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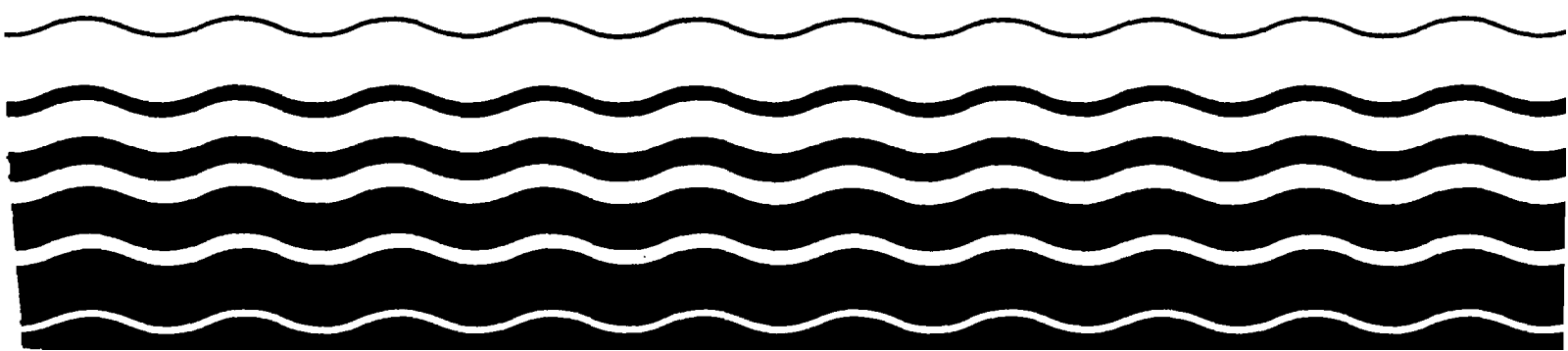
Ambient Water Quality Criteria for Nitrosamines



AMBIENT WATER QUALITY CRITERIA FOR

NITROSAMINES

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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ACKNOWLEDGEMENTS

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NITROSAMINES

CRITERIA

Aquatic Life

The available data for nitrosamines indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,850 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive freshwater aquatic life.

The available data for nitrosamines indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 3,300,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of N-nitrosodiethylamine and all other nitrosamines except those listed below, through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 8.0 ng/l, 0.8 ng/l, and 0.08 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 12,400 ng/l, 1,240 ng/l, and 124 ng/l, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of N-nitrosodimethylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 14 ng/l, 1.4 ng/l, and 0.14 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 160,000 ng/l, 16,000 ng/l, and 1,600 ng/l, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of N-nitrosodibutylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 64 ng/l, 6.4 ng/l, and 0.64 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 5,868 ng/l, 587 ng/l, and 58.7 ng/l, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of N-nitrosopyrrolidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time.

Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 160 ng/l, 16 ng/l, and 1.6 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 919,000 ng/l, 91,900 ng/l, and 9,190 ng/l, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of N-nitrosodiphenylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 49,000 ng/l, 4,900 ng/l, and 490 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 161,000 ng/l, 16,100 ng/l, and 1,610 ng/l, respectively.

INTRODUCTION

The nitrosamines belong to a large group of chemicals generally called N-nitroso compounds. Also included in this group are the structurally-related nitrosamides. Because they frequently coexist with N-nitrosamines in the environment, nitrosamides are addressed also in this document.

Synthetic production of N-nitrosamines is limited to small quantities, and the only nitrosamine produced in quantities greater than 450 kg/yr is N-nitrosodiphenylamine. It is used as a vulcanizing retarder in rubber processing and in the manufacture of pesticides. The general physical properties of diphenylnitrosamine are: molecular weight, 198.24 and a melting point of 66.5°C (Tanikaga, 1969). Other N-nitroso compounds are produced primarily as research chemicals and not for commercial purposes (U.S. EPA, 1976).

Nitrosamines are characterized by the functional group -N-N=O and nitrosamides are characterized by the functional group -C-N-N=O. Depending on the nature of the radical group, nitrosamines exist in several forms, including symmetrical dialkyl-nitrosamines, asymmetrical dialkyl-nitrosoamines, nitrosamines with functional groups, cyclic nitrosoamines and acylalkylnitrosamines with functional groups, cyclic nitrosamines and acylalkylnitrosamines or nitrosamides (Searle, 1973).

The nitrosamines vary widely in their physical properties and may exist as solids, liquids, or gases. They are soluble in water and organic solvents. Nitrosamines of low molecular weight are volatile at room temperature, and high molecular weight nitrosamines are steam volatile (U.S. EPA, 1976).

The most significant source of N-nitrosamines and N-nitrosamides in the environment is probably nitrosation of amine and amide precursors (Bogovski, et al. 1972). These reactions may occur in air, soil, water, food, and animal systems, when the precursors are present simultaneously (Mysliwy, et al. 1974; Fine, et al. 1977b; Rounbehler, et al. 1977; Mills, 1976). The extent of exposure to the general population of N-nitrosamines and N-nitrosamides is unknown. The most significant exposures, resulting from anthropogenic sources, are probably restricted to limited industrial areas (Fine, et al. 1977a,b,c).

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Aquatic Life Toxicology*

INTRODUCTION

The data base is limited to three fish and two invertebrate species; three acute tests with N-nitrosodiphenylamine were conducted using static tests and unmeasured concentrations. Feeding studies with N-nitrosodimethylamine and rainbow trout demonstrated a dose-related carcinogenic response. This response is similar to dose-related effects with mammals and numerous nitrosamines, including N-nitrosodimethylamine. Details of these later studies are available in the human health effects portion of this document. An additional study with a crayfish showed extensive degeneration of the antennal gland and other effects after a 6-month exposure to the same compound.

EFFECTS

Acute Toxicity

The acute value of n-nitrosodiphenylamine for Daphnia magna and the bluegill is 7,760 µg/l and 5,850 µg/l, respectively, (Table 1). This latter result is significantly different from that for the mummichog, a saltwater species, for which the 96-hour LC_{50} for N-nitrosodiphenylamine is 3,300,000 µg/l (Table 1). No explanation for this difference is apparent.

Residues

Bioconcentration of N-nitrosodiphenylamine by the bluegill (U.S. EPA, 1978) reached steady-state within 14 days and the bioconcentration factor was 217 (Table 2). Depuration rate was rapid so that the half-life of this compound in the tissues was less than 1 day.

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

Miscellaneous

Grieco, et al. (1978) fed Shasta strain rainbow trout N-nitrosodimethylamine in the diet for 52 weeks (Table 3). After this time the fish were placed on a control diet for an additional 26 weeks. No hepatocellular carcinomas were detected at 26 weeks after feeding began. At 52 weeks, however, a direct dose-related response of hepatocellular carcinoma occurred in trout fed 200, 400, and 800 mg dimethylnitrosamine/kg. A greater incidence of carcinomas was observed at 78 weeks, even though feeding was discontinued after 52 weeks. For further information and details on mammalian carcinogenesis of nitrosamines, the reader is referred to the human health effects portion of this document.

Another study, by Harshbarger, et al. (1971), exposed the crayfish, Procambarus clarkii, for 6 months to N-nitrosodimethylamine under renewal procedures. Microscopical studies revealed extensive degeneration in all parts of the antennal gland at 200,000 µg/l and hyperplasia of the tubular cells in the hepatopancreas at 100,000 µg/l.

Summary

Daphnia magna and the bluegill are the tested freshwater species with acute values for N-nitrosodiphenylamine of 7,760 and 5,850 µg/l, respectively. These results are quite different from that for the saltwater mummichog for which the acute value is 3,300,000 µg/l. The bluegill bioconcentrated the same compound to a factor of 217, but the tissue half-life was less than one day.

Chronic feeding studies with rainbow trout and N-nitrosodimethylamine demonstrated a dose-related response of hepatocellular carcinoma over a feeding range of 200 to 800 mg/kg. An aqueous exposure of crayfish to the same compound resulted in extensive antennal gland degeneration and other effects at concentrations of 100,000 to 200,000 µg/l.

CRITERIA

The available data for nitrosamines indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,850 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive freshwater aquatic life.

The available data for nitrosamines indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 3,300,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive saltwater aquatic life.

Table 1. Acute values for nitrosamines

Species	Method*	Chemical	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
		FRESHWATER SPECIES			
Cladoceran, *Daphnia magna*	S, U	N-nitroso-diphenylamine	7,760	7,760	U.S. EPA, 1978
Bluegill, *Lepomis macrochirus*	S, U	N-nitroso-diphenylamine	5,850	5,850	U.S. EPA, 1978
		SALTWATER SPECIES			
Mummichog, *Fundulus heteroclitus*	S, U	N-nitroso-diphenylamine	3,300,000	3,300,000	Ferraro, et al. 1977

* S = static, U = unmeasured

Table 2. Residues for nitrosamines (U.S. EPA, 1978)

Species	Tissue	Chemical	Bioconcentration Factor	Duration (days)
		FRESHWATER SPECIES		
Bluegill, *Lepomis macrochirus*	whole body	N-nitroso-diphenylamine	217	14

Table 3. Other data for nitrosamines

Species	Chemical	Duration	Effect	Result	Reference
		FRESHWATER SPECIES			
Crayfish, *Procambarus clarkii*	dimethylnitrosamine (N-nitrosodimethylamine)	6 mos	Antennal gland degeneration and hyperplasia of hepatopancreas	100,000-200,000 µg/l	Harshbarger, et al. 1971
Rainbow trout, *Salmo gairdneri*	dimethylnitrosamine (N-nitrosodimethylamine)	78 wks	Dose-related hepatocellular carcinomas	Feeding in diet at 200-800 mg/kg	Grieco, et al. 1978

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INTRODUCTION

The N-nitrosamines represent one group of those organic compounds characterized by a nitroso group (-N=O) attached to a nitrogen (N-nitroso compounds). Closely related to the N-nitrosamines are the N-nitrosamides. The formation of both groups of compounds from precursors in the environment, and in the animal or human body, occurs through a common mechanism (nitrosation). Both groups of compounds are typically highly toxic, again probably through common mechanisms. It is extremely unlikely that the human population would be exposed only to nitrosamines or only to nitrosamides since the precursors of both generally occur together. Thus, although this document is intended to refer specifically to N-nitrosamines, it has been considered prudent to follow the precedent of earlier literature (in which the term "nitrosamines" is frequently used synonymously with N-nitroso compounds) and to include some discussion of the N-nitrosamides.

It has also not proven possible to treat the health effects of N-nitrosamines without considering sources of both preformed N-nitrosamines and their precursors.

Sources of and Routes of Exposure to N-nitroso Compounds

Exogenous Sources: N-nitrosamines are widespread in the environment. Concentrations in the nanogram to microgram per unit volume or mass range have been recorded in air, water, soil, plants, and foodstuffs (Fine, et al. 1977a). Synthetic production is limited to small quantities: N-nitrosodiphenylamine is the only nitrosamine produced in quantities greater than 450 kg/yr. Other N-nitroso compounds are produced primarily as research chemi-

cals and not for commercial purposes (Walker, et al. 1976). The most probable source of environmental N-nitrosamines and N-nitrosamides is nitrosation of amine and amide precursors (Bogovski, et al. 1972).

Both nitrosating agents and nitrosatable compounds are ubiquitous in the environment from natural and man-made sources. The most widespread form of inorganic nitrogen is nitrate. Nitrate is a common constituent of plants and is the primary form which plants absorb from the soil. Nitrite is found only in low concentrations because of its greater reactivity. However, nitrate is readily converted to nitrite by microbial reduction, and, according to some evidence (Klubes and Jondorf, 1971), bacteria are capable of promoting the synthesis of nitrosamines from a secondary amine and nitrate without conversion of the latter to nitrite. Oxides of nitrogen may also act as nitrosating agents. It has been estimated that 20.7×10^9 kg of nitrogen oxides were emitted from industrial, commercial, and domestic sources in the United States during 1970 (U.S. EPA, 1977).

Nitrosatable compounds occur in great variety. Some are ubiquitous in nature either as components of living organisms (for example, amino acids such as proline, tryptophan, and arginine; cyclic amines such as purines and pyrimidines) or as products of the anaerobic decay of protein-rich organic matter (amines, ureas, etc.). Many agricultural chemicals are nitrosatable amino compounds (for example, the antisuckering agent, dimethyldodecylamine; the methylcarbamate insecticides). Amines are emitted from coking plants and petroleum refineries and, together with other forms of combined nitrogen, including nitrates, from sewage treatment plants, etc. Industrial amine production has been reviewed and summarized by Walker, et al. (1976).

Nitrosation of amide or amine precursors may occur in the air, soil, water and in some stored or preserved foods. The major requirement is probably the simultaneous presence of precursors (Mills, 1976).

Endogenous Sources: There is now conclusive evidence that nitrosation of amines and amides even in trace concentrations occurs in the gastrointestinal tract of both animals and man (Mysliwy, et al. 1974; Fine, et al. 1977b; Rounbehler, et al. 1977).

Nitrate may be ingested in the food, mainly as a preservative in cured meats. It can originate in the body from reduction of nitrate by bacteria containing the enzyme nitrate reductase. The major site is the oral cavity by bacterial reduction of nitrate in ductal saliva (Tannenbaum, et al. 1974), although other sites have been demonstrated or proposed, including the stomach, in human subjects with gastric hypoacidity (Sander and Schweinsberg, 1972), and the infected urinary bladder (Hawksworth and Hill, 1974). Recent studies (Tannenbaum, et al. 1978a) indicate that nitrite is also formed de novo in the upper portion of the human intestine, probably from ammonia or organic nitrogen compounds. As material passes through the intestine, some nitrite is converted to nitrate. Absorbed nitrate is recycled into saliva via the salivary glands, the stomach via the parietal glands, and the bladder via the urine. Absorbed nitrite is rapidly destroyed in the blood.

The amount of nitrosamine formed at any site is affected by many factors such as nucleophilicity of the amine, substrate concentration, and pH. A detailed discussion is provided by Mirvish (1975). Conditions in the stomach of monogastric animals following a meal (pH range 1 to 5) particularly favor nitrosation. Tannenbaum, et al. (1978a) suggest that nitrite originating in the intestine may react to form N-nitroso compounds in the cecum

and colon, which are relatively more acidic than the small intestine. Nitrosamine formation has also been shown to be possible in saliva even at neutral pH, although the amount formed is small (Tannenbaum, et al. 1978b). Some substances, such as thiocyanate, increase the rate of nitrosamine formation (Boyland, et al. 1971). Thiocyanate occurs in saliva, especially that of smokers, and in gastric juice. Others, such as ascorbic acid, inhibit the reaction (Mirvish, et al. 1972).

The situation with regard to inhaled potential nitrosamine precursors is considerably more speculative. Nitrous acid is rapidly formed when a mixture of nitric oxide (NO), nitrogen dioxide (NO_2), and water interact in systems of high surface-to-volume ratio (Wayne and Yost, 1951; Graham and Tyler, 1972). It therefore seems reasonable to expect that if these gases are inhaled as pollutants of ambient air, they will rapidly equilibrate in the lung to form nitrous acid. The neutral, buffered pH of the lung is not normally regarded as favorable to formation of N-nitroso compounds (although, as indicated above, nitrosamine formation in saliva has been observed at neutral pH). However, it has been suggested (U.S. EPA, 1976) that if nitric acid, sulfuric acid, or other common atmospheric acidic pollutants were inhaled in sufficient amount to produce a local acidity within the respiratory tract, nitrosation could occur by interaction between inhaled nitrogen oxides and tissue amines and amides. It is also said (U.S. EPA, 1976) to be theoretically possible for all the precursors necessary for nitrosamine formation to be generated in acid aerosol droplets in an atmosphere containing significant amounts of nitrogen oxides, sulfur oxides, and ammonium ion.

It is evident that the human population is exposed to both preformed N-nitroso compounds in the environment and to similar compounds formed endo-

genously from precursors in the environment. Assessment of the relative significance of various exposure pathways is clearly invalid unless both "nitrosamines" and their precursors are considered.

Ingestion from Water

Precursor chemicals of nitrosamines are ubiquitous in soils and water. The concentration of simple aliphatic amines is normally low (nanogram-to-milligram per kilogram amounts) since they are rapidly metabolized by microorganisms [National Academy of Sciences (NAS), 1978]. Many pesticides have been shown to be nitrosatable, and some, such as atrazine, are only slowly degraded and persist in soil and water. Nitrite concentrations in soil and water are normally low ($\leq$1 mg/kg nitrite N). However, the concentrations of nitrite (and its precursors, ammonia and nitrate) and nitrosatable compounds can be much greater in soils heavily fertilized with organic waste matter or in waters receiving runoff from agricultural areas or discharges of industrial or municipal wastewater containing substantial amounts of amines. Levels of nitrate in municipal drinking waters in the United States seldom exceed 10 mg/l nitrate N, although some smaller water supplies and private wells contain much more nitrate. Concentrations as high as 100 to 500 mg/l of nitrate N have been reported in polluted wells (NAS, 1977).

It has been amply demonstrated that nitrosamines are formed in soils, water, and sewage after addition of relatively large amounts of secondary or tertiary amines and nitrite or nitrate (Ayanaba, et al. 1973; Ayanaba and Alexander, 1974). N-nitrosodimethylamine has been found in a number of soil samples (Fine, et al. 1977c) at the 1 to 8 µg/kg (dry basis) level. Fine, et al. (1977c) speculate that this may have arisen from absorption of preformed nitrosodimethylamine from the air or absorption of dimethylamine with subsequent nitrosation. Another possible source is pesticide application.

Several pesticides (carbamates and N,N-disubstituted amides) have been shown to yield nitrosodimethylamine upon nitrosation (Mirvish, 1975). Others, such as the phenoxyacetic acid derivatives, are formulated as amine salts; some commercial preparations have been found to contain as much as 0.06 percent nitrosodimethylamine as a contaminant (Fine, et al. 1977a). Nitrosamines are readily leached through the soil profile by percolating water and thus may eventually contaminate surface and ground waters if formed in the soil (Dean-Raymond and Alexander, 1976). These authors have also found N-nitrosodimethylamine to be taken up from soil by spinach and lettuce; the percentage taken up from the soil varied from 0.02 to 5.1 with the experimental conditions. However, under natural conditions, nitrosamines are not commonly found in plants.

Significant concentrations of nitrosamines have been reported for a limited number of samples of ocean water, river water, and waste treatment plant effluent adjacent to or receiving wastewater from industries using nitrosamines or secondary amines in production operations. Nitrosodimethylamine has been reported at the 3 to 4 µg/l level in waste water samples (Fine, et al. 1977c). To what extent the nitrosamine arose from impurities in the amine process or from nitrosation in the waste treatment plant is not known. In water samples from wells characterized by both high nitrate levels and coliform counts, the concentration of volatile and nonvolatile nonionic nitrosamines was less than 0.015 µg/l (U.S. EPA, 1977). Volatile nitrosamines have not been detected in drinking water (Fine, et al. 1975). However, there is a unconfirmed report indicating existence of nonvolatile nitrosamines (including N-nitrosoatrazine) in New Orleans water at levels of 0.1 to 0.5 µg/l (Fine, et al. 1976).

Nitrosamines are rapidly decomposed by photolysis and do not persist for a significant time in water illuminated in sunlight. Thus, it is unlikely that they will be present in high (greater than 1 mg/l) concentrations in surface waters. However, in the absence of light they can be expected to persist (Tate and Alexander, 1976). No degradation of N-nitrosodimethylamine, N-nitrosodiethylamine, or N-nitrosodipropylamine was observed in lake water during a 3.5 month period (Tate and Alexander, 1975). Fine, et al. (1977a) have shown that nitrosodimethylamines can exist for extended periods of time in the aquatic environment.

Ingestion from Food

Many food constituents are either directly capable of conversion to N-nitroso compounds or give rise through chemical action or metabolic processes to nitrosatable products. Walters (1977) has listed some of these compounds. Amino acids such as proline, hydroxyproline, tryptophan, arginine, etc., are nitrosatable. The action of heat on other amino acids can give rise to degradation products, such as pipecolic acid, containing secondary amino groups. There is no evidence that proteins are nitrosated directly, but they release nitrosatable amino acids during food processing or digestion. Walters (1977) suggests that prolyl peptides may be more readily nitrosated than proline itself. A number of other tissue components, such as choline and phospholipids, contain tertiary amines and quaternary ammonium groups which can be dealkylated to secondary amines. Many of the purine and pyrimidine bases of the nucleic acids contain amino groups capable of forming N-nitroso derivatives, as do some vitamins, for example folic acid. Other nitrosatable compounds include caffeine in coffee, amines in tea, and orotic acid in milk. Some pesticides (for example, atrazine, carbaryl, fer-

bam, simazin) are nitrosatable, and hence their release in or on food represents another source of precursors of N-nitroso compounds (Elsperu and Lijinsky, 1973).

Nitrate and nitrite are also well supplied in the diet. The mean intake in food of nitrate plus nitrite in the United States has been calculated to be approximately 120 mg per day (White, 1975), although there must be considerable individual variability. According to these estimates, 86 percent of the nitrate comes from vegetables such as celery, potatoes, lettuce, melons, cabbage, spinach, and root vegetables; some, such as spinach and beets, contain 2,000 to 3,000 ppm of nitrate. Cured meat supplies nine percent of the nitrate. Only 0.2 percent of the nitrite is supplied by vegetables; 21 percent comes from cured meat (White, 1975).

Nitrate is secreted in the saliva, the mean amount being approximately 40 mg per day. Of this, about 10 mg per day is reduced to nitrite in the mouth by the oral flora (Tannenbaum, et al. 1974). These quantities, although internally derived, also represent inputs to the gastrointestinal tract. Ingestion of vegetables containing high levels of nitrate has been shown to lead to extremely high concentrations of nitrite in saliva, and these levels may persist for several hours (Tannenbaum, et al. 1976).

Preformed nitrosamines have been found in food, particularly in meats such as sausages, ham, and bacon which have been cured with nitrite. To date, analyses have been confined largely to the volatile N-nitroso compounds. N-nitrosodimethylamine has been found to be present in a variety of foods (including smoked, dried or salted fish, cheese, salami, frankfurters, and cured meats) in the 1 to 100 µg/kg range, but more usually in the 1 to 10 µg/kg range (Montesano and Bartsch, 1976). Other nitrosamines tentatively identified in meat products are N-nitrosodiethylamine, N-nitrosopiperi-

dine, and N-nitrosopyrrolidine (Montesano and Bartsch, 1976). N-nitrosopyrrolidine has been consistently found to be present in cooked bacon at the 10 to 50 µg/kg concentration level, but not in raw bacon (Fine, et al. 1977a). It apparently arises from N-nitrosoproline by decarboxylation during the cooking process (Lijinsky, et al. 1970). The source of nitrosamines in meat products is undoubtedly nitrosation; a report from a USDA Expert Panel on Nitrites and Nitrosamines (U.S. Dep. Agric., 1978), therefore recommends substantial reductions in the amounts of nitrate and nitrite used in cured meats.

Recently data have become available on human exposure to nitrosamines in beverages (Goff and Fine, 1979). Eighteen brands of domestic and imported beer contained N-nitrosodimethylamine at levels ranging from 0.4 to 7.0 µg/l, and six out of seven brands of Scotch whiskey were also shown to contain N-nitrosodimethylamine, at levels between 0.3 and 2.0 µg/l. Analysis was performed using gas chromatograph interfaced to a Thermal Energy Analyser (TEA).

It is necessary to note that studies prior to 1970 reporting the presence of nitrosamines in foods are open to question since the analytical methodology employed has been shown to be non-specific.

N-nitroso compounds are difficult to analyze for two reasons. First, they are usually present at ppb levels which require specialized instrumentation to confirm their positive identity. For example, high resolution mass spectrometry with peak matching or Thermal Energy Analyzer (TEA) is generally regarded as acceptable. Second, if the identity of the nitrosamine is established, proof must be provided that it was present in the environment and was not formed artificially during analysis. This is a difficult question to answer, since nitrosamines are generally found in the

presence of much larger concentrations of their precursors [International Agency for Research on Cancer (IARC), 1972, 1974, 1976, 1978].

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

No measured steady-state BCF is available for any of the following compounds, but the equation "Log BCF = (0.85 Log P) - 0.70" can be used (Veith, et al. 1979) to estimate the steady-state BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). the measured log P values were obtained from Hansch and Leo (1979). The adjustment factor of 3.0/7.6 = 0.395 is used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and

shellfish in order to obtain the weighted average bioconcentration factor for the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans.

Chemical	Meas. Log P	Estimated Steady State BCF	Weighted Average BCF
N-nitrosodimethylamine	-0.575	0.065	0.026
N-nitrosodiethylamine	0.48	0.51	0.20
N-nitrosodibutylamine	1.92	8.55	3.38
N-nitrosopyrrolidine	-0.19	0.138	0.055

A measured steady-state bioconcentration factor of 217 was obtained for N-nitrosodiphenylamine using bluegills (U.S. EPA, 1978). Similar bluegills contained an average of 4.8 percent lipids (Johnson, 1980). An adjustment factor of 3.0/4.8 = 0.625 can be used to adjust the measured BCF from the 4.8 percent lipids of the bluegill to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for N-nitrosodiphenylamine and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 217 x 0.625 = 136.

Inhalation

In theory there are several possible routes to the formation of nitrosamines in the atmosphere. These have been discussed in some detail (U.S. EPA, 1976, 1977). Due to the photolabile nature of nitrosamines, it seems unlikely that concentrations in ambient air would exceed a few ppb except very near sources of direct emissions of nitrosamines. This has since been confirmed by recent observations of Fine, et al. (1977a). N-nitrosodi-

methylamine was identified as an air pollutant near two chemical plants, one using the amine as a raw material and the other discharging it as an unwanted by-product. Typical levels at the first factory were 6 to 36 $\mu g/m^3$ on site, 1 $\mu g/m^3$ in the residential neighborhood adjacent to the factory, and 0.1 $\mu g/m^3$ two miles away. Typical daily human exposures were calculated to be 39 μg on site, 10 μg in the adjacent residential neighborhood, and 0.3 μg two miles away. Typical levels adjacent to the second site were 0.001 to 0.04 $\mu g/m^3$. However, nitrosamines were detected only twice at 40 collection points in New Jersey and New York City, and then only below the 0.01 $\mu g/m^3$ level. Fine, et al. (1977a) conclude that airborne N-nitroso compounds may not represent a daily widespread air pollution problem, but rather a localized problem associated with a particular segment of a specialized industry or with a particularly severe pollution level.

Many drugs and medicines contain secondary or tertiary amine groups. Model and animal experiments have demonstrated that these compounds can be readily nitrosated and thus suggest that they are precursors of N-nitroso compounds in vivo (Lijinsky and Taylor, 1977).

Tobacco and tobacco smoke contain both secondary amines and nitrosamines. Nitrosamines are not present in fresh tobacco, but are found during curing (Hoffman, et al. 1974). In relatively high concentrations (in the order of 100 mg/m^3), secondary amines and nitrogen dioxide can react rapidly to form nitrosamines; this reaction apparently occurs in tobacco smoke (U.S. EPA, 1977). The mainstream smoke from an 85 mm U.S. blended cigarette without a filter tip has been found to contain 0.084 μg N-nitrosodimethylamine, 0.030 μg N-nitrosomethylethylamine, 0.137 μg N-nitrosonornicotine, and traces of N-nitrosodiethylamine (Hoffman, et al. 1974). It can be estimated that the intake from smoking 20 cigarettes per day would therefore be

approximately 2 μg N-nitrosodimethylamine, 1 μg N-nitrosomethylethylamine, and 3 μg N-nitrosonornicotine. Walker, et al. (1976) have attempted to evaluate the exposure to nitrosamines of a non-smoker exposed to tobacco smoke. Assuming exposure to the smoke of five simultaneously burning cigarettes under crowded conditions with no ventilation, levels in air were calculated to be approximately 0.015 $\mu g/m^3$ N-nitrosodimethylamine, 0.004 $\mu g/m^3$ N-nitrosomethylethylamine, and 0.015 $\mu g/m^3$ N-nitrosonornicotine with traces of N-nitrosodiethylamine.

Dermal

N-nitroso-bis(2-hydroxyethyl)amine (N-nitrosodiethanolamine) has been reported to occur in cosmetic preparations, including facial creams, hand lotions, and hair shampoos, in concentrations ranging from 20 to 48,000 μg/kg (Fan, et al. 1977). The extent to which this compound is absorbed from the skin is unknown.

Commercial pesticide formulations available for home use have been found to contain as much as 0.06 percent N-nitrosodimethylamine as a contaminant (Fine, et al. 1977a). The contamination could have arisen during the manufacturing process or from nitrosation of dimethylamine by nitrate rust inhibitors added to prevent corrosion of the can. The main routes of exposure from home use of pesticides can be expected to be inhalation and absorption through the skin during spraying operations. Severn (1977), using data from three studies on inhalation and dermal exposure to pesticides during spraying of orchards, estimated that the intake from skin deposition, assuming 50 percent absorption, averaged about 325 times more than the intake via inhalation and concluded that the same ratio would hold for individuals performing hand spot-spraying. Inhalation, dermal, and/or oral exposure could also occur from careless use of these pesticides.

Fine, et al. (1977a) have calculated the daily exposure to preformed N-nitrosamines under worst-case conditions (Table 1). The intake from nitrite preserved foods assumes 100 g cooked bacon to be consumed daily. Air exposure is based on the highest concentrations measured on a factory site. For the general population, exposure information is very limited. It has been estimated that air, diet, and smoking all play a roughly equivalent role in direct human exposure, contributing a few micrograms per day, with direct intake from drinking water probably much less than 1 μg/day (U.S. EPA, 1976).

There is even greater uncertainty with regard to the significance of exposure to precursors. The chief source of nitrate exposure, except in the newborn, is ingested vegetables, unless rural well water high in nitrate is consumed. Food and water normally contribute approximately 100 μg/day. Inhalation may also contribute several hundred μg/day (U.S. EPA, 1977). On a daily basis, the major source of nitrite is saliva (Table 2). However, salivary nitrite is presented to the body as a continuous, low-level input, in comparison with the relatively high concentrations over short periods resulting from ingestion of cured meats. This may be significant since the rate of nitrosation is a function of the square of the nitrite concentration (U.S. EPA, 1977). Estimates of the contribution to the daily intake of N-nitroso compounds (as nitrosodimethylamine) have been attempted (NAS, 1978). Using blood levels of nitrosamines measured in one human subject before and after consuming a lunch consisting of spinach, cooked bacon, tomato, bread, and beer (Fine, 1977b), it was calculated that *in vivo* formation contributed 2.8 μg/day nitrosodimethylamine. For various reasons it is believed that the total amount of nitrosamine formed may have been considerably more than this. A second approach assumed that the rate of formation of nitrosamines is equal to 5 percent of the amount of nitrite present.

TABLE 1

Calculated Daily Human Exposure to N-nitroso Compounds**

	Daily intake (µg)				
	Nitrosodimethylamine + Nitrosodiethylamine	Nitrosopyrrolidine	Nitrosonornicotine	Other	
Nitrite preserved foods, 100 g.	1	5			
Tobacco smoke, 20 cigarettes	2		3		
Drinking water, New Orleans				8*	
Air, factory site	40			10*	
Herbicide formulation, 1 ml spill	640				

*Tentative, unconfirmed identification as N-nitroso compound.
**Source: Fine, et al. 1977a

TABLE 2

Calculated Average Daily Exposure to Nitrate and Nitrite**

Source	Nitrate		Nitrite	
	mg	%	mg	%
Vegetables	86.1	86.3	0.20	1.8
Fruits, juices	1.4	1.4	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	2.0	0.02	0.2
Water	0.7	0.7	0.00	0.0
Cured meats	9.4	9.4	2.38	21.2
Saliva	(30.0)*		8.62	76.8
Total	99.8	100	11.22	100

*Not included in total
**Source: White, 1975

This yielded an estimated daily production from precursors of 962 μg nitrosodimethylamine. However, this approach is likely to give a substantial overestimate. The conclusion appears inescapable that in vivo nitrosation provides a major contribution to the total body burden of N-nitroso compounds.

It must also be concluded that water supplies are a relatively minor source when compared with other potential sources of either preformed N-nitroso compounds or their precursors.

PHARMACOKINETICS

Distribution

Following intravenous injection into rats, nitrosamides (e.g., N-nitrosomethylurea, N-nitrosoethylurea) and nitrosamines (e.g., N-nitrosodimethylamine, N-nitrosomorpholine) are rapidly, and apparently uniformly, distributed in the body (Magee, 1972; Stewart, et al. 1974). Orally administered nitrosodiethylamine is found in the milk of lactating rats (Schoental, et al. 1974). Both nitrosamines (e.g., nitrosodiethylamine) and nitrosamides (e.g., N-nitrosoethylurea) can presumably cross the placenta since they are capable of inducing neoplasms in the offspring if administered to rats in late pregnancy (Magee, et al. 1976).

Metabolism

The nitrosamides are rapidly metabolized in the animal body. The half-lives of intravenously administered N-nitrosomethylurea and N-nitrosoethylurea in rats are about two minutes and five to six minutes, respectively. The metabolism of ^{14}C-labeled N-methyl-N'-nitro-N-nitroso-guanidine has been studied in some detail. Following an oral dose, most of the radioactivity was excreted in the urine within 24 hours and less than 3 percent in the feces. Less than 3 percent of the radioactivity remained in the body as acid-insoluble materials at 24 to 48 hours (Magee, et al. 1976).

The nitrosamines are metabolized less rapidly and persist in the body unchanged for a longer period than nitrosamides. The rate of metabolism depends upon the chemical structure. In the rat or mouse, administration of ^{14}C-labeled nitrosodimethylamine leads to about 60 percent of the isotope appearing as $^{14}CO_2$ within 12 hours. Corresponding figures for labeled nitrosodiethylamine and nitrosomorpholine are about 45 percent and 3 percent, respectively. For the three compounds, corresponding urinary excretions are 4, 14, and 80 percent, respectively. Metabolic products of dialkylnitrosamines found in the urine which contains the nitroso group are formed by ω-oxidation of the alkyl groups to give the corresponding alcohols and carboxylic acids (Magee, et al. 1976).

In vitro studies have demonstrated that the organs in the rat with the greatest capacity for metabolism of nitrosodimethylamine are the liver and kidney and that this compound is metabolized to a DNA-methylating agent by human liver slices at a rate slightly slower than, but comparable with, that of rat liver slices (Montesano and Magee, 1974).

The product(s) of metabolism of N-nitrosamines are thought to be responsible for the mutagenicity and/or carcinogenicity of many of these compounds. One hypothesis is that these active intermediates alkylate DNA at specific sites. Although the liver appears to be the major site of decomposition, other organs, such as kidney and lung, possess varying capacity to metabolize nitrosamines. The relative metabolic activity of different organs toward the same compound varies among species (Magee, et al. 1976).

Evidence to support the various proposed metabolic pathways of N-nitroso compounds is inconclusive. However, the in vitro studies of Montesano and Magee (1974) indicate that nitrosamines are metabolized similarly by human, guinea pig, and rat tissue.

EFFECTS

Acute, Subacute, and Chronic Toxicity

N-nitroso compounds are acutely toxic to every animal species and are also poisonous to humans.

The dialkyl and cyclic N-nitrosamines are characteristically hepatotoxins, producing hemorrhagic centrilobular necrosis. In experimental animals acute exposure to nitrosodimethylamine or nitrosodiethylamine produces liver lesions in 24 to 48 hours; deaths occur in three to four days, or the animals survive and apparently recover completely in about three weeks. Other organs than the liver are less severely affected; the main features are peritoneal and sometimes pleural exudates, which may contain a high proportion of blood, and a tendency to hemorrhage into the lungs and other organs. Kidney lesions, limited to the convoluted renal tubules, and testicular necrosis have been described in protein-deficient rats following treatment with nitrosodimethylamine (Magee, et al. 1976).

The livers of rats and other species chronically exposed to nitrosamines exhibit various pathological changes, including biliary hyperplasia, fibrosis, nodular parenchymal hyperplasia, and the formation of enlarged hepatic parenchymal cells with large nuclei (Magee, et al. 1976). Chronic administration of many nitrosamines induces tumors of the liver and other organs (see Carcinogenicity section).

The N-nitrosamides also induce a liver necrosis, but it is not as pronounced as that seen with the N-nitrosamines and is localized in the periportal areas. Unlike the nitrosamines, the nitrosamides cause severe tissue injury at the site of contact. The degree of local damage may be related to the rate at which the compound decomposes at the site since the damage is probably caused by a breakdown product rather than by the compound itself.

The systemic targets of the nitrosamides are mainly the organs of rapid cell turnover, including the bone marrow, crypt cells of the small intestine, and lymphoid tissues (Magee, et al. 1976).

The effects of human exposure to nitrosodimethylamine were first reported by Freund in 1937. The following description is from Weisburger and Raineri (1975).

> "Freund recorded the case of a young chemist engaged in the synthesis of dimethylnitrosamine, who presented with a number of syndromes eventually traced to occupational exposure. The patient had ill-defined pains in the abdomen, exhaustion, headaches, and distended abdomen. A second case, which involved an accidental single severe exposure due to a spill of nitrosamine, again led to abdominal fluid accumulation. During an exploratory laparotomy, ascitic fluid was found and the liver was enlarged. This patient failed to survive. Microscopic findings at autopsy revealed liver necrosis and areas of intense regenerative proliferation of the liver cells."

Further cases are now on record. Of two men accidentally exposed to nitrosodimethylamine used as a solvent in an automobile factory, one recovered after exhibiting signs of liver damage; the other died in a clinical accident, and a necroposy revealed a cirrhotic liver with regenerating nodules. Two of three men in an industrial research laboratory, working with nitrosodimethylamine over a period of ten months, showed signs of liver injury. One died of bronchopneumonia, and a necropsy found liver cirrhosis. The other developed a hard liver with an irregular surface, but recovered after exposure was terminated (Shank, 1975). The two individuals surviving this 1953 episode were still alive in 1976 (Weisburger and Raineri, 1975).

The acute toxicity of the N-nitroso compounds varies considerably. Single dose oral LD_{50} values in adult rats range from 18 mg/kg for N-nitrosomethylbenzylamine to more than 7,500 mg/kg for N-nitrosoethyl-2-hydroxyethylamine (Table 3). The acute oral LD_{50} in the rat for nitrosodiphenylamine, the only nitrosamine now produced in the U.S. in amounts greater than 450 kg/year, is given as 1,650 mg/kg [National Institute for Occupational

TABLE 3

Acute Oral LD_{50} Values (Druckrey, et al. 1967) and Relative Carcinogenic Potency Expressed as Log (LD_{50}) (Wishnok and Archer, 1976) in BD Rats. Classification of N-nitroso compounds follows that of Druckrey, et al. (1967)

Compound	LD_{50} (mg/kg)	$Log(1/D_{50})$**
Symmetrical dialkyl(aryl)nitrosamines:		
N-nitrosodimethylamine	40	2.27
N-nitrosodiethylamine	280	3.20
N-nitrosodi-n-propylamine	480	2.05
N-nitrosodi-iso-propylamine	850	0.97
N-nitrosodiallylamine*	800	-
N-nitrosodi-n-butylamine	1,200	1.61
N-nitrosodi-n-amylamine	3,000	0.59
N-nitrosodicyclohexylamine*	5,000	-
N-nitrosodiphenylamine*	3,000	-
N-nitrosodibenzylamine*	900	-
Asymmetrical alkyl(aryl)nitrosamines:		
N-nitrosomethylethylamine	90	2.32
N-nitrosomethylvinylamine	24	2.89
N-nitrosomethylallylamine	340	2.10
N-nitrosomethyl-n-amylamine	120	2.60
N-nitrosomethylcyclohexylamine	30	2.98
N-nitrosomethyl-n-heptylamine	-	1.53
N-nitrosomethylphenylamine	280	1.60
N-nitrosomethylbenzylamine	18	3.10
N-nitrosomethyl-(2-phenylethyl)amine	48	3.01
N,N'-dimethyl-N,N'-dinitrosoethylenediamine	150	2.40
N-nitrosoethylvinylamine	88	2.64
N-nitrosoethyl-iso-propylamine	1,100	1.49
N-nitrosoethyl-n-butylamine	380	2.11
N-nitrosoethyl-tert-butylamine*	1,600	-
N-nitroso-n-butyl-n-amylamine	2,500	1.00
Cyclic nitrosamines:		
N-nitrosopyrrolidine	900	1.41
N-nitrosoproline (ethyl ester)*	5,000	-
N-nitrosopiperidine	200	1.91
N,N'-dinitrosopiperazine	160	1.95
N-nitroso-N'-methylpiperazine	1,000	0.95
N-nitroso-N'-carbethoxypiperazine	400	1.91
N-nitrosoindoline	320	0.88
N-nitrosomorpholine	320	1.95
N-nitrosohexamethyleneimine	340	-
N-nitrosoheptamethyleneimine	280	-
N-nitrosooctamethyleneimine	570	-

TABLE 3 (Continued)

Compound	LD_{50} (mg/kg)	$Log(1/D_{50})$**
N--nitroso compounds with functional substituent groups:		
3-(N-nitroso-N-methylamino)-sulfolane	750	1.82
N-nitroso-N-phenylhydroxylamine	2,000	1.15
N-nitrosotrimethylhydrazine	95	2.24
N-nitrosoethyl-2-hydroxyethylamine	7,500	0.18
N-nitroso-bis(2-hydroxyethyl)amine	7,500	-
N-nitroso-bis(2-acetoxyethyl)amine	5,000	0.74
N-nitroso-n-butyl-(4-hydroxy-n-butyl)amine	1,800	1.51
N-nitrosomethyl-2-chloroethylamine	22	3.21
N-nitrosomethylcyanomethylamine	45	2.18
N-nitroso-bis(cyanomethyl)amine	163	1.95
N-nitrososarcosine	5,000	0.60
N-nitrosoethylsarcosinate	4,000	1.18
2-methyl-2(N-nitroso-N-methylamine)-pentan-4-one	2,100	1.04
Nitrosamides:		
N,n'-dinitroso-N,N'-dimethyloxamide	96	2.40
N-methyl-N-nitrosoacetamide	20	2.31
N-methyl-N-nitrosourethane	240	2.01
N-ethyl-N-nitrosourethane	-	1.96
N-methyl-N-nitrosourea	110	2.18
N,N'-dimethyl-N-nitrosourea	280	1.95
N-nitrosotrimethylurea	240	2.00
N-ethyl-N-nitrosourea	240	2.67
N-n-butyl-N-nitrosourea	1,200	2.10
Hydrazodicarboxylic acid bis (methyl-nitrosamide)	200	2.38
N-methyl-N'-nitro-N-nitrosoguanidine	420	2.51
N-nitrosoimidazolidone	250	2.26

*Non-carcinogenic in BD rats (Druckrey, et al. 1967)
**$1/D_{50}$ mean total carcinogenic dose

Safety and Health (NIOSH), 1976] or 3,000 mg/kg (Druckrey, et al. 1967). The relationship between structure and acute toxicity is not fully understood; however, for the dialkylnitrosamines acute toxicity appears to decrease with chain length (Shank, 1975). The predominantly hepatotoxic effects of these compounds are consistent with the hypothesis that the biologically active species is a metabolite and not the parent compound since the liver is generally the most active organ for metabolism. It is unlikely that under environmental conditions N-nitroso compounds would be present in sufficient quantity to provide an acutely toxic dose.

Teratogenicity

N-nitroso compounds can also be teratogens. The effects of experimental administration to pregnant animals have been studied systematically by Druckrey (1973a). In summary, whereas the N-nitrosamides were found to be teratogenic over an extended period of gestation, the N-nitrosamines were active only when administered late in pregnancy. Thus, near-LD_{50} levels of N-nitrosoalkylureas and N-nitrosoalkylanilines given to pregnant rats on day 9 or 13 of gestation produced malformations of the eye and brain in the offspring; similar levels of N-nitrosodimethylamine or N-nitrosodiethylamine did not (Napalkov and Alexandrov, 1968). Given at other periods of development, both N-nitrosamines and N-nitrosamides have been shown to be embryotoxic or carcinogenic (Druckrey, 1973b).

The two principal factors determining the response appear to be the state of differentiation of the various embryonic tissues and the metabolic competence of these tissues. Magee (1973) has adduced evidence suggesting that the lack of teratogenic and carcinogenic response to N-nitrosamines in early and mid-pregnancy is because the embryonic tissues have not yet acquired the competence for metabolic activation.

In Druckrey's studies (1973a), it was observed that some malformations, mainly those of the central and peripheral nervous systems, were associated with good survival times and that no tumors appeared at the sites of malformation. This led Druckrey to suggest that teratogenesis and carcinogenesis are two independent processes and that the molecular mechanisms of induction may be different.

Mutagenicity

The N-nitroso compounds include some of the most powerful chemical mutagens known. Montesano and Bartsch (1976) reported on the mutagenicity of 90 N-nitroso compounds, observed in direct mutagenicity assays and dominant lethal tests. Data on chromosome observations and tests in Drosophila melanogaster were also listed. As with other biological effects, there is a clear distinction between the mutagenic actions of N-nitrosamides and N-nitrosamines. N-nitrosamides are mutagenic in almost all test systems, due to nonenzymic formation of degradation products. N-nitrosamines, on the other hand, are not mutagenic in microbial test systems without metabolic activation.

Liver microsomal preparations from mouse, rat, hamster, and man are capable of activating nitrosamines. Czygan, et al. (1973), using human liver microsomes, found considerable variations in the capacity of the microsomes to activate N-nitrosodimethylamine to a mutagenic product. The cytochrome P-450 content showed proportional variations. (Cytochrome P-450 is the terminal enzyme in the microsomal system responsible for metabolism of foreign compounds). Czygan, et al. (1973) attributed the variations in cytochrome P-450 content to "diseases, therapy, or environmental pollutants." Czygan, et al. (1974) later demonstrated a positive correlation between the protein and choline content of the diet and the microsomal P-450 content, and con-

cluded that activation of nitrosamines can be influenced by nutritional factors. Extracts from organs other than liver are either ineffective or much less effective in activating nitrosamines to bacterial mutagens. Yet these organs may be the target for tumor induction in vivo by the same compounds. Thus, both nitrosodimethylamine and nitrosodiethylamine induce tumors in mouse lung and rat kidney; yet rat, mouse, and hamster lung microsomal preparations and mouse kidney preparations are ineffective in activating those compounds to mutagens in Salmonella typhimurium and Escherichia coli, respectively (Montesano and Bartsch, 1976).

Nitrosodimethylamine and nitrosodiethylamine have been reported to induce forward and reverse mutations in several bacterial species including S. typhimurium, E. coli, Neurospora crassa, gene recombination and conversion in Saccharomyces cerevisiae, "recessive lethal mutation" in Drosophila melanogaster, and chromosome aberrations in mammalian cells (Montesano and Bartsch, 1976). These compounds gave a negative response in the mouse dominant lethal test, probably due to the inability of the germ cells in the male to metabolize these compounds.

Not all N-nitroso compounds have been found to be mutagenic, although many have been tested only in microbial systems. Of the 23 N-nitrosamines listed by Montesano and Bartsch as having been tested in systems that included metabolic activation, six show no mutagenic activity. These include N-nitrosodiphenylamine, which is reported to give a negative response in both S. typhimurium and E. coli after activation with a rat liver microsomal preparation (Bartsch, et al. 1976; Nakajima, et al. 1974).

Carcinogenicity

Magee, et al. (1976) summarized data from studies through about 1975 on the carcinogenic activity of N-nitroso and related compounds. Of the 107

N-nitroso compounds (including 83 N-nitrosamines) listed, 87 (including 67 N-nitrosamines) are reported as having carcinogenic activity. Since that time more compounds have been tested and, to date, approximately 100 N-nitroso compounds are known to be carcinogenic in one or more species of experimental animals (Lijinsky and Taylor, 1977).

All animal species tested are susceptible, including the following: mice; rats; Chinese, Syrian, and European hamsters; gerbils; guinea pigs; rabbits; mink; dogs; pigs; and monkeys. Sensitivity varies with species. The African white-tailed rat, Mystromys albicaudatus, apparently remarkably free from spontaneous tumors, developed liver tumors after treatment with nitrosodiethylamine, although only after about 40 weeks of exposure to 50 to 100 mg/l in the drinking water; by comparison rats showed extensive hepatocellular carcinomas ten weeks after a ten-week exposure to 40 mg/l (Yamamoto, et al. 1972). Not all carcinogenic N-nitroso compounds have induced tumors in all species. The cyclic nitrosamine N-nitrosoazetidine (N-nitrosotrimethyleneimine) is reported to induce lung, liver, and kidney tumors in the rat and lung and liver tumors in the mouse, but induced no tumors under the test conditions used in the Syrian golden hamster. Toluene-p-sulfonylmethylnitrosamide is reported to have failed to induce tumors in the rat but produced lung tumors in the mouse (Magee, et al. 1976). The most recent addition to the list is N-nitrosodiphenylamine, previously thought to be a non-carcinogen. Cardy, et al (1979) have noted induction of neoplastic and non-neoplastic urinary bladder lesions in rats after two years of feeding N-nitrosodiphenylamine mixed in food at an average daily intake of 50 or 200 mg/kg body weight.

Not all N-nitrosamines have been found to induce tumors, although in most cases only one test species has been used, usually the rat. Those com-

pounds observed by Druckrey, et al. (1967) to give a negative response in rats are indicated in Table 3. Others include N-nitrosoethyl-(3-hydroxypropyl)-amine, N-nitroso-n-butylcarboxymethylamine, N-nitroso-n-butyl-(3-hydroxypropyl)amine, N-nitroso-n-butyl-(3-hydroxybutyl)amine, N-nitroso-t-butyl-(4-hydroxybutyl)amine (Okada, et al. 1976), and guvacoline (Lijinsky and Taylor, 1977). It is interesting to note that apparently all N,N-dialkylnitrosamines containing a tert-butyl group are noncarcinogenic (Heath and Magee, 1962). The list includes N-nitroso-l-proline, found in cured meats, particularly bacon. Although noncarcinogenic itself, nitrosoproline gives rise to the carcinogenic N-nitrosopyrrolidine during cooking (Lijinsky, et al. 1970). Aromatic nitrosamines are capable of transnitrosation, i.e., under suitable conditions, their nitroso group can be transferred to appropriate amine-type compounds. It is thus possible that noncarcinogenic transnitrosating agents could form new carcinogenic N-nitroso compounds in the stomach (Singer, et al. 1977).

The carcinogenic N-nitroso compounds are capable of inducing tumors in a wide variety of tissues, many compounds exhibiting a remarkable target organ specificity (organotropism) sometimes modified by the route of administration. Druckrey, et al. (1967) studied the effects of a large number of N-nitroso compounds following prolonged administration to adult rats of the BD strain (said to exhibit a spontaneous malignant tumor rate of one percent at 500 days). In general, daily doses were approximately 2.5 percent of the LD_{50} values listed in Table 3 and were administered in drinking water over the life span. Pilot experiments used higher dose rates (five percent or more of the LD_{50}), and some animals received the N-nitroso compounds by subcutaneous or intravenous injection or inhalation. The mean time to tumor

varied, with dose rate and compound, between 160 and 840 days. Druckrey, et al. (1967) made the following general observations (paraphrased from the English summary to their paper).

All symmetrically substituted dialkylnitrosamines produced carcinomas of the liver. The only exception was N-nitrosodi-n-butylamine, which produces carcinomas of the urinary bladder. Subcutaneous injection of this compound produced only bladder tumors. N-nitrosodiamylamine given subcutaneously selectively produced lung cancer.

Asymmetrical dialkylnitrosamines, especially those possessing a methyl group and with the second substituent group amyl, cyclohexyl, phenyl, benzyl or phenylethyl, and also N,N'-dimethyl-N,N'-dinitrosoethylene diamine, N-nitrosethylvinylamine, and N-nitrosoethyl-n-butylamine, selectively produced carcinomas of the esophagus following both oral and parenteral administration. N-nitrosomethylalkylamines induced malignant tumors of the kidney, particularly after intravenous injection.

The cyclic nitrosamines, N-nitrosopyrrolidine, N-nitrosomorpholine, and N-nitroso-N'-carbethoxypiperazine induced cancer of the liver. N-nitrosopiperidine and N,N'-dinitrosopiperazine produced carcinomas of the esophagus after both oral and intravenous administration but tumors of the nasal cavity, mostly esthesioneuroepitheliomas, after subcutaneous injection.

Nitrosamines with functional substituent groups also produced malignant tumors in different organs. 3-(N-nitroso-N-methylamino)-sulfolane and N-nitrososarcosine and its ethyl ester induced esophageal cancer. N-nitroso-n-butyl-(4-hydroxy-n-butyl)amine selectively induced carcinomas of the urinary bladder. N-nitrosoethyl-2-hydroxyethylamine and N-nitroso-bis-(2-hydroxyethyl)amine regularly produced liver tumors following chronic exposure but exhibited minimal toxicity in acute experiments (LD_{50}, 7,500 mg/kg).

Several N-nitrosamides produced carcinomas of the forestomach after oral administration or local sarcomas at the site of injection. Intravenous N-methyl-N-nitrosourethane selectively produced lung cancer. Methylnitrosoureas induced malignant tumors in the brain, spinal cord, and/or peripheral nervous system.

Lijinsky and his co-workers (1977) have systematically studied the effects of modification of chemical structure on the biological activity and organ specificity of the nitrosamines. They have found that minor changes can have a profound effect on which organ becomes the target organ. For example, chronic administration in the drinking water of N-nitrosohexamethyleneimine induces liver tumors in rats; N-nitrosoheptamethyleneimine produces lung tumors. Lijinsky (1977) has discussed his findings in relation to what is known of the mechanism of action of nitrosamines. His conclusion is that the major factor responsible for variations in biological activity is the reactivity of hydrogen atoms on carbon atoms adjacent to the nitroso group (alpha hydrogen atoms).

The response to a particular compound also varies among species. The following attempt to illustrate the diversity of responses is derived from Magee, et al. (1976). In most species, as in the rat, the predominant tumors following prolonged oral administration of dialkyl cyclic and many other N-nitrosamines are in the liver. Tumors in rats have been described as hepatomas and hepatocellular carcinomas, cholangiomas and cholangiocarcinomas, fibrosarcomas, and angiosarcomas. The tumor type(s) observed in mice depend upon both the strain and the compound. Nitrosodimethylamine produced mainly hemangiomatous tumors, with few parenchymal cell tumors. Nitrosodiethylamine induced mainly parenchymal tumors in seven strains of mice but predominantly hemangiosarcomas and hemangioendotheliomas in two strains.

Nitrosodiethylamine given to Syrian golden hamsters by the intragastric, intraperitoneal, or intradermal routes produced hepatocellular carcinomas that metastasized and were transplantable; continuous oral administration induced cholangiocarcinomas. However, following single or multiple subcutaneous injections, both adult and newborn hamsters developed mainly respiratory tumors and very few liver tumors. In the Syrian golden hamster, respiratory tract tumors induced by nitrosodiethylamine are confined mainly to the nasal cavities, larynx, and trachea irrespective of the route of administration. In the mouse, guinea pig, and rabbit, liver tumors following prolonged oral administration of nitrosodiethylamine are accompanied by adenocarcinomas of the lung.

In their first studies demonstrating the carcinogenicity of nitrosodimethylamine, Magee and Barnes (1956) reported that 19 of 20 rats continuously fed 50 mg/kg in the diet developed primary hepatic tumors within 40 weeks. However, they later (Magee and Barnes, 1959) found that in rats exposed for one week at 100 or 200 mg/kg in the diet, kidney tumors predominated over liver tumors. A single, near-LD_{50} dose (30 mg/kg body weight) of nitrosodimethylamine produced no progressive liver lesions nor liver tumors but a 20 percent incidence of kidney tumors. A single intraperitoneal injection given to newborn mice induced hepatocellular carcinomas (Toth, et al. 1964). A single dose to partially hepatectomized adult rats (Craddock, 1973) or to rats previously treated with a single dose of carbon tetrachloride (Pound, et al. 1973) induced liver tumors. Both treatments induce liver cells to divide, and these observations prompted Craddock (1973) to speculate that both injury to the genetic material and the occurrence of cell replication before the damage has been repaired are required

for carcinogenesis. However, the incidence of liver tumors following chronic administration of either nitrosodimethylamine or nitrosodiethylamine was the same in both intact and partially hepatectomized rats (Rajewski, et al. 1966). There seems to be no simple explanation as to why a single oral dose of nitrosodimethylamine, while ineffective in the adult mouse or rat, is capable of inducing liver tumors in the adult Syrian golden hamster (Tomatis and Cefis, 1967).

Some N-nitroso compounds administered during pregnancy induce cancer not only in the mother but also in the offspring. A single administration of N-nitrosoethylurea to pregnant rats resulted in malignant tumors of the vagina, uterus, or ovaries. Given on days 15 through 18 of gestation (but not before day 11), the compound produced brain and spinal cord tumors in the offspring. Ethylurea and nitrite given orally to pregnant rats also produced nervous system tumors. The sensitivity of the nervous system during prenatal development was estimated to be about 50 times that of adults (Druckrey, et al. 1969). Exposure during days 10 through 21 of gestation led to renal tumors in the offspring several months after treatment (Shank, 1975). The N-nitrosoamines, including nitrosodimethylamine, nitrosodiethylamine, nitrosomethylbutylamine, nitrosoethylvinylamine, and nitrosopiperidine, have induced tumors in the offspring of mice, rats, and Syrian golden hamsters only when administered during the last days of pregnancy. Subcutaneous, intraperitoneal, intravenous, and oral administration and inhalation exposure were equally effective (Tomatis, 1973). In rats the tumors observed were mainly neurogenic. However, Mohr, et al. (1966) observed tracheal papillomas in almost half the offspring of pregnant Syrian golden hamsters within 25 weeks of subcutaneous administration of N-nitrosodiethylamine on days 9 through 15 of gestation. In mice, treatment with nitrosodi-

ethylamine on day 16, 17, or 18 of gestation induced mainly lung tumors. It has been suggested that the inefficacy of the nitrosamines in early pregnancy is due to the lack in the fetus of enzyme systems necessary for metabolic activation (Druckrey, 1973b). Presumably, although active products are produced in the maternal tissues, they are generally too unstable to survive crossing the placenta and hence do not affect the fetus.

Exposure to N-nitrosamides during pregnancy may result in a risk not only to the immediate offspring but for at least two more generations of animals. An increased incidence of tumors has been reported in the F_1, F_2, and F_3 descendants of rats treated with N-nitrosomethylurethane or N-nitrosomethylurea during pregnancy (Montesano and Bartsch, 1976). There is not experimental evidence to indicate that N-nitrosamines pose a similar threat.

Nitrosodiethylamine has been found in the stomach contents of suckling rats following oral administration to the dam. The young rats subsequently developed multiple tumors (Schoental and Appleby, 1973).

The carcinogenic action of the N-nitroso compounds can be modified by appropriate treatment. The effect of partial hepatectomy or prior administration of carbon tetrachloride has already been mentioned. Other interactions have also been demonstrated. The intragastric administration of methylcholanthrene to mice (which would be expected to increase the activity of liver nitrosamine-metabolizing enzymes) together with intraperitoneal injection of nitrosodimethylamine resulted in increased incidence and decreased latency period to tumors as compared with mice treated with either compound alone (Cardesa, et al. 1973). Intratracheal instillation of ferric oxide and subcutaneous injection of nitrosodimethylamine in Syrian golden hamsters induced esthesioneuroepitheliomas of the nasal cavity, a type of

tumor not induced in hamsters by nitrosodimethylamine alone (Stenback, et al. 1973). Ferric oxide is frequently used as a carrier for introducing carcinogenic chemicals into the lung by intratracheal instillation. It is believed to facilitate the penetration and retention of the carcinogen in the lung tissue. However, in the present instance, ferric oxide can be considered a cocarcinogen. Other studies have shown enhanced bronchial metaplasia and tracheal papilloma formation in hamsters treated with nitrosodiethylamine by subseqeuent exposure to cigarette smoke, volatile acids, aldehydes, and methyl nitrite and increased incidence of lung tumors by subsequent intratracheal instillation of benzo(a)pyrene and/or ferric oxide particles (Magee, et al. 1976). The toxicity and carcinogenicty of various alkylnitrosoureas are said to be increased when administered with copper, nickel, or cobalt ions (Magee, et al. 1976). Magee, et al. (1976) cite examples of agents known to depress the activity of drug metabolizing enzymes and which have been reported to modify the action of N-nitrosamines. A protein-deficient diet protected against acute liver damage in rats and resulted in an almost twofold increase in the LD_{50}; however, the incidence of kidney tumors in survivors was 100 percent. Aminoacetonitrile, which inhibits the metabolism of nitrosodimethylamine both in vivo and in vitro, prevented its toxic and carcinogenic effect in rat liver. At the present time, these interactions appear to be of academic rather than practical interest.

Although there is a wealth of reported studies on the carcinogenicity of N-nitroso compounds, these tend to address structure-activity relationships or mechanisms of action; information on dose-response characteristics is sparse. Table 4 includes experimental data culled from studies in the published literature in which nitrosamines were administered over the lifetime

TABLE 4

Dose-response Data from Studies Involving Lifetime Exposure to Four N-nitrosamines

Animals with Malignant Benign Tumors of Listed Organs	Daily Dose (mg/kg Body wt.)	Animals with Tumors (Animals Exposed) Male	Female
Compound: N-Nitrosodimethylamine			
Vehicle: Diet	0	0(12)	0(29)
Species: Rat	0.67	1(19)	
Target Organ: Liver	0.12		0(18)
(Terracini, et al. 1967)	0.17	1(6)	
	0.30		4(62)
	0.60		2(5)
	1.2		15(23)
	6.0		10(12)
[1]Compound: N-Nitrosodiethylamine			
Vehicle: Drinking Water	0.075	5(60)	
Species: Rat	0.15	22(45)	
Target organ: Liver	0.30	63(80)	
(Druckrey, et al. 1963)	0.60	51(60)	
	1.2	36(40)	
Compound: N-Nitrosodi-n-butylamine			
Vehicle: Drinking Water	7.6	46(47)	
Species: Mouse	8.2		40(42)
Target organ: Urinary Bladder and/or Esophagus	29.1	45(45)	
(Bertram and Craig, 1970)	30.9		45(45)
[2]Compound: N-Nitrosopyrrolidine			
Vehicle: Drinking Water	0	0(61)	
Species: Rat	0.30	3(60)	
Target organ: Liver	1.0	17(62)	
(Preussmann, et al. 1977)	3.0	31(38)	
	10.0	14(24)	

TABLE 4 (cont.)

Animals with Malignant Benign Tumors of Listed Organs	Daily Dose (mg/kg Body wt.)	Animals with Tumors (Animals Exposed)	
		Male	Female
Compound: N-Nitrosodiphenylamine			
Vehicle: Diet	0	0(19)	0(18)
Species: Rat	50	0(46)	0(48)
Target organ: Urinary Bladder	200	16(45)	40(49)
(Cardy, et al. 1979)			

[1]It is assumed that these are male BDI rats.

[2]No sex difference.

of experimental animals at two or more daily dose levels which induced tumors in some but not all animals exposed. Although only tumors (benign and malignant) occurring in the principal target organ are listed, in all cases other organs were also affected. Some comments are necessary. The data of Druckrey, et al. (1963) are difficult to interpret since many animals were lost through intercurrent infections. Thus, of the 60 animals originally exposed to nitrosodiethylamine at the 0.075 mg/kg body weight level, 40 succumbed to a "pneumonia infection" during the first 600 days of the experiment and, by the time the first (and only) hepatic carcinoma had been identified in this group, there were only three survivors. The sex of the animals used in this study is not specified. However, it is probable that they were male BD II (albino) rats. In addition to tumors of the urinary bladder, Bertram and Craig (1970) report a very high incidence of esophageal tumors following administration of N-nitrosodi-n-butylamine. The incidence of bladder tumors in females was relatively low, but these tumors developed significantly later than in males. The authors speculate that, had not death from esophageal tumors intervened, both sexes would have had a uniformly high bladder tumor incidence. Preussman, et al. (1977) report that other dose response studies (initially with N-nitrosopiperidine) are under way or planned. In other studies with nitrosodimethylamine, mink, apparently the most sensitive species, developed tumors when fed 0.05 mg/kg body weight two days per week (NAS, 1978). An increase in the incidence of malignant liver and kidney tumors was found in male but not in female rats, and not in mice of either sex when the animals continuously inhaled air containing 200 $\mu g/m^3$ of dinitrosomethylamine for 17 months (mice) or 25 months (rats). A concentration of 5 $\mu g/m^3$ produced no increase in tumors (NAS, 1978). Preussman, et al. (1977) have attempted to derive "no-effect

levels" for rats for nitrosodimethylamine and other carcinogenic nitrosamines (although they themselves question the validity of such levels). Expressed as dietary levels, the estimates are: nitrosodimethylamine, 1 to 2 mg/kg; nitrosodiethylamine, <1 mg/kg; N-nitrosopyrrolidine, 3 to 5 mg/kg (corresponding to a daily intake of approximately 0.1, <0.1, and 0.3 mg/kg body weight, respectively).

Attempts have been made to derive some measure of the relative carcinogenic potency of N-nitroso compounds in the absence of complete dose-response information. The favored data base is the review of Druckrey, et al. (1967) of studies in which adult BD rats received small daily doses (usually orally) of 51 N-nitrosamines and 13 N-nitrosamides. Druckrey, et al. calculated the mean total carcinogenic dose required for production of tumors in 50 percent of the animals (D_{50}). Wishnok and Archer (1976) have used only those D_{50} values corresponding to a daily dose that was an approximately constant fraction (one to three percent) of the acute oral LD_{50} (a dose which gave a mean induction time for appearance of tumors of about 300 to 600 days), and, in order to have increasing carcinogenicity represented by increasing (and manageably small) numbers, have expressed carcinogenic potency as log $1/D_{50}$. Table 3 lists the values given by Wishnok and Archer (1976) for most of the carcinogenic N-nitroso compounds examined by Druckrey, et al. (1967). For the four N-nitrosamines for which dose-response data are available, the order of increasing potency as measured by log $1/D_{50}$ is: N-nitrosopyrrolidine (1.41); N-nitroso-n-butylamine (1.61); N-nitrosodimethylamine (2.27); N-nitrosodiethylamine (3.20). Analysis of experimental dose-response data places these compounds in the same order (Table 5). Despite this possibly fortuitous agreement, log ($1/D_{50}$) values can be regarded only as providing general guidance.

TABLE 5

Concentrations in Water Estimated to Induce no more than one Excess Cancer per 100,000 Individuals Exposed over a Lifetime

Compound	Estimated Concentration (ng/l)	Data Base
N-Nitrosodimethylamine	14	Rats (female) (Druckrey, et al. 1967)
N-Nitrosodiethylamine	8	Rats (male?) (Druckrey, et al. 1963)
N-Nitrosodi-n-butylamine	64	Mice (male) (Bertram and Craig, 1970)
N-Nitrosopyrrolidine	160	Rats (mixed sexes) (Preussman, et al. 1977)

Wishnok and Archer (1976) and Wishnok, et al. (1978), using the log ($1/D_{50}$) value as a measure of carcinogenicity, have attempted to relate carcinogenicity to the chemical and physical properties of N-nitroso compounds. Wishnok, et al. (1978) have derived an equation that takes into account not only chemical structure, but also the partition coefficient of the N-nitroso compounds and their electronic factors as expressed by Taft σ^* values of substituents on the α-carbon atoms. With certain explainable exceptions, which are discussed by Wishnok, et al., the equation appears to serve as a reasonably reliable method for assessing carcinogenicity.

There is no instance known of occupational exposure to specific nitrosamines having resulted in a cancer in man. The epidemiologic evidence for the association of N-nitroso compounds with human cancer is also very limited. These data have been reviewed by a panel convened by the National Academy of Sciences (1978), and the following is taken in its entirety from this report.

> A few epidemiological studies have attempted to associate environmental nitrates, nitrites, and nitroso compounds with human cancer. A problem common to all the early studies was the inability to measure with high specificity N-nitroso compounds in biological samples. For example, African studies associating esophageal cancer with a nitrosamine in a local alcoholic beverage (McGlashan, 1969) and a study relating carcinoma of the cervix with nitrosamine formation in the vagina of South African women (Harington, et al. 1973) were done without the advantage of mass spectroscopic confirmation that is needed to identify the nitrosamines.
>
> The International Agency for Research on Cancer has investigated the possible association between N-nitroso compounds in the diet and esophageal cancer in specific areas of Iran and France, where these tumors occur at a high rate, and in nearby areas where the tumor rates are not elevated (Bogovski, 1974). Complete studies of possible sources of exposure to the carcinogens have not been made, but 15 of 29 samples of cider contained 1 to 10 µg/kg DMN (nitrosodimethylamine) and two samples also contained DEN (nitrosodiethylamine) (less than 1 µg/kg). Correlations between dietary intake of N-nitroso compounds and incidence of esophageal cancer have not yet been made.

The Chinese conducted a similar study in the Anyang region, where it is claimed that approximately 20 percent of all deaths (not just cancer deaths) result from esophageal cancer (Coordination Group, 1975). Twenty-three percent of the food samples from areas with the highest cancer rates were reported to contain DMN, DEN, and methylbenzyl nitrosamine. However, confirmation of this analysis by gas chromatography and mass spectroscopy is required before the finding can be accepted. Dietary nitrite levels were higher in areas of high cancer incidence than in low incidence areas. Chickens in areas where there were high rates of esophageal cancer in humans also had a high incidence of similar tumors, suggesting an environmental etiology for the disease.

Zaldivar and Wetterstand (1975) demonstrated a linear regression between death rates from stomach cancer and the use of $NaNO_3$ as fertilizer in various Chilean provinces. Fertilizer use was presumed equitable to human exposure to nitrates and nitrosamines, but no actual exposure data were reported. Armijo and Coulson (1975) have shown similar correlations. These reports suggest that nitrate from fertilizer enters the diet in meat, vegetables, and drinking water, is reduced to nitrite by microbial action, and thus is available for *in vivo* nitrosation of secondary amines in the diet, to form carcinogenic nitrosamines, which induce stomach cancer. As yet, no scientific data have been gathered that support this hypothesized etiology, and the suggested causal relationship remains highly speculative.

Hill, et al. (1973) correlated differences in rates of stomach cancer with the nitrate content of drinking water in two English towns; but again, the evidence required to demonstrate a causative role for nitrate is not available. Gelperin, et al. (1975) compared death rates ascribed to cancer of the gastrointestinal tract and liver with nitrate levels of drinking water in three unmatched population groups in Illinois used in an infant mortality study. No significant differences in cancer rates were found among the three groups (the level of significance was not stated). It is doubtful, however, whether the available mortality data permitted an analysis that could have detected an effect in the high nitrate population.

Increased rates of stomach cancer have been observed in Japan in occupational groups and other populations characterized by an unusually high consumption of salt-preserved foods (Sato, et al. 1959); presumably, these foods are high in nitrate and perhaps in nitrite.

A statistical correlation is presented of the incidence of cancer mortality with estimated exposures of urban populations in the United States to various environmental and dietary factors. Again to quote the report (NAS, 1978): "Strong positive correlations were shown between the aggregate rate

of cancer mortality and components of the diet, particularly nitrite and protein; however, insufficient biological evidence is available to confirm the hypothesized causal pathway (involving formations of N-nitroso compounds from nitrite and amines, reacting in the stomach)." There is, in fact, direct evidence for formation of nitrosamines from precursors in the human stomach (see, for example, Fine, et al. 1977b); still in contention is the extent to which nitrosation occurs.

Although N-nitrosamines such as N-nitrosodimethylamine and N-nitrosomorpholine are rapidly and fairly evenly distributed throughout the bodies of rats after injection (Magee, 1972; Stewart, et al. 1974), the acute toxic damage they produce is more severe in the liver than elsewhere, and tumors following chronic exposure are confined mainly to the liver and kidney (Druckrey, et al. 1967). In vitro studies have shown that the liver and kidney possess the greatest capacity for metabolism of N-nitrosodimethylamine (Montesano and Magee, 1974). These observations are most readily explained on the assumption that carcinogenesis and other biological actions of nitrosamines are mediated by metabolic products. The lack of mutagenic activity exhibited by nitrosamines in bacterial test systems in the absence of a metabolic activating system (Montesano and Bartsch, 1976) supports this hypothesis, which is now generally accepted.

The N-nitrosamides differ from the N-nitrosamines in that they are chemically unstable at physiological pH and decompose nonenzymically, again into active metabolic products, upon contact with the tissues. They therefore tend to produce damage or tumors at the site of administration.

The nature of the metabolite(s) responsible for the carcinogenic activity of N-nitroso compounds is still in debate. Magee (1977) has adduced considerable evidence in support of the commonly accepted hypothesis that the

active agents are electrophilic alkylating agents which bind to DNA. The major product formed in rat liver after administration of nitrosodimethylamine or nitrosodiethylamine is the corresponding 7-alkylguanine. However, little correlation has been found between the occurence of 7-alkylguanine in DNA and the tumor-producing activity of the nitrosamines. A much better correlation has been demonstrated between the formation and persistence of O^6-alkylguanines and tumor incidence (Pegg and Nicoll, 1976). These authors postulate that the formation and persistence until cell division of certain promutagenic products such as O^6-methylguanine might be responsible for the initiation of tumors and that the differing abilities of various tissues to catalyze DNA repair might account for part of the differing susceptibilities of these tissues to the carcinogenic action of the N-nitroso compounds (Pegg and Nicoll, 1976). However, Lijinsky and coworkers found that a series of cyclic nitrosamines, while as carcinogenic as the aliphatic nitrosamines, gave rise to much smaller amounts of alkylated guanines; in some cases none could be detected (Lijinsky, 1977). For this and other reasons, Lijinsky concludes that the initial step cannot be a simple alkylation of DNA.

Many N-nitrosamines (and N-nitrosamides) are teratogenic, mutagenic, or carcinogenic. Evidence from experimental animals suggests that, as carcinogens, they are most effective by the oral route and when given as multiple small doses. However, some are capable of inducing tumors after a single dose, and they are also capable of inducing tumors in certain organs and tissues regardless of the route of administration, i.e., they are systemic carcinogens. In the rat, at least, every organ is probably susceptible to tumor induction by some nitrosamine. There is a strong relationship between

chemical structure and type of tumor induced. There are large differences in tumor response among species, both in type of tumor produced and in susceptibility.

The late fetus and neonate appear to be highly susceptible to the carcinogenic action of both N-nitrosamines and N-nitrosamides. The sensitivity of the nervous system to some N-nitrosamides during prenatal development is about 50 times that in the adult. A single exposure to some nitrosamides during pregnancy may result in development of tumors not only in the immediate descendants but in at least two succeeding generations. Although prolonged exposure to some nitrosamides is needed to elicit tumors in adult animals, a single dose of the compound will induce tumors in the newborn.

The epidemiological studies to date have been inadequate to establish any correlation between exposure to N-nitroso compounds or their precursors and human cancer as valid causal relationships. Nevertheless, the ability of N-nitrosamines to induce tumors in a wide range of species other than man, together with the fact that human liver tissue is capable of forming alkylating and mutagenic metabolites, suggest strongly that it is improbable that humans are refractory to the carcinogenic action of these compounds.

CRITERION FORMULATION

Existing Guidelines and Standards

Current Levels of Exposure

For the general population, exposure information is very limited. It has been estimated that air, diet, and smoking all play roughly equivalent roles in direct human exposure, contributing a few micrograms per day, with direct intake from ingested water probably much less than 1 µg/day (U.S. EPA, 1976).

There is even greater uncertainty with regard to the significance of exposure to precursors. The chief source of nitrate exposure, except in the newborn, is ingested vegetables, unless rural well water high in nitrate is consumed. Food and water normally contribute approximately a few hundred milligrams per day. Inhalation may also contribute several hundred micrograms per day (U.S. EPA, 1977). On a daily basis, the major source of nitrite is saliva. However, salivary nitrate is presented to the body as a continuous, low-level input, as contrasted with the relatively high concentrations over short periods resulting from ingestion of cured meats. This may be significant since the rate of nitrosation is a function of the square of the nitrite concentraton (U.S. EPA, 1977).

The concentrations of nitrite (and its precursors, ammonia and nitrate) and nitrosatable compounds can be much greater in soils heavily fertilized with organic waste matter or in waters receiving runoff from agricultural areas or discharges of industrial or municipal waste waters containing substantial amounts of amines. Levels of nitrate in municipal drinking water in the U.S. seldom exceed 10 mg/l nitrate N, although some private supplies contain much more.

Significant concentrations of nitrosamines have been reported for a limited number of samples of ocean water, river water, and waste treatment plant effluent adjacent to or receiving wastewater from industries using nitrosamines or secondary amines in production operations. Nitrosodimethylamine has been reported at the 3-4 ng/l level in these samples. Nitrosamines, however, are rapidly decomposed by photolysis and do not persist for a significant time in water exposed to sunlight.

Although it is difficult to analyze this wide spectrum of exposure potential, it must be concluded that ingested water is a relatively minor source of exposure when compared with other potential sources of either preformed N-nitroso compounds or their precursors.

Special Groups at Risk

Because of the ubiquitous nature of nitrosatable compounds and nitrosating agents in the environment (food, air, drugs, tobacco, water, soil) special risk groups would have to include those individuals who are exposed to multiple exposures. To quantify this, however, is almost impossible at this point because of the need to create exposure scenarios for which the bounding factors are unknown or relatively wide ranging.

Basis and Derivation of Criterion

Both N-nitrosamines and N-nitrosamides exhibit acute toxicity, teratogenicity, mutagenicity, and/or carcinogenicity. For most, it is the latter capability which demands consideration in the context of human exposure since the toxicological evidence is such that they must be treated as potential human carcinogens. Thus, nitrosamines are included in a list from the American Conference of Governmental Industrial Hygienists (ACGIH, 1977) "In-

dustrial Substances Suspect of Carcinogenic Potential for Man." No Threshold Limit Value (TLV) is given. The guidelines which follow are based upon the assumption that N-nitrosamines are human carcinogens.

Adequate dose-response data to permit an assessment of the carcinogenic risk to man are available from studies involving lifetime exposure of rats or mice to five nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine, N-nitrosopyrrolidine, and N-nitrosodiphenylamine) in their drinking water or food (see Table 4). These data have been used to derive estimates of the concentrations in water which, if used as the source for man of drinking water and edible fish and shellfish, would increase the risk of a tumor by not more than one in 100,000 individuals exposed for the duration of their life span. The methods of extrapolation are discussed in the Human health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document. The water criteria shown in Table 5 are based on parameters listed in the Appendix.

Table 3 lists one measure of the relative carcinogenic potential of a number of N-nitrosamines. The value of N-nitrosodiethylamine (3.20) is exceeded only by that for N-nitrosomethyl-2-chloroethylamine (3.21) and approached only by the values for N-nitroso-methylbenzylamine (3.10) and N-nitrosomethyl-(2-phenylethyl) amine (3.01). Hence, N-nitrosodiethylamine can reasonably be considered to be one of the most carcinogenic nitrosamines. It is, therefore, appropriate to recommend a water criterion value for the nitrosamine class based on the value obtained for N-nitrosodiethylamine. If sufficient evidence exists to indicate that some nitrosamines may be less potent carcinogens than N-nitrosodiethylamine, then a separate criterion should be derived. This has been done in four cases. In addition, if there

is sufficient experimental evidence that a particular nitrosamine is not carcinogenic to mammals, then a noncarcinogenic-based criterion should be allowed.

Criteria have been derived by considering only the excess cancer risk imposed by exposure to contaminated drinking water, fish, and shellfish. However, the average daily intake of preformed nitrosamines from other sources (air, diet, and smoking) is estimated to be on the order of a few micrograms per day (U.S. EPA, 1976). There is an additional and, at the present time, ill-defined contribution to the body burden from the in vivo nitrosation of precursors. This contribution has been variously estimated to range from a few micrograms to several hundred micrograms daily (NAS, 1978). Thus, present evidence suggests that control of exposure to N-nitrosamines should take into account both preformed nitrosamines and their precursors in the environment.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including, where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." Nitrosamines are suspected of being human carcinogens. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of nitrosamines in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of nitrosamines corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of

10^{-5}, for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6}, or 10^{-7} as shown in the table below.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1) ng/l			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 grams fish and shellfish (2)				
N-nitrosodimethylamine	0	0.14	1.4	14.0
N-nitrosodiethylamine	0	0.08	0.8	8.0
N-nitrosodi-n-butylamine	0	0.64	6.4	64
N-nitrosopyrrolidine	0	1.60	16.0	160
N-nitrosodiphenylamine	0	490	4,900	49,000
Consumption of fish and shellfish only.				
N-nitrosodimethylamine	0	1,600	16,000	160,000
N-nitrosodiethylamine	0	124	1,240	12,400
N-nitrosodi-n-butylamine	0	58.7	587	5,868
N-nitrosopyrrolidine	0	9,190	91,900	919,000
N-nitrosodiphenylamine	0	1,610	16,100	161,000

(1) Calculated by applying either a linearized multistage model (N-nitroso-di-n-butylamine, N-nitrosopyrrolidine, and N-nitrosodiphenylamine) or a time to tumor model (N-nitrosodimethylamine and N-nitrosodiethylamine), as discussed in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document, to the animal bioassay data presented in the Appendix and in Table 4. Since the extrapolation models are linear at low doses, the additional lifetime risk is directly proportional to the water concen-

traton. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately zero percent of the exposure of these first four nitrosamines results from the consumption of aquatic organisms which exhibit an average bioconcentration potential near zero. The remaining 100 percent of these nitrosamines' exposure results from drinking water. In the case of N-nitrosodiphenylamine 31 percent of the exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 136 l/kg. The remaining 69 percent reflects exposure from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of nitrosamines, (1) occurring from the consumption of both drinking water and aquatic life grown in waters containing the corresponding nitrosamines concentrations, and (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding nitrosamines concentrations. Because data indicating other sources of nitrosamines exposure and their contributions to total body burden are inadequate for quantitative use, the figures reflect incremental risks associated with the indicated routes only.

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APPENDIX

Derivation of Criterion for Dimethylnitrosamine

Druckrey et al. (1967) summarized a series of experiments in which a large series of nitrosamine compounds were given to BD rats for a lifetime. He found that the incidence of liver tumors increased with daily dose, d, and that the median time when tumors were observed, t_{50}, was less at higher doses and the relationship between d and t_{50} was $d(t_{50})^{2.3} = k$, where k is a constant equal to 0.81×10^4 mM/kg/day when t_{50} is expressed in units of days.

The water quality extrapolation model uses dose units of mg/kg/day and time units of fractions of a lifetime. Converting k to these units by using 728 days (two years) as the lifetime and a molecular weight of 74 mg/mM gives the following:

$$k = \frac{0.81 \times 10^4 \text{mM/kg/day} \times 74 \text{ mg/mM}}{(728)^{2.3}} = 0.15661$$

Therefore the parameters of the dose-response model are:

$n_t/N_t = 0.5$ $\qquad$ $dt^n = 0.15661$

$n_c/N_c = 0$ $\qquad$ $R = 0.026$ l/kg

$w = 0.35$ kg

With these parameters, the carcinogenic potency factor for humans, B_H, is 25.88 $(\text{mg/kg/day})^{-1}$. The result is that the water concentration should be less than 14 ng/l in order to keep the individual lifetime risk below 10^{-5}.

Derivation of Criterion for Diethylnitrosamine

Druckrey, *et al.* (1963) administered diethylnitrosamine to BD rats via drinking water in nine dose groups ranging from 0.075 to 14.2 mg/kg/day.

They found that the incidence of liver tumors increased with daily dose, d, and that the median time when tumors were observed, t_{50}, was less at higher doses and the relationship between d and t_{50} was $d(t_{50})^{2.3} = k$, where k is a constant. The value of the constant was not given in the 1963 publication, but a later paper by Drucker, et al. (1967) stated that $k = 0.35 \times 10^4$ mM/kg/day.

When this is converted to the units of mg/kg/day for dose and fractions of a lifetime (which is 728 days) for time, the value of k becomes:

$$k = \frac{0.35 \times 10^4 \text{ mM/kg/day} \times 102 \text{ mg/mM}}{(728)^{2.3}} = 0.09328.$$

Therefore, the parameters of the dose-response model are:

$n_t/N_t = 0.5$ $\quad$ $dt^n = 0.09328$

$n_c/N_c = 0$ $\quad$ $R = 0.20$ l/kg

$w = 0.35$ kg

With these parameters the carcinogenic potency factor for humans, B_H, is 43.46 $(\text{mg/kg/day})^{-1}$. The result is that the water concentration should be less than 8.0 ng/l in order to keep the individual lifetime risk below 10^{-5}.

Derivation of Criterion for Dibutylnitrosamine

Bertram and Crain (1970) administered dibutylnitrosamine via drinking water to C57BL/6 mice at dose levels of about 8 and 30 mg/kg/day until the animals became moribund or died. They found that dibutylnitrosamine induced tumors of the bladder and esophagus in both sexes. Using the bladder and/or esophageal tumor induction in males, the parameters of the extrapolation are:

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0	a
7.6	46/47
29.1	45/45

l_e (low dose) = 630 days

le (high dose) = 414 days

Le = 630 days

L = 630 days

w = 0.028 kg

R = 3.38 l/kg

With these parameters the carcinogenic potency factor for humans, q_1^*, is 5.43 $(mg/kg/day)^{-1}$. The result is that the water concentration should be less than 64 ng/l in order to keep the individual lifetime risk below 10^{-5}.

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[a]Specific incidence was not reported. Very low spontaneous incidence in controls was stated.

Derivation of Criterion of N-Nitrosopyrrolidine

Preussman, et al. (1977) found a dose-related incidence of hepatocellular carcinomas in Sprague-Dawley rats in a lifetime feeding study of N-nitrosopyrrolidine at levels of 0.3, 1.0, 3.0, and 10 mg/kg/day. The parameters of the extrapolation are:

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0.0	0/61
0.3	3/60
1.0	17/62
3.0	31/38
10	14/24
le = 728 days	w = 0.350 kg
Le = 630 days	R = 0.055 l/kg
L = 728 days	

With these parameters the carcinogenic potency factor for humans, q_1^*, is 2.13 $(mg/kg/day)^{-1}$. The result is that the water concentration should be less than 160 ng/l in order to keep the individual lifetime risk below 10^{-5}.

Derivation of Criterion of N-Nitrosodiphenylamine

Cardy, et al. (1979) found in a lifetime feeding study of F344 rats that n-nitrosodiphenylamine induced transitional-cell carcinomas of the urinary bladder in both sexes at significant incidences over matched controls. Using the female data the parameters of the extrapolation are:

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0	0/18
50	0/48
200	40/49
le = 700 days	w = 0.250 kg
Le = 700 days	R = 136 l/kg
L = 700 days	

With these parameters the carcinogenic potency factor for humans, q_1^*, is 4.92×10^{-3} $(mg/kg/day)^{-1}$. The result is that the water concentration should not exceed 49 µg/l in order to keep the lifetime human cancer risk below 10^{-5}.

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