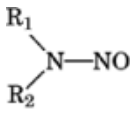
Kirk-Othmer Encyclopedia of Chemical Technology.

N-NITROSAMINES

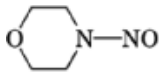
N-Nitrosodialkylamines (*N*-nitrosamines) were first characterized in the late nineteenth century (1, 2). Although they have been used as synthetic intermediates and solvents, and possess interesting structural and spectroscopic properties, their potential uses have been eclipsed by their toxicity and especially their genotoxicity (3–7). Most of the *N*-nitrosamines tested are carcinogenic in animals. Nitrosamines have induced tumors in every animal species tested (8). Although there is no direct causal evidence, humans are probably susceptible to cancers induced by nitrosamines, and several specific nitrosamines are strongly suspected to be human carcinogens (9–12). The primary focus of research on these compounds in the 1990s is therefore on biochemistry, utility as experimental mutagens and carcinogens, and on potential human exposure to them (13–28).

1. Properties

Many of the chemical, physical, and biological properties of more than 20 selected *N*-nitrosamines have been summarized (29). *N*-Nitrosamines **(1)** encompass a wide range of structural types because the single feature common to them is the NNO functionality, and there are few restrictions on the groups that can be attached to the remaining two valences on the amine nitrogen, where R_1 or R_2 can be alkyl, aryl, or mixed. When R_1 or $R_2 = CH_2X$, X may be H, alkyl, aryl, halogen, or alkoxy. When either R_1 or $R_2 = \text{aryl}$, various substituents may be attached to the rings. When R_1 or $R_2 = H$, or when $X = OH$, the resulting primary *N*-nitrosamines or α-hydroxy-*N*-nitrosamines are generally unstable (30–34). Examples of other structural types, including derivatives of cyclic amines such as *N*-nitrosomorpholine [59-89-2] **(2)** or *N*-nitrosopyrrolidine [930-55-2] **(3)**, have also been characterized (7, 8, 29, 35–42).



(1)



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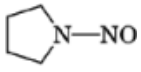
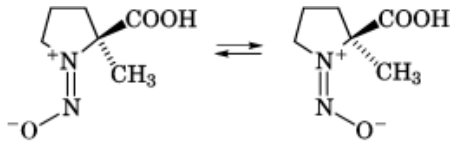


(3)

Table 1. Characteristic Spectral Properties of *N*-Nitrosamines

Property	Functionality involved	Value
uv–vis λ, nm	NNO	230–235, 330–375
ir absorption, cm^{-1}	N–N stretch	1040–1160
	N–O stretch	1430–1500
nmr absorption, ppm	α-CH (*E*)	5.8–6.9 (CCl_4)
		6.1–6.32 (C_6H_6)
	α-CH (*Z*)	6.3–6.6 (CCl_4)
		6.6–6.8 (C_6H_6)
mass spectral fragmentation, m/z	entire molecule	M, M-17, M-30, M-31

N-Nitrosamines typically are light yellow volatile solids or oils. The electron delocalization in the NNO functionality sufficiently restricts rotation around the N–N bond that the *E* **(4)** and *Z* **(5)** isomers of unsymmetrically substituted examples can often be separated (43).

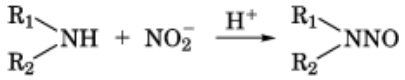


(4) **(5)**

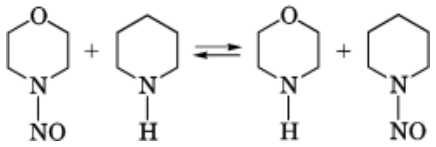
The spectroscopic properties of the *N*-nitrosamines, especially the nmr and mass spectra, vary widely depending on the substituents on the amine nitrogen (44–47). The nmr spectra are affected by the *E–Z* isomerism around the N–N partial double bond and by the axial–equatorial geometry resulting from conformational isomerism in the heterocycles (44, 45). Some general spectral characteristics for typical dialkylnitrosamines and simple heterocyclic nitrosamines are given in Table 1.

1.1. Synthesis

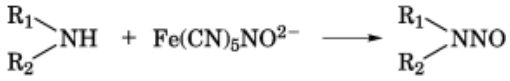
The classic laboratory synthesis of *N*-nitrosamines is the reaction of a secondary amine with acidic nitrite [14797-65-0] at ca pH 3. The primary nitrosating intermediate is N_2O_3 arising from nitrous acid [7782-77-6] (48).



Primary and tertiary amines also form *N*-nitrosamines (41, 49, 50). Although these nitrosations are generally slower and give lower yields than is the case with secondary amines, there are exceptions including some drugs such as aminopyrine [58-15-1] (49, 51, 52). There are a number of known catalysts for nitrosation reactions including halides, pseudohalides, sodium thiocyanate [534-18-9], or formaldehyde [50-00-0] (53, 54). Nitrosations of higher amines such as dihexylamine [143-16-8] are enhanced by micelle formation (55). Secondary amines can be nitrosated at nonacidic pH by N_2O_3 or N_2O_4, or by other NO donors such as nitrosyl chloride [2696-92-6] (56, 57). *N*-Nitrosamines can be formed under some conditions by transnitrosation which is the transfer of the NO group from a nitrosamine to an amine (58, 59).



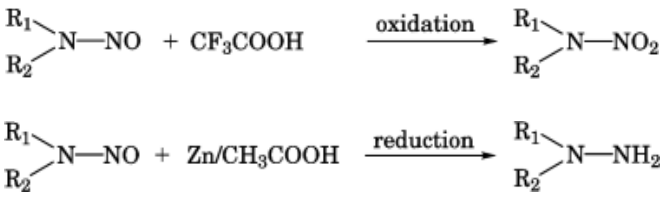
Efficient nitrosations of amines with inorganic nitrosyl compounds also have been reported (60).



Inhibition of nitrosation is generally accomplished by substances that compete effectively for the active nitrosating intermediate. *N*-Nitrosamine formation *in vitro* can be inhibited by ascorbic acid [50-81-7] (vitamin C) and α-tocopherol [59-02-9] (vitamin E) (61, 62), as well as by several other classes of compounds including pyrroles, phenols, and aziridines (63–65). Inhibition of intragastric nitrosation in humans by ascorbic acid and by foods such as fruit and vegetable juices or food extracts has been reported in several instances (26, 66, 67).

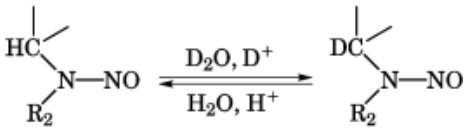
1.2. Reactions

The chemistry of the *N*-nitrosamines is extensive and will be only summarized here (8, 35, 42). Most of the reactions of the nitrosamines, with respect to their biological or environmental behavior, involve one of two main reactive centers, either the nitroso group itself or the C–H bonds adjacent (α) to the amine nitrogen. The nitroso group can be removed readily by a reaction which is essentially the reverse of the nitrosation reaction, or by oxidation or reduction (68, 69).

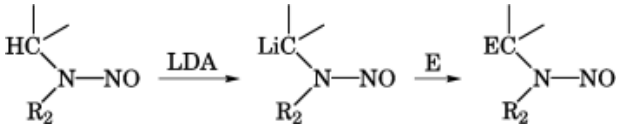


The effects of uv radiation on *N*-nitroso compounds depend on the pH and the medium. Under neutral conditions and in the absence of radical scavengers, these compounds often appear chemically stable, although the *E–Z* equilibrium, with respect to rotation around the N–N bond, can be affected (70). This apparent stability is due to rapid recombination of aminyl radicals and nitric oxide [10102-43-9] formed during photolysis. In the presence of radical scavengers nitrosamines decay rapidly (71). At lower pH, a variety of photoproducts are formed, including compounds attributed to photoelimination, photoreduction, and photo-oxidation (69). Low concentrations of most nitrosamines, even at neutral pH, can be eliminated by prolonged irradiation at 366 nm. This technique is used in the identification of *N*-nitrosamines that are present in low concentrations in complex mixtures (72).

Reactions at the α-carbons have been of considerable interest because it is at these positions that enzymatic oxidation, which is believed to initiate the events leading to carcinogenic metabolites, generally occurs (5, 7, 8, 73). The α-hydrogens exchange readily as shown in the following where D represents ^{2}H. This exchange apparently results from stabilization of an anionic intermediate by electron delocalization (74, 75).



This property has been exploited in syntheses of *N*-nitrosamine derivatives by the reaction of electrophiles (E) with α-lithiated intermediates. These intermediates are prepared by hydrogen–lithium exchange using lithium diisopropylamide [4111-54-0] (LDA) (76, 77).



2. Handling and Disposal

N-Nitrosamines are potentially hazardous and should be handled in designated hoods and with protective clothing. Nitrosamines can be destroyed by treatment with aluminum–nickel alloy under basic conditions (78, 79).

3. Analytical and Test Methods

The potential exposure of humans to *N*-nitrosamines through diet, occupational or other sources, or their possible formation *in vivo*, has created a continuing need for the detection and confirmation of low levels of *N*-nitrosamines in complex mixtures of organic chemicals or biological matrices. Many of the *N*-nitrosamines that are recognized as environmentally significant are sufficiently volatile and stable for analysis by gas chromatography (gc). The less volatile *N*-nitrosamines can be analyzed by high performance liquid chromatography (hplc) (35). A variety of detection techniques such as polarography, spectrophotometric cleavage of the NNO bond followed by detection of the resulting nitrite, alkali flame detection, electrolyte conductivity (Coulson detector), and mass spectrometry, have been used, but most of these techniques are not sufficiently sensitive, selective, or accessible for routine analysis of low levels of nitrosamines present in complex mixtures (80). The most significant contribution to *N*-nitrosamine analysis has been the thermal energy analyzer. This detector, which responds to nitric oxide released from the *N*-nitroso compounds by pyrolysis, is sensitive and highly selective for the NNO functionality and can be used for both gc and hplc, although it is somewhat limited as an hplc detector (81, 82). Some classes of compounds, eg, alkyl nitrites and C-nitroso compounds, give false-positive responses, but various screening methods, such as the destruction of nitrosamines by uv radiation, can usually distinguish these types of molecules from *N*-nitrosamines (70, 83, 84). Photohydrolytic systems based on release of nitric oxide by uv irradiation are used as alternative hplc detectors (82, 85). A method which is potentially useful for nonvolatile *N*-nitroso compounds and based on acidic denitrosation has been described (86).

Confirmation of the identities of nitrosamines generally is accomplished by gas chromatography–mass spectrometry (gc/ms) (46, 87). High resolution gc/ms, as well as gc/ms in various single-ion modes, can be used as specific detectors, especially when screening for particular nitrosamines (87) (see Analytical methods; Trace and residue analysis).

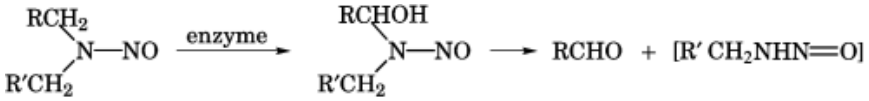
4. Health and Safety Factors

4.1. Toxicity

Many *N*-nitrosamines are toxic to animals and cells in culture (4, 6–8, 88). *N*-Nitrosodimethylamine [62-75-9] (NDMA) is known to be acutely toxic to the liver in humans, and exposure can result in death (89). Liver damage, diffuse bleeding, edema, and inflammation are toxic effects observed in humans as a result of acute and subacute exposure to NDMA. These effects closely resemble those observed in animals dosed with NDMA (89, 90).

4.2. Carcinogenicity

Some of the toxicological properties of a selected group of nitrosamines are listed in Table 2. The number of nitrosamines that have been tested for carcinogenicity exceeds 200. If related *N*-nitroso compounds such as nitrosamides or nitrosoureas are included, the number of tested compounds is well over 300 (5, 7, 8, 29, 35, 38, 91). Most are carcinogenic, although the potency varies dramatically within the series (7, 75). The mean dose for the formation of tumors by *N*-nitrosodiethylamine (NDEA), for example, is only ca 0.0006 mol/kg body weight, whereas *N*-nitrosoproline is considered noncarcinogenic (7, 92). Carcinogenicity has been observed both with single, relatively large doses and with long-term chronic exposure to lower doses (7, 93). The *N*-nitrosamines generally are organ selective (7, 8, 94). *N*-Nitrosodimethylamine, for example, is primarily a liver carcinogen, *N*-nitrosomethylbenzylamine is primarily an esophageal carcinogen, and *N*-nitrosobutyl(4-hydroxybutyl)amine is primarily a bladder carcinogen. Other target organs include the nose, bladder, pancreas, lungs, and kidneys (7, 8, 95). Most nitrosamines are not direct-acting carcinogens, but require metabolic activation in order to exert their carcinogenic effect. The potency and the organ selectivity of the *N*-nitrosamines are therefore determined by complex interactions involving molecular structures and the spectrum of metabolizing enzymes in the test animal (96, 97). There are consequently species- and sex-related differences in both potency and organ selectivity (7, 8, 98, 99). *N*-Nitroso-*N*-methyl(2-oxopropyl)amine, for example, is an esophageal carcinogen in the rat and a pancreatic carcinogen in the hamster (100, 101). The principal enzymes involved in nitrosamine metabolism are the cytochrome P450 enzymes, with P450 2E1 being the most widely studied (102). Other known or suspected nitrosamine-metabolizing enzyme systems include sulfotransferases, alcohol dehydrogenases, and peroxidases (61, 102–104). The mechanisms involved in nitrosamine carcinogenesis are not completely understood in detail, especially with respect to organ selectivity or for nitrosamines that have no α-hydrogens (*N*-nitrosodiphenylamine [86-30-6]) or are oxidized at other positions (*N*-nitrosodiethanolamine or *N*-nitroso-*N*-methyl(2-oxopropyl)amine)). The following sequence, however, is generally accepted for the metabolic activation of most simple dialkyl *N*-nitrosamines to electrophilic intermediates (5, 7, 8, 31, 32, 87, 105–108). Other metabolic pathways include denitrosation, oxidation at sites other than the α-hydrogens, and chain-shortening (99, 109–112).



(6) (7)

$$[R'CH_2NHN{=}O] \rightleftharpoons [R'CH_2N{=}NOH] \xrightarrow{H^+} [R'CH_2N{=}NOH_2^+] \xrightarrow{?} [R'CH_2N^+{}_2]$$

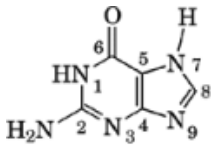
(7) (8) (9) (10)

Table 2. Toxicological Properties of Some Representative *N*-Nitrosamines in the BD Rat[a]

Compound	CAS Registry Number	LD_{50}	$\log(1/D_{50})$[b]	Principal target organ
N-nitrosodimethylamine (NDMA)	[62-75-9]	40	2.3	liver
N-nitrosodiethylamine (NDEA)	[55-18-5]	280	3.2	liver, esophagus
N-nitrosodiethanolamine (NDELA)	[1116-54-7]	7500	0.005	liver
N-nitrosodipropylamine	[621-64-7]	480	0.05	liver, esophagus
N-nitrosodiisopropylamine	[601-77-4]	850	2.1	liver
N-nitrosopyrrolidine (NPYR)	[930-55-2]	900	1.0	liver
N-nitrosomorpholine (NMOR)	[59-89-2]	320	1.9	liver
N-nitrosodicyclohexylamine	[947-92-2]		[c]	
N-nitrosoproline (NPRO)	[7519-36-0]		[c]	
N-nitrosomethyl(benzyl)amine	[937-40-6]	18	3.1	esophagus
N-nitrosopiperidine	[100-75-4]	200	1.9	liver, esophagus
N-nitrosonornicotine	[16543-55-8]		[d]	

[a] BD rat represents a particular strain used to test carcinogenicity of some *N*-nitroso compounds. Refs. 7 and 62.
[b] D_{50}: dose causing tumors in 50% of the test animals; increasing values for $\log(1/D_{50})$ represent higher carcinogenicity (51).
[c] Not carcinogenic to the BD rat.
[d] Not investigated in the BD rat. Suspected human carcinogen (12).

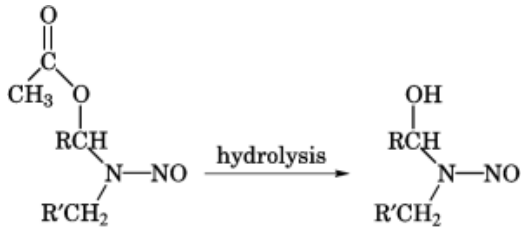
There is substantial evidence for the initial enzymatic α-oxidation to **(6)** and for the alkylation of nucleic acids by the resulting electrophiles (5, 7, 31, 32, 108, 113–116). The intervening intermediates (**7–10**) are hypothesized largely by analogy with the known behavior of primary nitrosamines (30, 116) and from reactive synthetic α-oxidized nitrosamines (33, 34). Oxidation of nitrosamines in microsomes may lead to intermediates (**9**) and (**10**) *in vitro*, and these may also be responsible for alkylation of nucleic acids *in vivo* (106, 107). Microsomes are cell particles of the smallest size with a piece of endoplasmic reticulum attached, and contain the predominant biotransformation systems, including the cytochrome P450 enzymes. Tumors are thought to arise from mispairing during subsequent replications of the alkylated DNA. Most alkylation appears to occur at the N-7 position of DNA guanine **(11)**, but alkylation at the O-6 position of guanine is more closely correlated with tumor formation than is alkylation at N-7 (114).



(11)

4.3. Mutagenicity

The *N*-nitrosamines, in general, induce mutations in standard bacterial-tester strains (117). As with carcinogenicity, enzymatic activation, typically with liver microsomal preparations, is required. Certain substituted *N*-nitrosamine derivatives **(12)** induce mutations without microsomal activation (31, 33, 34). Because the α-acetoxy derivatives can hydrolyze to the corresponding α-hydroxy compounds, this is consistent with the hypothesis that enzymatic oxidation leads to the formation of such unstable α-hydroxy intermediates **(13)** (118). However, for simple *N*-nitrosamines, no systematic relationship has been found between carcinogenicity and mutagenicity (117, 119–123).



(12) (13)

4.4. Human Exposure

N-Nitrosamines have been reported in pesticide preparations (124), corrosion inhibitors (125), lubricating fluids and cosmetics (qv), ie, *N*-nitrosodiethanolamine (126, 127), sunscreens (128), rubber products including baby-bottle nipples and pacifiers (115, 129), foods including cheese, processed meats, beer (qv), cooked bacon, and powdered milk (42, 130–133), and tobacco products (52, 134–136) (see Insect control technology; Corrosion and corrosion control; Lubrication and lubricants; Food toxicants, naturally occurring). In addition to exposure by preformed *N*-nitroso compounds there is exposure by nitrosation of amines in the mouth, stomach, or other sites in the body. Formation of nitrosamines inside the body has been demonstrated unambiguously in humans and in experimental animals. Amine- and nitrite-fed animals develop tumors that are identical with those expected from the corresponding *N*-nitrosamines (137). The formation *in vivo* of *N*-nitrosamines from amines and nitrite or nitrite precursors can be directly observed for nonmetabolized nitrosamines such as *N*-nitrosoproline (138) and when nitrosamine metabolism is blocked (53, 139, 140). Nitrosation in humans can be monitored by urinary levels of *N*-nitrosoproline or *N*-nitrosothiazolidine carboxylic acid (nitrosothioproline) (92, 141–144). In the case of *N*-nitrosoproline, the precursors (proline and nitrate) can be administered systematically (92). These results have stimulated a large number of studies concerning possible relationships between nitrosation in the body and elevated cancer risk (138, 145–149), and whether this nitrosation can be blocked or inhibited, especially by dietary components (26, 66, 67, 138, 150).

Nitrite is also formed in the body. Saliva, for example, contains efficient nitrate-reducing bacteria and consequently provides a constant low (ca 7 pg/mL) level of nitrite, that increases rapidly following ingestion of nitrate (151, 152). Nitrate synthesis involves production of nitric oxide by a large number of cell types including endothelial cells, which line cavities and vessels, macrophages, which engulf and digest cells and microorganisms, neurons, and liver cells (153–156). The nitric oxide can react with oxygen to form N_2O_3, which is a nitrosating agent at neutral or near-neutral pH (48, 57, 157, 158). There is thus the possibility of nitrosamine formation at sites in the body other than the stomach.

There is insufficient evidence to unequivocally link nitrosamine exposure to elevated risk for human cancer (159). There are, however, a number of specific cases, especially with respect to the tobacco-related nitrosamines, in which exposure to *N*-nitroso compounds is of concern. The strongest evidence in this context is probably that relating to oral cancer rates among habitual users of smokeless tobacco (snuff). Oral cancer rates among this group are significantly elevated over those of nonusers, and *N*-nitrosonornicotine, and 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone [64091-91-4], both of which are potent animal carcinogens, are present along with less potent *N*-nitroso compounds in smokeless tobacco (52, 160–163). Urinary metabolites of these compounds can be detected in users. Both DNA and protein adducts, which are evidence for the presence of the active electrophilic metabolites of the nitrosamines, have been detected in human samples (12, 164–166). This evidence, although remaining circumstantial, is nonetheless becoming increasingly persuasive (149, 150, 164, 166).

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General References

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