacturer of these chemicals has produced a series of chlorinated biphenyls and chlorinated polyphenyls over the years which were numbered to identify each product. The first two digits indicated the type of material: 12 - chlorinated biphenyls; 25 - blend of chlorinated biphenyls and chlorinated triphenyls (75:25); 44 - blend of chlorinated biphenyls and chlorinated triphenyls (60:40); and 54 - chlorinated triphenyls. The last two digits indicated the approximate weight percentage of chlorine in the product. Thus, the product numbered Aroclor 1242 contains mainly trichlorobiphenyls, Aroclor 1248 contains mainly tetrachlorobiphenyls and Aroclor 1254 contains mainly pentachlorobiphenyls.

Some of the physical and chemical properties of Japanese and US products are given in Tables 1 and 2.

Specific data on the impurities in these products are not available, but colour is an indication of purity: the darker the product, the lower the purity (Monsanto Co., 1970). The raw materials used in the synthesis of polychlorinated biphenyls determine to a large degree the type of impurity or contaminant in the commercial product. Fractionated samples of some non-US products have shown them to contain the tetra- and pentachlorodibenzofurans and the hexa- and heptachloronaphthalenes as contaminants (Interdepartmental Task Force on PCBs, 1972).

2. Production, Use, Occurrence and Analysis

Several review articles on polychlorinated biphenyls have been published (Fishbein, 1973; Hubbard, 1964; Interdepartmental Task Force on PCBs, 1972).

2.1 Production and use[1]

The polychlorinated biphenyls were first described in the chemical

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

literature in 1881. Commercial manufacture of these chemicals in the US is believed to have begun in 1929; and although a number of US companies have registered trademarks for polychlorinated biphenyls, recent information indicates that there is only one US producer of polychlorinated biphenyls. If these chemicals are sold at present by other companies, they are either manufactured by this one US producer or imported.

Polychlorinated bi-, ter- and polyphenyls are produced by chlorination of the appropriate aromatic hydrocarbon using either iron or ferric chloride as a catalyst. The composition of the end-product is determined by the quantity of chlorine present (Hubbard, 1964).

It was estimated in 1970 that since the commercial introduction of polychlorinated biphenyls in 1929, 454 million kg of these chemicals had been sold in North America. Between 1960 and 1970, production of polychlorinated biphenyls increased steadily from 17.3 million kg in 1960 to 85 million kg in 1970. In 1971 the US producer voluntarily agreed to restrict sale of polychlorinated biphenyls to those applications which minimize their introduction into the environment; thus, production dropped to 18.2 million kg, with an estimated output of 11.4-13.6 million kg for 1972. In general, the majority of these chemicals produced are the lower chlorinated grades (Interdepartmental Task Force on PCBs, 1972).

US imports of polychlorinated biphenyls were 290,836 kg in 1971 (US Tariff Commission, 1972) but only 160,985 kg in 1972 (US Tariff Commission, 1973). US exports of polychlorinated biphenyls increased steadily from 8% of production (1.6 million kg) in 1963 to 16% of production (6.2 million kg) in 1970. In 1971, 25% of total production was exported, but this amounted to only 4.4 million kg (Interdepartmental Task Force on PCBs, 1972).

It has been estimated that US production in 1971 represented roughly one half of the total world production of polychlorinated biphenyls. The following countries have also been reported to produce these chemicals: Czechoslovakia, the Federal Republic of Germany, France, Italy, Japan, Poland, Spain, the United Kingdom and USSR (Anon., 1973; Interdepartmental Task Force on PCBs, 1972). Argentina, Brazil and India have been reported to be possible producers (Anon., 1972).

Production began in Japan in 1954, and by the end of 1971 approximately 55.4 million kg had been produced. Approximately 60% of Japan's total exports have gone to the US, with the remaining 40% going to Europe; between 1962 and 1971, exports of these chemicals totalled 4.5 million kg. Imports, solely from the US, totalled 0.6 million kg from 1967 to 1971. The production of polychlorinated biphenyls is now prohibited in Japan.

Prior to 1971, polychlorinated biphenyls were used in a variety of applications, primarily because of their excellent thermal and chemical stability. However, in 1971, Monsanto, the sole US producer of these chemicals, voluntarily decided to limit their use to those closed electrical applications from which possible release to the environment is minimized. Before that time about 40% of the polychlorinated biphenyls used in the US went into applications which led to loss into the environment, e.g., in plasticizers, hydraulic fluids and lubricants, surface coatings, inks, sealants, adhesives, pesticide extenders and microencapsulation of dyes for carbonless duplicating paper. The remaining 60% of US sales was used mainly in electrical transformers and capacitor applications; of all applications prior to 1971, the largest uses were in capacitors and transformers and in plasticizer applications, including carbonless duplicating paper. Today polychlorinated biphenyls are used in the US only in closed-system electrical applications which minimize introduction into the environment (Hubbard, 1964; Interdepartmental Task Force on PCBs, 1972).

2.2 Occurrence

(a) Occupational exposure

Although polychlorinated biphenyls may have been used in pesticide formulations in conjunction with various pesticides, no information on human exposure during crop or forest spraying is available.

The US Occupational Safety and Health Administration health standards for air contaminants require that an employee's skin exposure to chlorodiphenyl (42% chlorine) and chlorodiphenyl (54% chlorine) does not exceed eight-hour time-weighted averages of 1 mg/m^3 and 0.5 mg/m^3, respectively, in the workplace air during any eight-hour workshift for a forty-hour work week (US Code of Federal Regulations, 1974).

Masuda et al. (1972) reported that polychlorinated biphenyls occurred in carbonless copying paper in Japan; average amounts of 30 µg were found remaining on the skin after handling such paper. Lister & Bennett (1972) reported that the major manufacturers of this paper in the UK did not use polychlorinated biphenyls.

(b) Air and rain

Polychlorinated biphenyls may enter the air during the destruction of manufactured articles containing polychlorinated biphenyls in waste-disposal burners, through the gradual wear and weathering of polychlorinated biphenyl-containing products or through leaks from sealed systems. It has been estimated that some 400 thousand kg polychlorinated biphenyls were released into the atmosphere in 1970 in the US from waste-disposal burners (Nisbet & Sarofim, 1972) and that 1000-2000 thousand kg entered the atmosphere from the vaporization of plasticizers (Panel on Hazardous Trace Substances, 1972). In comparison, loss by evaporation or leakage from closed systems would appear to be insignificant. Carnes et al. (1973) have also demonstrated polychlorinated biphenyls in ash from industrial burners; in addition, some chlorinated dibenzofurans and dibenzodioxins may also be released through oxidation processes during incineration.

Measurement of the monthly airborne fallout of polychlorinated biphenyls in 8 sites in Sweden during 1970-71 revealed levels of 550-10,500 ng/m^2/month (Södergren, 1972). Air samples collected during February-April 1973 from a 20-metre high tower in Bermuda, or in June 1973 on the open sea off the island, contained polychlorinated biphenyl levels of 0.21-1.6 ng/m^3 in the vapour phase. Higher levels (2.1-9.4 ng/m^3) were found in Rhode Island where peaks resembled chromatograms of Arochlor 1242, which contains mainly tri-, tetra- and pentachlorobiphenyls (Bidleman & Olney, 1974).

Bevenue et al. (1972) reported levels of 60 ng/l polychlorinated biphenyls in rainwater in Hawaii during 1971-72.

(c) Soil and water

No data on polychlorinated biphenyl residues in various soils appear to be available. Holden (1970) reported concentrations of <0.1-14 ppm polychorinated biphenyls (wet weight) similar to Aroclor 1254 in 15 samples of

sewage sludge intended for disposal in the sea off the Firth of Clyde in Scotland. Analyses by other laboratories of sewage sludge intended for disposal off the Thames and Mersey estuaries showed that 0.2 ppm and 0.1-5 ppm (wet weight) polychlorinated biphenyls were present, respectively (Holden, 1970). These figures are equivalent to a total discharge of about 1 thousand kg polychlorinated biphenyls per year. Analyses at 9 sewage treatment plants in California in 1970 revealed that concentrations of 0.2-12 ppb occurred in sewage pumped into the Pacific Ocean (Schmidt et al., 1971).

In the US, polychlorinated biphenyl concentrations of about 0.02-2.5 µg/l were reported in Milwaukee river water in 1969-70; the highest concentrations occurred near chemical plants (Veith & Lee, 1971). In Green Bay, Lake Michigan, Veith (1972) found concentrations of polychlorinated biphenyls (as Aroclor 1254) of <0.01-0.45 µg/l in various river waters entering the bay.

Measurements made during the summer of 1972 showed that the average concentrations of polychlorinated biphenyls in North Atlantic sea-water were 35 ng/l at the surface and 10 ng/l at a depth of 200 metres; the polychlorinated biphenyl concentration in Sargasso Sea surface waters averaged 27 ng/l (Harvey et al., 1973). However, Bidleman & Olney (1974) found lower values, <1-19.3 ng/l, in Sargasso Sea surface waters; the peaks resembled chromatograms of Aroclor 1254 or 1260, which contain mainly penta-, hexa- and heptachlorobiphenyls.

According to a review by the Panel on Hazardous Trace Substances (1972), polychlorinated biphenyls have been found at concentrations of 10-100 ng/l in drinking-water in Japan.

On July 3 1973, the US Environmental Protection Agency proposed a list of toxic pollutants which includes polychlorinated biphenyls (US Environmental Protection Agency, 1973). If this list is adopted, effluent standards restricting or prohibiting discharges of these chemicals into streams may come into effect.

(d) Animals and plants

A review of levels of polychlorinated biphenyls found in fish and birds

in Japan has been published recently (Doguchi, 1973).

The concentrations of polychlorinated biphenyls in vertebrate food chains increases in such a way that the top predators (e.g., birds of prey, sharks and seals) may have total concentrations of polychlorinated biphenyls 10^7-10^8 times that in the ambient environment.

Studies of zooplankton from the North Atlantic shelf and slope areas revealed polychlorinated biphenyl concentrations in these organisms of 0.07-3 ppm (dry weight) and 0.02-0.64 ppm (wet weight) in the 2 areas, respectively (Risebrough et al., 1972). In the Gulf of Mexico, concentrations of <3-1000 µg/kg (wet weight) have been found in zooplankton (Giam et al., 1973); and, in plankton in the Gulf of St. Lawrence, concentrations of 0.09-3 mg/kg (wet weight) have been found (Ware & Addison, 1973).

In small predatory fish, polychlorinated biphenyl levels of 8 µg/kg were found in pike (fresh tissue) taken from an unpolluted lake in Finland, whereas 0.3 mg/kg were found in herring taken from the open sea (Hattula, 1972). Zitko (1971) found concentrations of 0.02-1 mg/kg polychlorinated biphenyls in eel, salmon, herring, mackerel, mussel, cod, hake and plaice, and traces in ocean perch, taken from the St John River system, New Brunswick, or from the Nova Scotia banks. Similar levels (0.36-1.54 mg/kg) were found in tuna from the Atlantic coast of North America (Zitko & Choi, 1971). Much higher levels (up to 7 mg/kg Aroclor 1254) have been found in shrimp and crab caught in accidentally-polluted bays, e.g., Escambia Bay, Florida (Duke et al., 1970) and in fish from inland lakes: for example, 26 mg/kg were found in a 12-year old trout caught in Cayuga Lake in Ithaca, New York (Bache et al., 1972) in which case the gas chromatogram was similar to that of Aroclor 1254. Stalling & Mayer (1972) have also reported high levels of polychlorinated biphenyls (>20 mg/kg) in inland lake and river fish in the US.

Numerous reports concerning polychlorinated biphenyl residues in predatory birds in Canada, Denmark, Finland, Sweden, the UK and the US have appeared. Jensen et al. (1969) reported the presence of up to 190 mg/kg polychlorinated biphenyls (whole body-weight) in 4 white-tailed eagles found between March-June in 1965 and in 1966. Risebrough et al. (1968)

identified up to 65 mg/kg polychlorinated biphenyls (wet weight) in the carcasses of 4 peregrine falcons which died after having been trapped for falconry. Residues of 30 and 900 mg/kg were found in the livers of 2 herons in the UK (Prestt et al., 1970). Levels of 1.3-272 mg/kg polychlorinated biphenyls (wet weight) (comparable to Clophen A 60) were found in the livers of terrestrial predatory birds in Denmark (Karlog et al., 1971), and levels of 93-470 mg/kg (wet weight) were found in the livers of cormorants found dead in 1970 in the Netherlands (Koeman, 1973).

Various concentrations of polychlorinated biphenyls have been recorded in birds' eggs. Concentrations in the eggs of cormorants, gulls and ducks from the Bay of Fundy, Canada, ranged from 6-44 mg/kg (Zitko & Choi, 1972). Lower levels, about 1-5 mg/kg (wet weight), have been reported in the UK (Prestt et al., 1970) and in the US (Greichus et al., 1973).

Polychlorinated biphenyl residues of up to 310 mg/kg have also been reported in the fat of seals living off the coast of Sweden (Jensen et al., 1969).

(e) Food

Sea-food or food packaged in grey cardboard packaging appear to have been the two main sources of dietary polychlorinated biphenyls. Small residues of polychlorinated biphenyls may also occur in milk and meat products, since silage stored in upright silos which have polychlorinated biphenyl wall sealants may contain about 0.1 mg/kg polychlorinated biphenyls (Savage et al., 1973). Fries (1972) detected 19 mg/kg in milk fat taken from cows at farms which had polychlorinated biphenyl-treated silos.

Cereals packed in cardboard containing 10 mg/kg polychlorinated biphenyls have been shown to contain about 0.2-0.3 mg/kg polychlorinated biphenyls (Trout, 1972). One sample of cashew nuts packaged in cardboard drums was reported to have contained 10 mg/kg polychlorinated biphenyls (Bailey et al., 1970), and analysis of various types of food packed in paperboard with or without a plastic barrier showed polychlorinated biphenyl levels of <0.1-4.3 mg/kg (Stanovick et al., 1973).

Polychlorinated biphenyls have also been demonstrated in human milk in

Japan at concentrations of 0.03 mg/kg (whole milk) and 1.1 mg/kg (fat basis) (Oura et al., 1972); and in the Federal Republic of Germany concentrations of 0.1 mg/kg (whole milk) and 3.5 mg/kg (fat basis) (Tombergs, 1972) have been found. However, none could be detected in the milk of women in Texas and New Guinea (Dyment et al., 1971), although the mean polychlorinated biphenyl level in samples of human milk in California was 60 µg/kg (Risebrough & Brodine, 1970). Thus, since the daily milk intake of breast-fed infants is 150 g/kg bw, the amount of polychlorinated biphenyls ingested would be in the order of 9 µg/kg bw/day. This figure is slightly above the maximum daily intake which would occur in adults eating 150 g/week of fish containing 15 mg/kg polychlorinated biphenyls (e.g., coho salmon from Lake Michigan) or 80 g of fish per day in Japan, where values of up to 5 mg/kg may be present (Panel on Hazardous Trace Substances, 1972).

The US Department of Agriculture has analyzed polychlorinated biphenyls in selected food commodities (US Department of Agriculture, 1972): fish contained from traces to 35.3 mg/kg (average, 1.87 with 54% positive samples); cheese contained from traces to 1.0 mg/kg (average, 0.25 and 6% positive samples); milk contained from traces to 27.8 mg/kg (average, 2.27 and 7% positive samples); whereas eggs contained from traces to 3.74 mg/kg (average, 0.55 and 29% positive samples). Of the total number of commodity samples examined, only 19% were positive with respect to contamination by polychlorinated biphenyls, reflecting an average concentration of 1.14 mg/kg of sample.

Twenty-two of 720 composite samples from the US Food and Drug Administration's (FDA) market-basket surveys have been found to contain polychlorinated biphenyls ranging in concentration from traces to 0.36 mg/kg. This maximum value was due to migration of polychlorinated biphenyls from greyboard containers and dividers for packaged shredded wheat. Estimates of the mean daily intake of polychlorinated biphenyls in the American diet range from 5-10 µg per day (National Environmental Research Center, 1974).

During the 'Yusho incident' in Japan, a poisoning accident caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyl (Kanechlor 400), the minimum toxic intake for humans was estimated to be 200 µg/kg bw/day (Kuratsune et al., 1972).

The FDA has established temporary tolerances for polychlorinated biphenyls in foods, animal feeds and food-packaging materials. These tolerances are as follows: 2.5 mg/kg (ppm) in milk (fat basis); 2.5 mg/kg (ppm) in manufactured dairy products (fat basis); 5 mg/kg (ppm) in poultry (fat basis); 0.5 mg/kg (ppm) in eggs; 0.2 mg/kg (ppm) in finished animal feed for food-producing animals (except in feed concentrates, feed supplements and feed premixes); 2 mg/kg (ppm) in animal feed components of animal origin, including fishmeal and other by-products of marine origin and in finished animal feed concentrates, supplements and premixes intended for food-producing animals; 5 mg/kg (ppm) in fish and shellfish (edible portion); 0.2 mg/kg (ppm) in infant and junior foods; 10 mg/kg (ppm) in paper food-packaging material intended for or used with human food, finished animal feed and any components intended for animal feeds (US Federal Register, 1973).

2.3 Analysis

This subject has been reviewed by Fishbein (1973) and by Jensen et al. (1973).

One of the main problems in the analysis of polychlorinated biphenyls is that during their extraction from the media, other organochlorine pesticides (which may have similar retention times on chromatographs) are also eluted. Simpler chromatograms can be obtained by using separation techniques prior to gas chromatography. Such clean-up methods include TLC, paper and column chromatography; and solvent partition or saponification and dechlorination of other compounds with alcoholic KOH have also been used. p,p'-DDE can be oxidized to 4,4'-dichlorobenzophenone without affecting the polychlorinated biphenyls (Collins et al., 1972).

Some widely-used methods and their modifications are described in the Pesticide Analytical Manual (US Department of Health, Education and Welfare, 1968), by Armour & Burke (1970) and by Porter et al. (1970). Volume III of the Pesticide Analytical Manual (US Department of Health, Education and Welfare, 1970) and Biros et al. (1970) give a method for analyzing residues in human tissues.

Quantitation of polychlorinated biphenyls from electron-capture

chromatograms has been discussed by Zitko et al. (1971). However, for a fast, simple determination, the polychlorinated biphenyl sample can be converted to decachlorobiphenyl and compared with an Aroclor 1254 standard (National Environment Research Center, 1974). Some difficulties encountered with the use of standards in the quantification of polychlorinated biphenyls have been described by Beezhold & Stout (1973).

Confirmation of identity can be made by combined gas chromatography-mass spectrometry, which, in addition, yields the number of chlorine atoms per molecule (Bagley et al., 1970; Biros et al., 1970; Widmark, 1967). However, rearrangement of the chlorine atoms or phenyl groups occurring in the electron-impact-induced fragmentation limits the use of mass-spectrometry for structural studies. Nuclear-magnetic-resonance (NMR) spectroscopy has been used to resolve the structure of some polychlorinated biphenyl isomers (Sissons & Welti, 1971). Characterization of the components of technical polychlorinated biphenyl mixtures has been reported by Tas & Kleipool (1972) using pure standards, GLC retention times on 3 different columns, infra-red spectra and NMR spectroscopy.

Methods for the determination of polychlorinated biphenyls in food, plant or animal tissue in Japan have been described by Wakimoto et al. (1973).

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 12 6-week old male dd mice were administered Kanechlor 300, 400 or 500 in the diet at concentrations of 100, 250 or 500 ppm for 32 weeks. Six control mice received the basal diet. Gross examination after 32 weeks of treatment showed that 7/12 mice given 500 ppm Kanechlor 500 developed liver nodules, and histopathological examination showed the presence of hepatocellular carcinomas in 5/12 mice. No metastases or tumours in other organs were seen. Amyloid degeneration of the liver was seen in the other groups, especially in those at the 100 ppm dosage level. No changes occurred in the 6 controls (Ito et al., 1973; Nagasaki et al., 1972). [See also Table 3.]

TABLE 3

HISTOPATHOLOGICAL FINDINGS IN THE LIVERS OF MALE dd-MICE TREATED WITH PCBs FOR 32 WEEKS

PCBs in diet (ppm)		Effective no. of mice	Amyloid degeneration	Liver nodules	
				Nodular hyperplasia	Hepatocellular carcinoma
Kanechlor	500	12	0/12 -	7/12(58.3%)	5/12(41.7%)
500	250	12	2/12(16.7%)	0/12 -	0/12 -
	100	12	3/12(25.0%)	0/12 -	0/12 -
Kanechlor	500	12	0/12 -	0/12 -	0/12 -
400	250	12	3/12(25.0%)	0/12 -	0/12 -
	100	12	10/12(83.3%)	0/12 -	0/12 -
Kanechlor	500	12	1/12 (8.3%)	0/12 -	0/12 -
300	250	12	4/12(33.3%)	0/12 -	0/12 -
	100	12	10/12(83.3%)	0/12 -	0/12 -
Control		6	0/6 -	0/6 -	0/6 -

From Ito et al. (1973)

Two groups of 50 male BALB/cj mice were fed 300 ppm Aroclor 1254 in the diet for 11 months or for 6 months followed by the control diet for 5 months. Two additional groups of 50 mice were fed the basal diet. At the end of 11 months 1/24 surviving mice treated for 6 months with Aroclor 1254 had a hepatoma, while of the mice surviving the continuous treatment 9/22 developed hepatomas. In addition, adenofibrosis was observed in all 22 mice fed Aroclor 1254 continuously for 11 months (Kimbrough & Linder, 1974).

Rat: In groups of 10 male and 10 female Sherman rats fed 0, 20, 100, 500 or 1000 ppm Aroclor 1260, or 0, 20, 100 or 500 ppm Aroclor 1254, for 8 months, several rats died before 6 months at the 2 high-dose levels. Lesions described as adenofibrosis of the liver occurred in 2/10 males fed 1000 ppm Aroclor 1260 and in 1/10, 1/10 and 4/10 females fed 100, 500 and 1000 ppm Aroclor 1260. A higher incidence of this lesion occurred in rats fed Aroclor 1254, the incidences in males and females being 10/10 and 9/10 at 500 ppm and 1/10 and 7/10 at 100 ppm, respectively (Kimbrough et al., 1972).

A group of 10 male and 10 female Donryu rats was fed a diet containing 38.5-616 ppm Kanechlor 400 for 400 days. The initial concentration of 38.5 ppm was increased by factors of 2 in varying steps during the first 125 days until 616 ppm was reached. After 56 days at this level the concentration was reduced to 462 ppm for the remainder of the study except during two 28-day rest periods. A control group of 5 males and 5 females received a basal diet. In 6/10 treated females, multiple adenomatous nodules of the liver were observed; such lesions did not occur in male rats nor in the controls. No hepatocellular carcinomas were seen (Kimura & Baba, 1973).

Groups of 30 male Wistar rats were fed for 52 weeks on diets containing 100, 500 or 1000 ppm Kanechlor 300, 400 or 500. One group received a control diet. Each Kanechlor produced cholangiofibrosis in a proportion of the rats when fed at 1000 ppm in the diet, while lower dosage levels were ineffective. Hepatic nodular hyperplasia was found with all compounds, the incidence increasing with the degree of chlorination and with concentration in the diet (Ito et al., 1974). [See also Table 4.]

TABLE 4

HISTOPATHOLIGICAL FINDINGS IN THE LIVERS OF MALE WISTAR RATS TREATED WITH PCBs

PCBs in diet (ppm)		Experimental period (weeks)	Effective no. of rats	Fibrosis	Cholangiofibrosis	Nodular hyperplasia	Amyloidosis
Kanechlor	1000	49.3	13/30	±	4/13(30.8%)	5/13(38.5%)	0/13 -
500	500	52	16/30	-	0/16 -	5/16(31.3%)	0/16 -
	100	52	25/30	-	0/25 -	3/25(12.0%)	0/25 -
Kanechlor	1000	40	10/30	++	2/10(20.0%)	3/10(30.0%)	0/10 -
400	500	27.8	8/30	-	0/8 -	0/8 -	0/8 -
	100	39.6	16/30	-	0/16 -	2/16(12.5%)	2/16(12.5%)
Kanechlor	1000	52	15/30	-	2/15(13.3%)	0/15(6.7%)	0/15 -
300	500	52	19/30	-	0/19 -	0/19 -	0/19 -
	100	52	22/30	-	0/22 -	1/22(4.5%)	0/22 -
Control		52	18/20	-	0/18 -	0/18 -	0/18 -

From Ito et al. (1974)

3.2 Other relevant biological data

(a) Animals

The LD_{50}'s of orally administered Aroclor 1254 and 1260 in Sherman rats are 4-10 g/kg bw (Kimbrough et al., 1972) and that of Aroclor 1242 4.25 g/kg bw in male Wistar rats (Bruckner et al., 1973).

In DDD mice, the various components of a single oral dose of Kanechlor 400 were equally absorbed and distributed, mainly in the skin, liver and kidney. The tetrachlorobiphenyls, the major components of Kanechlor 400, were almost completely eliminated after 3-4 weeks; but the penta- and hexachlorobiphenyls, the minor components, were still retained after 9-10 weeks (Yoshimura & Oshima, 1971). In male Wistar King rats, 3 days after a single oral dose of 25 mg ^{3}H-Kanechlor 400, radioactivity was distributed mainly in the liver, skin and adipose tissue. During the first 4 weeks, 70% of the radioactivity was excreted in the faeces and 2% in the urine (Yoshimura et al., 1971).

Fifty male Sherman rats were fed 500 ppm Aroclor 1254 in the diet for 6 months, and 5 rats were killed 0, 1, 2, 3, 4, 6, 8 and 10 months after exposure to Aroclor 1254 had ceased. In the rats killed 10 months after the end of exposure, about 1200 ppm and 22 ppm polychlorinated biphenyls (PCBs) were present in the adipose tissue and liver, respectively. The compounds found were those containing mainly 5, 6 or 7 chlorine atoms (Kimbrough et al., 1973).

Preliminary work by Goto et al. (1973) on the metabolism of orally administered 2,2'-, 2,4-, 2,3-, 3,4-, 3,3'- and 4,4'-dichlorobiphenyls in male rats indicates that the main metabolites are dichlorodihydroxybiphenyls, which are excreted via the urine and faeces as such and not as conjugates.

In male Wistar rats 64% of a single oral dose of 25 mg 3,4,3',4'-tetrachlorobiphenyl (TCB) was excreted unchanged in the faeces during the first 14 days after dosing, and a metabolite thought to be the 2- or 5-hydroxy-3,4,3',4'-TCB was also found in small quantities (3%) (Yoshimura & Yamamoto, 1973). Similarly, the metabolites of 2,4,3'4,'-TCB were identified in the

faeces of rats as free 5- or 3-hydroxy-2,4,3',4'-TCB (Yoshimura et al., 1973). Gardner et al. (1973) showed that in rabbits oral administration of 2,5,2',5'-TCB resulted in 3 urinary metabolites (3- and 4-hydroxy-2,5,2'5'-TCB and trans-3,4-dihydro-3,4-dihydroxy-2,5,2',5'-TCB), possibly formed through an arene oxide (epoxide).

Low levels of PCBs in the diet, or i.p. injections of PCBs, increase the microsomal mixed-function oxidase activity of the liver in rats (Bruckner et al., 1973; Norbach & Allen, 1972). PCBs can also be absorbed through the skin in rabbits (Vos & Beems, 1971).

In Sherman rats given doses of 10 or 50 mg/kg bw/day Aroclor 1254 on days 7-15 of pregnancy, the average PCB concentrations in foetuses taken by Caesarean section on day 20 of pregnancy were 0.63 and 1.38 mg/kg, respectively, compared with <0.12 mg/kg in controls (Curley et al., 1973a). Transplacental transfer was also demonstrated in rabbits (Grant et al., 1971).

Vos & de Roij (1972) demonstrated immunosuppressive activity of PCBs on both the humoral and cellular response of the immune system.

When Aroclor 1254 was fed to pheasants either as a single dose of 50 mg or as 17 weekly doses of 12.5 or 50 mg, up to 82% was absorbed from the gastrointestinal tract and up to 50 mg/kg (wet weight) were found in their eggs (Dahlgren et al., 1971). In cows fed 200 mg/day Aroclor 1254 for 60 days, the average concentration in milk fat was 60 mg/kg (measured between the 40th and 60th days of feeding) (Fries et al., 1973). PCBs are also excreted in human milk (see section 2.2 (e)).

Two-day old cockerels were given a diet containing 400 mg/kg Phenoclor DP 6, Clophen A60 or Aroclor 1260 for 60 days, and concentrations of 120-2900 mg/kg and 40-700 mg/kg PCBs were found in the liver and brain, respectively, between the 12th and 58th days (Vos & Koeman, 1970). A maximum of 50 mg/kg Aroclor 1254 was found in eggs of white Leghorn hens fed for 12 weeks on a diet containing 50 mg/kg PCBs (Platonow & Reinhart, 1973).

In a series of papers describing an outbreak of poisoning involving 2 million chickens in Northern Japan, Kohanawa et al. (1969a,b) first reported that a 'dark oil', a by-product of a rice bran, may have been involved.

The pathological changes seen in the poisoned chickens included oedema of the subcutaneous tissue and lungs, hydropericardium, peritoneal ascites and yellowish mottling of the liver. Histologically, oedema of skeletal muscle, epicardium and lungs and focal necrosis of the liver were observed. Similar pathological changes could be induced in 20 experimental chickens fed on a commercial diet made up by the manufacturer with the dark oil, which was shown to contain Kanechlor 400 (Koga et al., 1970, 1971; Shoya et al., 1969).

(b) Man

Storage in human adipose tissue of the general population

Two samples of human adipose tissue were found to contain 200 and 600 mg/kg PCBs, but the source of these samples was not reported (Biros et al., 1970). Price & Welch (1972) estimated that 36% of the general population had levels of 1-2 mg/kg (wet weight) PCBs, ranging from penta- to deca-chlorobiphenyls, in their adipose tissue. In a Human Monitoring Survey of the exposure to pesticides experienced by the general population, Yobs (1972) found measurable amounts of PCBs (1-2 mg/kg) in 26% and >2 mg/kg in 5% of 637 samples of human adipose tissue taken from the general population of 18 states in the US and of Colombia. Bjerk (1972) reported average levels of 0.9 mg/kg (1.6 mg/kg fat basis) in adipose tissue taken from 40 humans dying in the Oslo area. In Japan, mean PCB concentrations in 25 samples were found to be 0.5 and 0.8 mg/kg, using 2 different separation techniques (Curley et al., 1973b). Slightly higher concentrations (averages, 2.6 mg/kg in males and 1.8 mg/kg in females) were reported in 98 adipose tissue samples taken from humans living in Tokyo. Maximums of 5.1 mg/kg occurred in 70-79-year old men and 2.4 mg/kg in 60-69-year old women (Doguchi, 1973).

3.3 Observations in man

Kuratsune et al. (1972) reported an incident of accidental poisoning by PCB involving 1057 persons in Japan who ingested rice-oil which had been contaminated by Kanechlor 400 during the manufacturing process. From that group, 4 autopsy reports, 3 in adults and 1 in a stillborn, are available.

The autopsy of one 48-year old Japanese female, who was diagnosed as

having liver cirrhosis, showed multilobular cirrhotic changes in the liver and many nodules of hepatic-cell carcinoma (Kikuchi, 1972). In the other 3 cases, there were various skin lesions associated with exposure to PCB, and chromatographic analysis showed PCB to be present in the mesenteric fatty tissue and skin in all 4 cases (Kikuchi et al., 1969, 1971). The skin of the stillborn child from an exposed mother showed disturbances of pigmentation; histologically, the changes were the same as those of PCB poisoning. The presence of PCB in the tissues of this stillborn child demonstrates transplacental passage (Kikuchi et al., 1969), and further evidence of such passage was provided by Miller (1971) and by Kuratsune et al. (1972) who reported on several infants born to mothers included in these cases of poisoning.

4. Comments on Data Reported and Evaluation[1]

4.1 Animal data

A limited number of PCBs have been tested. Kanechlor 500 and Aroclor 1254 are carcinogenic in mice inducing benign and malignant liver-cell tumours following oral administration, the only route tested. In rats, Kanechlor 500, 400 and 300 induced multiple hyperplastic liver nodules following oral administration.

4.2 Human data

In the absence of epidemiological studies the available case report does not allow an evaluation to be made.

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[1] See also the section, "Animal Data in Relation to the Evaluation of Risk to Man" in the introduction to this volume (p. 15).

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VINYL CHLORIDE*

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: 75-01-4

Chlorethene; chlorethylene; chloroethene; chloroethylene; ethylene monochloride; monochloroethene; monochloroethylene

VC; VCM; Vinyl chloride monomer; Vinyl C monomer

1.2 Chemical formula and molecular weight

$H_2C=CHCl$ C_2H_3Cl Mol. wt: 62.5

1.3 Chemical and physical properties of the pure substance

(a) Description: A colourless, flammable gas under normal conditions of temperature and pressure but usually handled as a liquid under pressure

(b) Melting-point: $-153.7^{\circ}C$

(c) Boiling-point: $-13.9^{\circ}C$

(d) Density: d_4^{20} 0.9121

(e) Refractive index: n_D^{10} 1.4066

(f) Solubility: Slightly soluble in water (<0.11% w/w at $25^{\circ}C$ (Hardie, 1964)); soluble in ethanol; very soluble in ether, carbon tetrachloride and benzene

(g) Volatility: Vapour pressure is 2660 mm Hg at $25^{\circ}C$.

(h) Chemical reactivity: Vinyl chloride monomer polymerizes in light or in the presence of a catalyst. On combustion, it is degraded mainly to hydrogen chloride, carbon monoxide, carbon

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* Considered by the Working Group in Lyon, 18-24 June, 1974. The Working Group was aware of many ongoing studies; however, this document is based upon published data or data already accepted for publication which were available at that time.

dioxide and to traces of phosgene (O'Mara et al., 1971). It loses HCl on treatment with strong alkalis at high temperatures (Miller, 1969).

(i) Flash-point: $-78^{o}C$

1.4 Technical products and impurities

Vinyl chloride monomer is available commercially in cylinders or in bulk and is generally supplied as a liquid under pressure. The technical grade is 99.9% pure (Condensed Chemical Dictionary, 1971). Specifications for a typical commercial product call for maximums in mg/kg by weight of the following impurities: unsaturated hydrocarbons - 10; acetaldehyde - 2; dichloro compounds - 16; water - 15; hydrogen chloride - 2; nonvolatiles - 200; iron - 0.4. Phenol at levels of 25-50 mg/kg by weight is used as a stabilizer to prevent polymerization.

2. Production, Use, Occurrence and Analysis

Two review articles on vinyl chloride monomer have been published (Hardie, 1964; Keane et al., 1973).

2.1 Production and use[1]

The first synthesis of vinyl chloride monomer appears to have been made in 1835 by Regnault (Regnault, 1835). It has been produced commercially in the United States for at least 46 years (US Tariff Commission, 1928). Addition of hydrogen chloride to acetylene, formerly the most important route of synthesis, has been displaced by a method using the halogenation of ethylene; almost 90% of the vinyl chloride monomer produced in the US in 1971 was made from ethylene. In this process, ethylene is reacted with hydrogen chloride and oxygen to give ethylene dichloride, which is subsequently cracked to produce vinyl chloride monomer and hydrogen chloride.

In 1971, 10 US producers reported a total vinyl chloride monomer production of 1,969 million kg (US Tariff Commission, 1973). Preliminary

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

data indicate that in 1972 US production amounted to 2,310 million kg (US Tariff Commission, 1974c) and in 1973 to 2,428 million kg (US Tariff Commission, 1974b). US imports of vinyl chloride monomer have been negligible; but in 1972, US exports were reported to have been 282 million kg, over half of which was exported to Norway, Belgium and Spain (US Department of Commerce, 1972).

World production of vinyl chloride monomer in 1971 in various areas is estimated as follows (in millions of kg): Western Europe - 2,497; United States - 1,969; Japan - 1,275; Eastern Europe - 817; other areas - 499; total - 7,057. Countries producing vinyl chloride monomer (listed in decreasing order of estimated annual production in recent years) and the numbers of producers in each country are as follows: Japan (15), the Federal Republic of Germany (5), Italy (3), France (4), Belgium (3), the United Kingdom (4), The Netherlands (2), Brazil (3), Spain (3), Turkey (3), Taiwan (2), Argentina (2), Sweden (1), Mexico (1), USSR (2), South Korea (1), Finland (1), Czechoslovakia (1), Venezuela (1), Egypt (1), Thailand (1), Rumania (1), Chile (2), Greece (1) and Australia (1) (Keane et al., 1973).

In 1971, 77 million kg of vinyl chloride monomer were imported into the Common Market countries, as compared to an export volume of 52 million kg. Belgium/Luxembourg, Italy and The Netherlands were not exporters, whereas the Federal Republic of Germany and France were not importers; however, the situation is subject to rapid change due to the establishment of new plants and with changing short-term regional needs (European Communities, 1971). Japan exported 323 million kg of vinyl chloride monomer in 1972, principally to Taiwan (52%) and to the Republic of South Korea (30%).

At least 97% of the nearly 1,600 million kg of vinyl chloride monomer consumed in the US in 1971 was for the production of vinyl chloride homopolymer and co-polymer resins. The remainder was used in a variety of applications, the most significant of which are in the production of methyl chloroform; as an additive to specialty coatings; and, in several products, as a component of certain propellant mixtures. Hardie (1964) has reported that vinyl chloride monomer has been used as a refrigerant, as an

extraction solvent for heat-sensitive materials and in the production of chloroacetaldehyde, an intermediate in the synthesis of sulpha drugs; however, no evidence was found that vinyl chloride monomer is presently being used for these purposes.

The polyvinyl chloride resins made from vinyl chloride monomer find their largest markets in the building and construction industries. Other important outlets are their use in household products, in consumer goods, in electrical applications, in packaging and in transportation (see Appendices A and B for additional information on the uses of polyvinyl chloride and vinyl chloride-vinyl acetate co-polymers).

The consumption patterns for vinyl chloride monomer in Western Europe and Japan are believed to be similar to that in the US. An estimated 5% of the polymers made in Japan in 1972 were co-polymers of vinyl chloride monomer with other monomers.

In early April 1974, the US Environmental Protection Agency (EPA) announced that no new pesticide products containing vinyl chloride monomer as a propellant would be registered for use, and this body subsequently suspended from sale all pesticide aerosols containing vinyl chloride monomer for use in the home, in food-handling establishments, in hospitals or in other enclosed areas. Twenty-eight products were reported to be affected by the decision (US Environmental Protection Agency, 1974). In the USSR, vinyl chloride monomer has been banned for use in aerosol propellants, because of its hazardous effects (Grikitis, 1967).

In April 1974, three US manufacturers recalled from the market aerosol hairspray products containing vinyl chloride monomer, and the US Food and Drug Administration (FDA) was reported to have written to other manufacturers requesting that they identify products containing vinyl chloride monomer (Anon., 1974b).

In May 1974, the US Consumer Product Safety Commission issued an order proposing to ban the sale of all self-pressurized products containing vinyl chloride monomer which were intended for household use.

2.2 Occurrence

Vinyl chloride monomer is not known to occur in nature.

(a) Occupational exposure

Cook et al. (1971) reported that the air concentration of vinyl chloride monomer in a polymerization reactor prior to ventilation is of the order of 7800 mg/m^3 (3000 ppm); that during the scraping procedure, 130-260 mg/m^3 (50-100 ppm); and that close to the hands during scraping, 1560-2600 mg/m^3 (600-1000 ppm). Lange et al. (1974a) found a concentration of 1560-2600 mg/m^3 (600-1000 ppm) vinyl chloride monomer in a polymerization reactor after washing. Concentrations of 50-800 mg/m^3 (20-312 ppm) vinyl chloride monomer have been found in the working atmosphere in a plant producing polyvinyl chloride (Filatova & Gronsberg, 1957); air concentrations of vinyl chloride monomer in working places in polyvinyl chloride-producing factories were reported by these authors to range from 100-800 mg/m^3 (40-312 ppm) with peaks up to 87,300 mg/m^3 (34,000 ppm). Anghelescu et al. (1969) have reported values from 112-554 mg/m^3 (44-216 ppm). It has been estimated that 20,000 US workers, past and present, have been exposed to the chemical in manufacturing plants (Heath et al., 1974).

It has been reported that, on a time-weighted average, the concentration of vinyl chloride monomer to which coagulator operators are exposed ranges from 130-650 mg/m^3 (50-250 ppm). The close relationship between the concentrations of vinyl chloride monomer in air to which subjects have been exposed and that in their expired air after exposure has ceased suggests that monitoring of vinyl chloride monomer in air and breath analysis are both valid means of estimating the exposure of individuals to vinyl chloride monomer (Baretta et al., 1969).

Vinyl chloride monomer has been recognized as a potentially dangerous contaminant in manufacturing plants. In the USSR a maximum allowable concentration (MAC) of 30 mg/m^3 (12 ppm) has been recommended generally (Schottek, 1969), and in the Federal Republic of Germany a MAC of 260 mg/m^3 (100 ppm) has been introduced (Deutsche Forschungsgemeinschaft, 1973). In the US a maximum of 1300 mg/m^3 (500 ppm) in working atmospheres was allowed

until April 1974 when the Occupational Safety and Health Administration (OSHA) of the US Department of Labor instituted a temporary emergency standard of 130 mg/m^3 (50 ppm) vinyl chloride monomer in the working atmosphere (US Code of Federal Regulations, 1974a). On May 6 1974, the OSHA proposed a permanent standard which would set employee exposure at no detectable level, as determined by a sampling and analytical method capable of detecting concentrations of 1 ppm vinyl chloride monomer with an accuracy of 1 ppm ± 50%. This proposal also calls for monitoring, control methods, medical surveillance and records and reports (US Code of Federal Regulations, 1974b).*

Recently, it has been reported that polyvinyl chloride leaving certain manufacturing plants may contain 200-400 mg/kg (ppm) vinyl chloride monomer; on delivery to the customer this level is about 250 mg/kg (ppm); and after processing, levels of 0.5-20 mg/kg (ppm) are reached, depending on the method of fabrication (Anon., 1974e). Another source found 100 mg/kg (ppm) residual vinyl chloride monomer in polyvinyl chloride dispersions (Wilkinson et al., 1964).

(b) Air and rain

In early May 1974, an official of the EPA estimated that US plants were discharging 90 million kg vinyl chloride monomer into the atmosphere annually (Anon., 1974c). The EPA is also reported to have found 6% losses of vinyl chloride monomer from polyvinyl chloride-producing plants and concentrations of 2.6-5.2 mg/m^3 (1-2 ppm) in the air near these plants (Anon., 1974d). On May 30 1974, that body requested US companies making vinyl chloride monomer and polyvinyl chloride to provide process, emission and air quality data on their plants for possible use in establishing air pollution control standards (Anon., 1974f).

It has been reported (US Federal Register, 1974) that the use of aerosol products in enclosed spaces, even in short bursts (e.g., 30 seconds), could result in air concentrations of vinyl chloride monomer as high as 1000 mg/m^3 (400 ppm) and that these levels could persist for several hours after spraying.

*See also p. 310

(c) Food

In May 1973, a branch of the US Treasury Department banned the use of polyvinyl chloride for the packaging of alcoholic beverages (Anon., 1973b). This action was a result of studies reported by the FDA indicating that levels of up to 20 mg/kg (ppm) vinyl chloride monomer were present in alcoholic beverages packaged in this material (Anon., 1973a).

2.3 Analysis*

Unsaturated polymerizable compounds such as vinyl chloride monomer can be separated and identified as mercuric acetate adducts by thin-layer chromatography on silica gel (Braun & Vorendohre, 1964). Methods have also been described for the separation and determination of mixtures of halogenated compounds containing vinyl chloride monomer (Hollis, 1966; Hollis & Hayes, 1962), and one is available for the determination of residual vinyl chloride monomer in polyvinyl chloride dispersions (Wilkinson et al., 1964).

Infra-red spectroscopy using a long-path cell has been used for time-weighted monitoring in factory atmospheres (Baretta et al., 1969). Although the limit of detection was given in this report as 5 ppm, another source (Stewart et al., 1965) gave limits of detection of 0.26-26 mg/m^3 (0.1 and 10 ppm) vinyl chloride monomer in breath but stated that a sample 5-10 times 10 ppm is necessary for positive confirmation of vinyl chloride monomer. The analysis made by Stewart et al. (1965) involved chromatography and infra-red spectroscopy.

Vinyl chloride monomer can be detected at levels as low as 130 mg/m^3 (50 ppm) by a Tilley Refrigerant Leak Detector Lamp (Christie et al., 1965); it can also be determined by gas chromatography (Galipern et al., 1968; Hinshaw, 1966; Popova et al., 1967) and by gas-liquid chromatography (Hollis & Hayes, 1962). Porous polyaromatic polymer bead columns have been employed to concentrate gas samples at room temperature prior to their gas-chromatographic determination (Hollis, 1966; Williams & Umstead, 1968). The presence of vinyl chloride monomer in air samples has also been determined by colorimetry (Gronsberg, 1966).

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*See also p. 310

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

3.1 Carcinogenicity and related studies in animals

(a) Inhalation and/or intratracheal administration

Mouse: A total of 360 Swiss mice (180 males and 180 females) was exposed to concentrations of 10000, 6000, 2500, 500, 250 or 50 ppm vinyl chloride monomer (VCM) 4 hours daily on 5 days per week for 30 weeks. A total of 139 mice (95 males and 44 females) died within 34 weeks; 42 had adenomas and/or adenocarcinomas of the lung, 15 had mammary adenocarcinomas and 3 had angiosarcomas of the liver. In 28 (19 males and 9 females) of the 80 male and 70 female untreated controls which died within this time, no tumours were observed. The lifetime studies were still in progress at the time of reporting (Maltoni & Lefemine, 1974a). [See also Table I.] Further details of the experiments still in progress are available (Maltoni & Lefemine, 1974b).

Angiosarcomas of the liver have also been reported in 2 mice exposed by inhalation to 50 ppm VCM in a further experiment carried out in the US (US Federal Register, 1974).

Rat: A group of 26 male Ar/IRE Wistar rats was exposed to an atmospheric concentration of 3% v/v (equivalent to 30,000 ppm) commercial-grade VCM (99% pure) 4 hours per day on 5 days per week for 12 months. Altogether 17 rats survived or were killed from 40-54 weeks after the initial exposure, at which latter time the experiment was terminated. Skin tumours developed in all 17 rats (14 epidermoid carcinomas, 2 mucoepidermoid carcinomas, 1 papilloma); in addition, lung tumours developed in 7 rats (5 adenocarcinomas, 1 adenoacanthoma, 1 squamous-cell carcinoma) and osteochondromas in 5 rats. The skin tumour development was confined to the region of the submaxillary and parotid glands, and the bone tumours developed in the metacarpal and metatarsal regions of the four limbs. No tumours were observed in 25 untreated controls killed at an unstated time (Viola et al., 1971). [Maltoni & Lefemine (1974a) examined slides from the experiment and concluded that the skin tumours were Zymbal gland tumours and that the lung tumours were metastases of these.]

TABLE 1*

Incidences of tumours in mice exposed to VCM for 30 weeks and dying within 34 weeks

Concentrations of VCM (ppm) given 4 hrs/day on 5 days/week	Total no. of animals at start	Survivors at 34 weeks	Adenomas & adenocarcinomas of the lungs	Mammary adeno-carcinoma	Angio sarcoma of the liver	Others
10,000	60	26	12	5	0	3
6,000	60	32	12	4	1	2
2,500	60	37	6	2	0	0
500	60	40	8	1	2	0
250	60	44	4	2	0	0
50	60	42	0	1	0	0
Controls	150	122	0	0	0	0

* From Maltoni & Lefemine (1974a)

Groups of 30 male and 30 female Sprague-Dawley rats, 13-21 weeks old, were exposed by inhalation to 10000, 6000, 2500, 500, 250 or 50 ppm VCM 4 hours daily on 5 days per week for a period of 17 weeks. Carcinomas of the Zymbal gland developed in 3/24 rats of the highest dose group and in 1/12 rats of the 6,000 ppm group that died before the 59th week. No tumours of this organ were observed in the 183 untreated rats which survived up to this time. Rats of the same strain (69-96 per group) were also treated for 52 weeks at the same dose levels, and the following tumours developed within 130 weeks in various organs: carcinomas of the Zymbal gland in 28/282 rats treated with the 4 highest doses and nephroblastomas (24/349 rats) and angiosarcomas of the liver (45/349 rats) among the groups treated with the 5 highest dose levels. A total of 9 angiosarcomas was observed in organs other than the liver. The liver and kidney tumours metastasized to other organs. No tumours were observed within 130 weeks in 96 rats treated with 2,500 ppm vinyl acetate 4 hours daily on 5 days a week, nor in 68 untreated controls. [See also Table 2.] In a group of 60 Sprague-Dawley rats treated with 30,000 ppm VCM 4 hours daily on 5 days a week for 43 weeks, 2 Zymbal gland carcinomas developed within 34 weeks (Maltoni & Lefemine, 1974a). Further details of this experiment still in progress are available (Maltoni & Lefemine, 1974b).

(b) Other experimental systems

Prenatal exposure: Two groups of 30 female Sprague-Dawley rats were treated by inhalation between the 12th and 18th day of pregnancy with 10,000 or 6,000 ppm VCM for 4 hours daily. Of the resulting offspring, 4/54 and 2/32, respectively, had died by the 68th week after birth, and 1 subcutaneous angiosarcoma was observed in each group. The experiment was still in progress at the time of reporting (Maltoni & Lefemine, 1974a).

3.2 Other relevant biological data

(a) Animals

In 10 male and 10 female rats exposed by inhalation to 500 ppm VCM 7 hours per day on 5 days per week for 4.5 months, an increase in liver weight, "some central lobular granular degeneration in the liver and interstitial and tubular changes in the kidneys" were reported to have occurred

TABLE 2*

Incidence of tumours in rats exposed to VCM for 52 weeks and surviving up to 130 weeks

Concentration of VCM (ppm) given 4 hrs/day on 5 days/week	Total no. of animals at start	Survivors at 130 weeks	Zymbal gland tumour	Nephro-blastoma	Angio-sarcoma of the liver	Angio-sarcoma at other sites	Others
10,000	69	-	16	4	7	0	6
6,000	72	-	7	4	13	2	1
2,500	74	-	2	6	14	3	1
500	67	-	3	4	7	2	1
250	67	1	0	6	4	2	2
50	64	3	0	0	0	0	0
Controls	68	1	0	0	0	0	0

* From Maltoni & Lefemine (1974a)

in animals killed at that time. An increase in liver weight was also noticed in rats exposed to 200 or 100 ppm VCM 7 hours daily on 5 days per week for a period of 26-29 weeks. In rabbits inhaling concentrations of 200 ppm VCM 7 hours per day on 5 days per week, for 138-144 exposures within 204 days, "central lobular granular degeneration of the liver" was observed histologically in both male and female animals. No histological changes were observed in rats, guinea-pigs, rabbits or dogs inhaling 50 ppm VCM 7 hours per day on 5 days per week for 6 months (Torkelson et al., 1961).

Viola (1970) reported degeneration of the bone and connective tissue in male Wistar rats exposed to a concentration of 30,000 ppm VCM 4 hours per day on 5 days per week for up to 12 months. These lesions are similar to the acro-osteolysis of the hand in workers exposed to VCM. Degenerative changes were observed in the liver (interstitial hepatitis, necrosis, proliferation of Kupfer cells and fibrosis), brain (neuronal and glial cell degeneration) and kidney (tubular nephrosis and chronic interstitial nephritis). Basalaev et al. (1972) also reported degenerative changes of the bone in rats and rabbits exposed to 30-40 mg/m^3 (12-15 ppm) VCM 4 hours daily for a period of up to 6 months.

(b) Man

Workers exposed to VCM have been found to develop various lesions (Anon., 1974a), including sclerotic changes of the skin, circulatory disturbances, osteolysis, thrombocytopenia, marked fibrosis of the portal tract and impaired liver function.

The occurrence of acro-osteolysis in workers engaged in the polymerization of VCM is frequently associated with Raynaud's syndrome. The acro-osteolysis is generally located in the distal phalanges of the hands, but other sites are also affected. The ages of the affected people ranged from 20-47 years and the exposure time from 5-42 months. The incidences of cases so far reported by various authors does not appear to exceed 5% of personnel involved in the polymerization of VC, and the disease seems mainly to affect autoclave cleaners (Anghelescu et al., 1969; Chatelain & Motillon, 1967; Cordier et al., 1966; Dinman et al., 1971; Harris & Adams, 1967; Lange et al., 1974a; Marin et al., 1967; Viola, 1971; Wilson et al., 1967).

Among 338 workers not involved in the cleaning of autoclaves but only in the handling of the newly-produced polyvinyl chloride (PVC), no signs of acro-osteolysis were observed (Chatelain & Motillon, 1967). No cases of acro-osteolysis were observed in a total of 1178 workers handling PVC powder (Cordier et al., 1966; Wilson et al., 1967).

In 48 workers exposed to 140-1200 mg/m^3 VCM during the production of PVC in a Russian factory, some workers were reported to have had signs of irritation of the respiratory tract and hepatitis. Twenty-three workers were found to have an increased haemoglobin level in their blood (Tribuch et al., 1949).

Suciu et al. (1967) studied 168 workers involved in the polymerization of VCM, in whom they reported narcotic symptoms, asthenic nervous symptoms, Raynaud's syndrome and liver enlargement. The incidence of Raynaud's syndrome was 6% and that of liver enlargement 30%. Lange et al. (1974a) also reported liver disturbances in 11 patients employed in the manufacture of PVC; and in 5 liver biopsies marked periportal fibrosis and slight parenchymal damage were apparent.

In an investigation of 98 men employed for up to 25 years in 2 factories in which the polymerization of VCM was carried out, it was found that the time-weighted average concentrations of VCM in the atmosphere ranged from 400 mg/m^3 (155 ppm) in 1950 to 26 mg/m^3 (10 ppm) at the end of the study, although average concentrations of 800 mg/m^3 (300 ppm) VCM had been encountered prior to the introduction of continuous monitoring in 1950. It was found that "several" individuals, after approximately 20 years of exposure to time-weighted average concentrations of 800 mg/m^3 (300 ppm) VCM in the early part of their careers, showed changes in liver function as measured by retention of bromsulphalein and by the icterus index (Kramer & Mutchler, 1972).

Twenty male autoclave cleaners working in one PVC-producing factory in the Federal Republic of Germany were subjected to a series of liver function tests, their livers were examined by laparoscopy and histological examination of liver biopsies was carried out. Of these men, 19 had elevated bromsulphalein retention times, 16 had thrombocytopenia, 7 had splenomegaly

and 6 had hepatomegaly. In almost all cases there was histological evidence of portal fibrosis, and in 14 cases fibrosis of the liver capsule was observed during laparoscopy. In addition, 4 of the workers had signs of acro-osteolysis (Marsteller et al., 1973).

3.3 Observations in man

The first association of exposure to VCM with the development of cancer was made by Creech & Johnson (1974) who reported 3 cases of angiosarcoma of the liver in men working with this substance.

By following up medical records, reviewing pathological material and by systematic medical screening, Heath et al. (1974) have discovered to date 13 such cases, including the 3 cases reported by Creech & Johnson, among men employed at 4 VCM-polymerization plants. The first case involved a worker dying in 1961. In 2 of the cases, angiosarcomas were also present in tissues other than the liver. All 13 cases have occurred in white males aged 36-60 years, with a mean age at the time of diagnosis of 48.2 years. The length of time between first exposure to VCM and diagnosis of the tumour ranged from 12-29 years (mean, 20.3 years), and the mean total duration of work involving exposure to VCM was 18 years.

Heath et al. (1974) reported that data from the National Cancer Institute's Third National Cancer Survey (1969-71) indicate that only 25-30 cases of angiosarcoma of the liver should be expected each year among the entire US population. These authors also estimated the total VCM work population in the US, past and present, to be about 20,000. On this basis, the number of cases which would be predicted to occur over a period of 10 years would be 0.03. The ratio of the observed to expected number of cases (relative risk) is thus in the order of 400. However, at the plant most fully investigated, which at present has a work force of 270 men directly engaged in VCM polymerization activities, 7 of the 13 cases have been found. For these 7 cases the authors found no evidence to show that exposure to other potentially hepatotoxic material outside the plant played a role in the development of this tumour, and there was no history of acro-osteolysis.

At the same plant, medical records were reviewed for cases of non-malignant liver diseases, and a total of 4 such cases has been identified

to date, all in workers exposed to VCM. All were white males, and the average age was 46.5 years at the time of first diagnosis; work involving exposure to VCM began on average 20.8 years prior to diagnosis. Liver biopsies were performed in all 4 cases, and portal fibrosis was seen in each, accompanied by the observation in 3 cases of discrete nodules of subcapsular fibrosis. The latter feature was pathologically indistinguishable from the subcapsular lesions observed in 1 of the 7 tumour cases.

Suciu et al. (1967), Lange et al. (1974a) and Marsteller et al. (1973) have reported similar disorders, including periportal fibrosis, splenomegaly, hepatomegaly, slight parenchymal damage and fibrosis of the liver capsule in workers exposed to VCM (see also pp. 302-304).

In Western Europe 3 cases of fatal angiosarcoma of the liver have so far been reported, 2 in the Federal Republic of Germany (Lange et al., 1974b) and 1 in the UK (Lee & Harry, 1974). All 3 workers had been involved in VCM polymerization processes, and exposure to VCM ranged from 11-20 years.

4. Comments on Data Reported and Evaluation*

4.1 Animal data

Vinyl chloride monomer (VCM) is carcinogenic in mice and rats following exposure by inhalation. The tumours in mice were mainly lung tumours, mammary carcinomas and angiosarcomas (malignant haemangioendotheliomas) of the liver. Angiosarcomas of the liver and other organs, Zymbal gland carcinomas and nephroblastomas occurred in exposed rats. Preliminary studies have suggested that VCM also produces subcutaneous angiosarcomas in the offspring of rats that have been exposed during pregnancy.

4.2 Human data

In view of the extreme rarity of angiosarcoma of the liver in the general population, the observation of 16 cases in workers exposed to vinyl chloride monomer during the polymerization process is evidence of a causal relationship.

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* This evaluation is based upon published data or data accepted for publication up to 26 June 1974.

APPENDIX A

VINYL CHLORIDE POLYMERS

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: PM 9002-86-2

Chloroethylene polymer; polyvinyl chloride; polyvinylchloride; PVC

Bakelite*; Blacar; Boltaron; Breon; Carina; Corvic; Dacovin; Ekavyl; Etinox; Flamenol; Flovic; Geon*; Halvic; Hostalit; Kaylite; Kayrex; Koroseal; Lacqvyl; Lucoflex; Lucovyl; Marvinol; Niacet; Norvinyl; Opalon; Pevikon; Plioflex; Pliovic; Quirvil; Ravinyl; Rhodopas; Rhovyl; Rucoblend; Ryertex - Omicron; Scon; Seilon PVC; Sicron; TPC; ttp; Ultron; Upalon; Vestolit; Vinnol; Vinoflex; Vipla; Viplavil; Vybak; Vycell; Vygen; Vyram; Vyron; Welvic; Yardley

1.2 Chemical formula and molecular weight

$\{CH_2CHCl\}_n$ Mol. wt: 60,000-150,000

1.3 Chemical and physical properties of the pure substance

(a) Description: White powder

(b) Stability: Polyvinyl chloride is relatively unstable to heat and light in the absence of added stabilizers. HCl gas is a decomposition product of degradation.

(c) Solubility: Solvents for unmodified polyvinyl chloride of high molecular weight are: cyclohexanone, methyl cyclohexanone, dimethyl formamide, nitrobenzene, tetrahydrofuran, isophorone and mesityl oxide. Solvents for lower polymers are: dipropyl ketone, methyl amyl ketone, methyl isobutyl ketone, acetonylacetone, methyl ethyl ketone, dioxane and methylene chloride (Merck & Co., 1968).

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* These trade names are also used for other types of polymers.

1.4 Technical products and impurities

A wide variety of vinyl chloride homo- and co-polymers is available, with varying properties designed for specific applications. Consequently, the specifications vary widely.

Polyvinyl chloride resins for the production of rigid plastics contain essentially no plasticizer: the polymer may be a homo-polymer or a co-polymer made with low levels of co-monomers such as acrylates, vinyl acetate or ethylene. The co-monomers are used to aid in the processing of the resulting polymer.

Most of the flexible and semi-rigid polyvinyl chloride currently used contains plasticizer, generally at a level of 10-100% of the resin weight. The plasticizers most commonly used are dialkyl esters of dibasic aliphatic acids, such as dioctyl phthalate. Polyester plasticizers (e.g., adipic acid-glycol polyesters) and epoxy plasticizers (e.g., epoxidized soybean oil) are also widely used.

Other compounding materials, such as pigments, fillers and light- and heat-stabilizers, are also used in the production of commercial polyvinyl chloride.

Polyvinyl chloride resins are also used in the form of plastisols (polyvinyl chloride resin dispersed in plasticizer), organosols (polyvinyl chloride resin dispersed in plasticizers and a balanced mixture of solvents and diluents) and lattices (polyvinyl chloride resin dispersed in water with small amounts of surfactants).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

A method for the synthesis of polyvinyl chloride was reported in 1872 (Baumann, 1872). Commercial homo-polymers of vinyl chloride were introduced in 1933 (Darby & Sears, 1968).

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

Vinyl chloride polymers are presently produced by one of four processes: suspension, emulsion, bulk or solution polymerization. All are free-radical, exothermic processes. In the US in 1972 approximately 78% of homo-polymer and co-polymer output was produced by the suspension polymerization process.

In 1972, 22 US manufacturers produced 1,962 million kg of polyvinyl chloride resins of all types (US Tariff Commission, 1974a); it is estimated that approximately 685 million kg of the total were co-polymers. Preliminary data indicate that 1973 production amounted to 2,008 million kg (US Tariff Commission, 1974b).

US exports of all polyvinyl chloride resins (homo-polymer and co-polymer combined) are estimated to have been 69 million kg in 1972; imports were negligible.

More than 30 companies in Western Europe manufacture vinyl chloride polymers in plants located in Austria, Belgium, the Federal Republic of Germany, Finland, France, Greece, Italy, The Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom. Total Western European production was reported to have been 2,290 million kg in 1970 and 1971 (Organisation for Economic Cooperation & Development, 1972), and it is estimated to have been approximately 2,950 million kg in 1972.

Polyvinyl chloride resins are produced by 20 companies in Japan. Production in 1972 was approximately 1,078 million kg; in the same year, imports were 22 million kg, while exports totalled 49 million kg (Anon., 1973c).

Apart from these three major producing areas there are also about 20 producing facilities in Eastern Europe, namely in Bulgaria, Czechoslovakia, the German Democratic Republic, Hungary, Poland, Rumania, the USSR and Yugoslavia. About 15 companies produce polyvinyl chloride in Argentina, Brazil, Chile, Colombia, Mexico and Venezuela; there are about 20 companies in Formosa, India, Iran, Israel, Pakistan, the Philippines, Thailand and Turkey; and in Africa there are 4 companies, located in Algeria, Egypt and South Africa. Polyvinyl chloride is also produced in Canada and Australia (Anon., 1971).

In 1972, US consumption of polyvinyl chloride resins was as follows: building and construction industries (42%), household uses (15%), consumer goods (12%), electrical applications (11%), packaging (9%) and transportation (6%), with miscellaneous uses accounting for the remainder. Since 1968, the major uses have been in the building and construction industries and in household goods and packaging.

The major uses for polyvinyl chloride resins in building and construction industries are in piping and conduits, including water pipes, in flooring, in windows and other rigid structures, in pipe fittings, in sidings and as swimming-pool liners. The primary applications in household products are for upholstery, wall coverings, garden hoses and appliances. Consumer goods utilizing polyvinyl chloride resins include gramophone records, footwear, toys, outerwear, sporting goods and baby pants. Electrical applications consist primarily of wire and cable coatings. The major uses of polyvinyl chloride in packaging are in film, sheets, bottles, coatings and bottle-cap liners and gaskets; however, in the US, its use for the packaging of alcoholic beverages has been banned because of migration of vinyl chloride monomer into the alcohol. In the transportation industry major uses include upholstery and seat covers, automotive tops and automotive floor mats. Miscellaneous applications include its use in laminates, in medical tubing and in stationery supplies.

Of the total UK production in 1973 (400 million kg) the use of polyvinyl chloride in millions of kg for various applications was reported as follows: pipes and fittings for water pipes and electrical conduits - 100 million kg; electrical cables - 46 million kg; hard flooring - 30 million kg; packaging (foil and film) - 24 million kg; bottles - 13 million kg; bottle closures - 5 million kg; gramophone records - 21 million kg; car upholstery, roof interiors and wirings - 20 million kg; footwear - 20 million kg; conveyor belting - 6 million kg; total - 285 million kg (Anon., 1974e).

In Japan in 1972, polyvinyl chloride consumption is estimated to have been as follows: piping (28%), film (15%), fittings (11%), plate (9%), sheet (9%), wire and cable coatings (8%), leather (7%) and extruded products (6%).

2.2 Occurrence

Vinyl chlorine polymers are not known to occur in nature.

2.3 Analysis

No information was available to the Working Group.

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* Footnote to pp. 296, 297

At the time of submitting this volume to press, the Occupational Safety and Health Administration of the US Department of Labor had proposed a new permissible exposure limit for vinyl chloride monomer which will require that no employee may be exposed to concentrations greater than 2.6 mg/m^3 (1 ppm) averaged over any 8-hour period, and that no employee may be exposed to concentrations greater than 5 ppm averaged over any period not exceeding 15 minutes. The method of monitoring and measurement should have an accuracy of not less than ±50% from 0.25-0.5 ppm, ±35% from 0.5-1.0 ppm and ±25% over 1 ppm; such methods are available in the "NIOSH Manual of Analytical Methods". The effective date of the amendment to section 1910.93q of the US Code of Federal Regulations will be 1 January 1975 and the permissible exposure limit will apply to fabrication, monomer and polymer industries (US Federal Register 1974, 39 (no. 194), 35890-35898).

APPENDIX B

VINYL CHLORIDE-VINYL ACETATE CO-POLYMERS

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. No.: PM 9003-22-9

VA/VCl; VAc/VCl; PVAc/VCl

Airflex*; Bakelite*; Gelva*; Resyn*; Vinyon; Vipla

1.2 Chemical formula and molecular weight

$\{CH_2CHCl\}_n$ in combination with $\{CH_2CH\}_n$
$OOCCH_3$

Mol. wt: approx. 100,000

1.3 Chemical and physical properties of the pure substance

(a) Description: White powder

(b) Stability: Vinyl chloride-vinyl acetate co-polymers are relatively unstable to heat and light in the absence of added stabilizers. HCl gas is a decomposition product of degradation.

1.4 Technical products and impurities

Low levels of vinyl acetate are co-polymerized with vinyl chloride monomer to obtain specific properties for different applications in the resulting polymer. Depending upon the potential use destined for the polymer, the vinyl acetate level may vary from 2-20%, with an average of 11-12%.

Vinyl chloride-vinyl acetate resins are available both as solid resins and as emulsions. Solid resins, for the formulation of rigid plastics, contain little or no plasticizer; however, when they are formulated into products they generally contain pigment, filler and light- and heat-

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* These trade names are also used for other types of polymers.

stabilizers. Flexible and semi-rigid vinyl chloride-vinyl acetate resins contain plasticizers, e.g., dibasic aliphatic acid esters, polyesters and epoxy plasticizers.

Vinyl chloride-vinyl acetate polymer emulsions usually consist of neutral polymer dispersions in water, containing 50-60% solids and including small amounts of surfactants. Vinyl chloride-vinyl acetate co-polymers are also used in the manufacture of organosols (co-polymers dispersed in plasticizer and a balanced mixture of solvents and diluents) and of plastisols (co-polymers dispersed in plasticizer).

2. Production, Use, Occurrence and Analysis

2.1 Production and use[1]

Vinyl chloride-vinyl acetate co-polymers were introduced commercially in 1934 (Darby & Sears, 1968). Vinyl chloride-vinyl acetate dispersion co-polymers are manufactured by free-radical-initiated suspension and emulsion polymerization techniques. Solution polymerization is used for the manufacture of some special coating resins. It is believed that in the US approximately 78% of vinyl chloride-vinyl acetate co-polymer resins are made by suspension processes.

It was estimated that in 1971 the 15 US manufacturers produced 281 million kg of vinyl chloride-vinyl acetate co-polymer resin.

More than 10 companies in Western Europe manufacture vinyl chloride-vinyl acetate co-polymers in plants located in the Federal Republic of Germany, France, Italy, Switzerland and the United Kingdom. Total Western European production in 1972 is estimated to have been approximately 400 million kg.

In Japan there are 9 producers of vinyl chloride-vinyl acetate co-polymers. Approximately 11.8 million kg of co-polymer were manufactured in 1972, and 1.1-2.3 million kg of vinyl chloride-vinyl acetate solution co-polymers were imported from the US.

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

In the US the major use for vinyl chloride-vinyl acetate co-polymers is in the production of calendered flooring and of gramophone records. Other applications include injection molding, rigid sheet production and special coatings.

Co-polymers containing 2-8% acetate are used for calendering, since they have better processing characteristics than did the vinyl chloride homo-polymers available in the past. Calendered products include film and sheeting, floor tiles and coated fabrics. The better homo-polymers developed in recent years are competing with the co-polymers in the rigid sheet and floor-tile markets.

Co-polymers containing 10-16% acetate are used for compression molding of gramophone records because of their good flow properties. Some of these co-polymers are also used in injection molding for pipe fittings and industrial parts, and they are also used in blends with vinyl chloride homo-polymers for rigid extrusions (e.g., piping and siding).

Minor quantities of co-polymer dispersion resins are believed to be used in the form of latex for special applications (e.g., vinyl wall coverings). Solutions of co-polymers containing 8-12% acetate in solvents such as cyclohexanone and tetrahydrofuran are used in surface coatings of tins and metals, and for maintenance coatings.

Small amounts of the co-polymers are also used to produce specialty fibres: in 1971, the 2 US producers of such fibres are estimated to have manufactured approximately 1.36 million kg. This consisted mostly of staple (used in making heat-sealable papers, e.g., for tea bags), but some mono-filaments (used in automobile interiors) were also produced.

In Japan, vinyl chloride-vinyl acetate co-polymers are used for the manufacture of flooring, coatings, adhesives and gramophone records.

2.2 Occurrence

Vinyl chloride-vinyl acetate co-polymers are not known to occur in nature.

2.3 Analysis

No information was available to the Working Group.

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CORRIGENDA TO VOLUMES 1 - 6

Volume 1

p.5			*insert after line 6* Mrs I. Peterschmitt, Unit of Chemical Carcinogenesis
p.47	3.3(b)	line 2	*replace* 425 persons *by* 267 persons
	3.3(b)	line 4	*replace* associated with lead *by* related to lead
p.72	3.3(b)	line 4	*after* certificates *add* mentioning bladder tumours
	3.3(b)	line 4	*replace* 0.13 *by* 0.45
	3.3(b)	line 5	*delete from* The morbidity was.... *to the end of the para*
p.145	1.1	line 8	*insert* α *after* 7a *and after* 10a
p.160	3.1(a)	line 4	*replace* 13/3 *by* 13/13
p.161	3.1(c)	line 4	*replace* intraperitoneal *by* subcutaneous

Volume 2

p.20			*after the table replace* Morgan & Cralley[MC] (1963) *by* Morgan & Cralley[MC] (1973)
p.35	4.1	line 5	*after* less than 0.5 μm diameter and *add* more than
p.42	reference 10		*after* Gilson, J.C. *add* Timbrell, V.
p.138	para 2	line 3	*replace* 19/21 *by* 19/121
p.174	4.1 para 2	line 2	*replace* Iron-dextran *by* Iron-dextrin

Volume 4

p.7	title		*replace* of carcinogenic *by* of the carcinogenic
		line 2	*replace* of carcinogenic *by* of the carcinogenic
p.23			*after reference 3 add* Veys, C.A. (1972) Aromatic amines - the present status of the problem. Ann. occup. Hyg., 15, 11-15

Volume 4 (cont'd)

p.27	1.3(e)	*replace* d *by* n
p.57	1.2(a)	*in the chemical formula replace* $_{18}$ *by* $_{19}$
p.58	1.2(b)	*in the chemical formula replace* $_{16}$ *by* $_{17}$
	1.2(c)	*in the chemical formula replace* $_{20}$ *by* $_{21}$
p.61	2(b)	*replace para 2 by* In the UK Carcinogenic Substances Regulations 1967 Statutory Instrument (1967) No. 879, magenta is listed as a controlled substance in relation to the protection of persons engaged in its manufacture.
p.67	lines 6 & 7	*delete*
p.83	3.1(b) line 10	*delete from* In a later study... *to end of para*
P.145	1.1	*replace* 54-07-3 *by* 540-73-8

Volume 5

p.22	reference 32	*replace* Abbott, D.t. *by* Abbott, D.C.
p.84	line 2	*after* dichloroethane *add* tetrachlorodiphenylethane
p.97	table	*in last line 3rd column replace* 69/90 *by* 60/90

Volume 6

p.239		*replace* BHC (technical trades) *by* BHC (technical grades)

CUMULATIVE INDEX TO IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISK OF CHEMICALS TO MAN

Numbers underlined indicate volume, and numbers in italics indicate page. References to corrigenda are given in parentheses.