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# Carcinogenic *N*-Nitrosamines. Formation, Properties, and Analysis

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Literature data on the formation of carcinogenic *N*-nitrosamines in the environment, their physicochemical properties, their action on the human organism, the methods for their microanalysis, and also their content in the atmosphere, water sources, soil, and industrial agricultural and food products are described systematically and surveyed. The principal features of this comparatively new class of powerful chemical carcinogens are indicated.
The bibliography includes 284 references.

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## I. INTRODUCTION

A distinct tendency towards an increase in the pollution of the environment as a consequence of the intense technological activity by man can be traced during the last decade. A constant increase in the amount of bound nitrogen in the biosphere as a result of the increase in the scale of industrial manufacture, the consumption of fuel, the use of nitrogen-containing fertilisers and pesticides, the ejection into the atmosphere of nitrogen oxides, ammonia, amines, etc. has been noted.

The pollution of various objects by compounds with a high toxicity and carcinogenic and mutagenic properties is especially undesirable. Among substances of this type, the large group of *N*-nitroso-compounds, in which aliphatic and certain cyclic *N*-nitrosamines (NA) exhibit a high carcinogenic activity, can be distinguished. These compounds have been known for more than 100 years but due attention has not been paid to their investigation. Certain NA have been used in industry as solvents, intermediates for the synthesis of substituted hydrazines, etc. In the middle of the 1950's, it was observed that *N*-nitrosodimethylamine induces liver tumours in animals and in subsequent years it was established that about 200 different nitroso-compounds exhibit a carcinogenic activity.[1-5] About 1500 publications annually have been devoted recently to the study of the properties of this comparatively new class of carcinogens and especially of their biological activity.[6]

NA are fairly stable and are capable of circulating for a long time in the environment, which results in the pollution of various objects. It has been established that NA are contained in water, air, soil, food and industrial products, agricultural pesticides and herbicides, medicinal drugs, etc. and that the compounds most often detected are *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPyr), and *N*-nitrosopiperidine (NPip). *N*-Nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosoethylmethylamine (NEMA), *N*-nitrosomorpholine (NMor), *N*-nitrosothiazolidine (NThia), etc. are more rarely encountered.[3-5,7-11]

An important feature of NA compared with other classes of carcinogens is the possibility of their ready formation directly in various objects as a result of nitrosation. Amines and many other nitrogen-containing substances as well as different nitrosating agents are widely distributed in water, air, food products etc.

Data on the biological activity of NA and their presence in objects in the environment have been published in a series of reviews and monographs.[2-4,8,12-20] This review deals with processes leading to the formation of NA, the chemical reactions determining their properties and transformations, and methods for their analysis and surveys data on the content of these compounds in various objects.

## II. METHODS OF SYNTHESIS OF *N*-NITROSAMINES

The main process leading to the formation of NA is nitrosation. The compounds nitrosated can be various mono-, di- and polyamines as well as other nitrogen-containing compounds. Secondary and tertiary amines as well as other compounds containing the $(CH_3)_2N$ and $(C_2H_5)_2N$ groups are most readily and rapidly nitrosated. The nitrosating agents are usually nitrous acid derivatives XNO (X = halogen, $NO_2$, $NO_3$ $OH_2^+$, or OR) as well as the nitrosonium cation, etc.[21-25] The nitrite ion and free $HNO_2$ are converted into active nitrosating agents in an acid medium:[21,23,25-30]

$$2NO_2^- \xrightarrow{H^+} 2HNO_2 \rightleftarrows H_2ONO^+ + NO_2^- \rightleftarrows N_2O_3 + H_2O\,.$$

At low pH, there is a possibility of the formation of the nitrosonium cation:

$$N_2O_3 + 3H_2SO_4 \rightarrow 2NO^+ + 3HSO_4^- + H_3O^+\,.$$

In the presence of nitrogen halides, $HNO_2$ is capable of giving rise to the corresponding nitrosyl halides;[21,31]

$$HNO_2 + HCl \rightleftarrows NOCl + H_2O\,.$$

Nitrosating agents can be arranged in the following sequence in order of the decrease in their activity: $NO^+ > H_2ONO^+ > NOCl > N_2O_3$.[21,25,32] In aqueous solutions of nitrites and nitrous acid at pH below 5, various nitrosating agents and their mixtures can be present.[21,23,33]

### 1. Nitrosation of Amines

The nitrosation of secondary amines is usually used for the synthesis of NA and is the main cause of their formation in various objects.[12,21,22,26,29,34] Nitrosation by various agents takes place in water, mixtures of water with organic solvents, in organic solvents, in the gas phase, and also directly in various objects over a wide temperature range.[4,17,22,29,30,33-39] In solutions, this reaction ensures a high yield of NA:[21,22,29,36,40,41]

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!NH + NOY \rightarrow \left[\begin{matrix} R \\ R' \end{matrix}\!\!>\!NHNO\right]^+ Y^- \rightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!NNO + HY\,.$$

Under certain conditions, secondary amines are capable of being converted into the corresponding NA on treatment with not only active nitrosating substances but even sodium nitrite. In aqueous solutions of $NaNO_2$ and a secondary amine, the following reactions are possible under the influence of sunlight, UV light, or γ-radiation:[42-45]

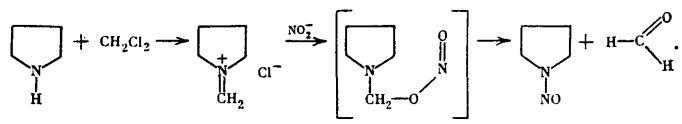
$$2NO_2^- + H_2O \rightarrow N_2O_3 + 2HO^-,$$

$$N_2O_3 + \underset{R'}{\overset{R}{>}}NH \xrightarrow{HO^-} \underset{R'}{\overset{R}{>}}N{-}NO + H_2O + NO_2^-,$$

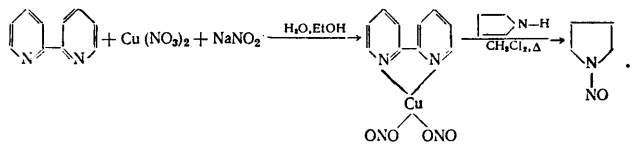
$$2NO_2^- + 2H_2O \rightarrow N_2O_4 + 2HO^- + 2HO^-,$$

$$N_2O_4 + \underset{R'}{\overset{R}{>}}NH \xrightarrow{HO^-} \underset{R'}{\overset{R}{>}}N{-}NO + H_2O + NO_3^-.$$

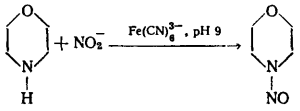
Certain secondary amines are nitrosated in methylene chloride with sodium nitrite,[30,35,46] for example:[35,46,47]



A series of metal salts promote the reaction of secondary amines and nitrites in neutral and basic media.[22,29,47-49] Thus pyrrolidine interacts fairly readily with $NaNO_2$, 2,2′-bipyridyl, and copper nitrate:[49]



Instead of 2,2′-bipyridyl, it is possible to use pyridine although with less success.[49] The reaction of morpholine with $NaNO_2$ is promoted by hexacyanoferrate(III):[21,49]



Secondary amines interact analogously with sodium nitroprusside:[22,50,51]

$$\underset{R'}{\overset{R}{>}}NH + Fe(CN)_5NO^{2-} \xrightarrow{OH^-} \underset{R'}{\overset{R}{>}}NNO.$$

Presumably the promoting action of hexacyanoferrate(III) on the nitrosation reaction is caused by the preliminary formation of the nitroprusside ion. This has been confirmed to some extent by the interaction of hexacyanoferrate(II) with nitrite:[49]

$$Fe(CN)_6^{4-} + NO_2^- \rightarrow Fe(CN)_5NO_2^{4-} + CN^-,$$

$$Fe(CN)_5NO_2^{4-} + 2H^+ \rightarrow Fe(CN)_5NO^{2-} + H_2O.$$

The nitrosation of secondary amines in the presence of certain aldehydes, for example, formaldehyde takes place in an alkaline medium.[4,28,47,52]

The nitrosation of secondary amines by gaseous nitrogen oxide is of practical interest from the standpoint of the formation of NA in various objects. Air usually contains $N_2O_3$ and $N_2O_4$ which are capable of interacting with secondary amines dissolved in water or organic solvents and also those present in various objects:[29,36,27,42,53]

$$2\,\underset{R'}{\overset{R}{>}}NH + 2N_2O_4 \rightarrow \underset{R'}{\overset{R}{>}}N{-}NO + \underset{R'}{\overset{R}{>}}N{-}NO_2 + HNO_3 + HNO_2.$$

On treatment with gaseous $N_2O_3$ nd $N_2O_4$, NA are formed in aqueous solutions over a wide pH range, even at pH > 5, which can be explained by the high rate of nitrosation of secondary amines compared with hydrolytic reactions.[29,36,42] Analogous results have been obtained in the nitrosation with gaseous nitrosyl chloride.[21,36,42] NA are also formed in air from secondary amines and gaseous nitrogen oxides.[36,37,54-57] It is believed that this reaction is initiated by photodissociation and takes place via a radical mechanism:[36,37,42,56,58]

$$HONO \xrightarrow{h\nu} NO^{\cdot} + HO^{\cdot} \xrightarrow{\underset{R'}{\overset{R}{>}}NH} H_2O + \underset{R'}{\overset{R}{>}}N^{\cdot} \xrightarrow{NO^{\cdot}} \underset{R'}{\overset{R}{>}}N{-}NO.$$

Certain organic compounds containing the $NO_2$ or ONO groups are also capable of nitrosating secondary amines. Aliphatic polynitro-compounds are known to be capable of acting as "$NO_2$-producing substances".[32,50] It has been shown that tetranitromethane, 2,2-dinitropropanol, and 2-bromo-2-nitropropane-1,3-diol afford NMor on heating with morpholine.[59] The mechanism of this reaction has been inadequately elucidated.

The nitrosating activity of nitrous acid esters has been more fully investigated:[31,42,60-65]

$$R''{-}ONO + HY \rightarrow \left[\begin{matrix} R''{-}ONO \\ | \\ H \end{matrix}\right]^+ Y^- \rightarrow R''{-}OH + Y{-}NO,$$

$$\underset{R'}{\overset{R}{>}}NH + Y{-}NO \rightarrow \underset{R'}{\overset{R}{>}}N{-}NO + HY.$$

On exposure to UV irradiation, this reaction can have a radical mechanism.[12,31,61] The thioesters of nitrous acid react analogously.[60,62,65-68]

The transnitrosation reaction, which is not used for the synthesis of NA but can occur in various objects and in the organism, is of significant interest. Various nitroso-compounds, especially those which have no carcinogenic activity or their carcinogenic activity is weak, can act as nitrosating agents and transnitrosation can result in the formation of active carcinogens.[69-72] A series of nitroso-compounds which are quite effective in this reaction, including heterocyclic and aromatic compounds and substituted ureas and urethanes, have been discovered. As a rule, they are all readily obtained by nitrosating substances containing $\rangle$NH group and have a fairly labile NO group.[69,72,73] The nitrosation reactions with participation of the non-carcinogenic nitrosodiphenylamine (NDPhA), used in the rubber industry, have been studied in fair detail. The conditions for the occurrence of this reaction both in various solutions and in the organism have been discovered and a probable process mechanism has been proposed:[70,71,73]

$$(C_6H_5)_2N{-}NO + HY \rightarrow \left[(C_6H_5)_2N\begin{matrix} H \\ NO \end{matrix}\right]^+ Y^- \rightarrow (C_6H_5)_2NH + Y{-}NO,$$

$$\underset{R'}{\overset{R}{>}}NH + Y{-}NO \rightarrow \underset{R'}{\overset{R}{>}}N{-}NO + HY.$$

The reaction is accelerated in the presence of $SCN^-$ and $I^-$ ions.[70,73] When solutions of NDPhA are heated, the following reactions occur:[21,71]

$$2\,(C_6H_5)_2N{-}NO \xrightarrow{\Delta} (C_6H_5)_2N{-}N(C_6H_5)_2 + 2NO,$$

$$\underset{R'}{\overset{R}{>}}NH + NO \xrightarrow{O_2 \text{ or catalyst}} \underset{R'}{\overset{R}{>}}N{-}NO.$$

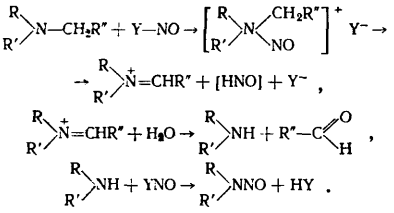
The reaction of NDPhA with dibutylamine results in the formation of NDPA, that with morpholine affords NMor and that with tetramethylthiuran disulphide gives rise to NDMA.[71] Mono- and di-isonitrosopiperazines are also effective in transnitrosation reactions.[41,73]

Transnitrosation with nitrosoaminoacids, which are readily formed in various objects and in the organism, has been investigated.[70-73] Thus the interaction of nitrosoproline or hydroxynitrosoproline with morpholine in animal organisms has led to the detection of NMor.[69]

The possibility of nitrosating secondary amines by nitrates after the conversion of the latter into $NO_2^-$, $NO_2$, or NO is of practical interest. The microbiological and chemical reduction of nitrates occurs widely both in objects in the environment and in the organism.[7,13,18,33,34,42,45,47,74,75] The presence of high concentrations of nitrates in the soil, in water, in mineral fertilisers, in food products, in various types of animal feed, etc. makes a significant contribution to the pollution of various objects by nitrosamines.

Numerous studies have demonstrated that NA are formed from secondary amines and various nitrosating agents directly in human and animal organisms.[8,12,17,18,53,76-82]

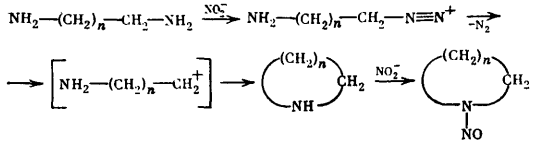
The nitrosation of various tertiary amines, which takes place in a complex manner, has been fairly widely investigated. The majority of workers believe that the nitrosation is preceded by a cleavage stage:[27,34,41,63,83,87]



This mechanism has been demonstrated by isolating the reaction mixture and identifying the NA, the corresponding aldehyde, and the [HNO] conversion product—nitrous oxide.[41,84] Tertiary amines are nitrosated with more difficulty than secondary amines; even under optimum conditions, the yield of NA is significantly lower and rarely reaches 40–50%.[32,41,83,84,87,88] However, it has been noted that trimethylamine and also trimethylamine oxide form NDMA fairly rapidly.[27,41,84,89,90] The ease of nitrosation of tertiary amines decreases appreciably with increase in the number of carbon atoms in the amine.[21,41,84,87,89,90] As a rule, a series of side products are formed together with the NA under severe conditions.[41,83,87,88] The formation of NA from tertiary amines has been demonstrated in various objects and also directly in the organism.[13,34,41,76,84,89,90]

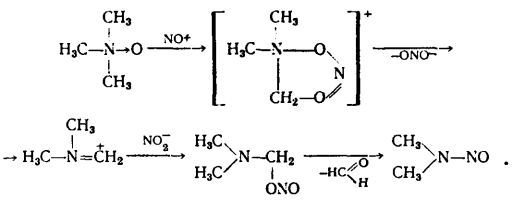
The interaction of nitrosating agents with primary amines has been less thoroughly investigated. These reactions proceed via various fairly complex mechanisms and the yield of the NA is in most cases insignificant.[12,21,32,91,92]

Certain primary di- and poly-amines, including biologically active compounds such as putrescine, cadaverine, spermine, and spermidine can give rise to cyclic NA on nitrosation:[4,5,91,93]



Putrescine reactions with nitrous acid to produce NPyr in yields up to 1.6%.[92-94] For comparison, we may note that the yield of NDBA in the nitrosation of n-butylamine under the same conditions is only 0.01%.[92,94] The nitrosation of primary amines in various objects and in the organism is possible, but this gives rise to only small amounts of NA.[4,91-93]

The nitrosation of quaternary ammonium compounds takes place at elevated temperatures and high concentrations of the starting materials, the yield of the NA being insignificant.[95-97] The mechanism of these processes has been inadequately investigated. It is assumed that tetramethylammonium salts are less capable of being nitrosated than dimethylamine oxide:[84,85,95,97]

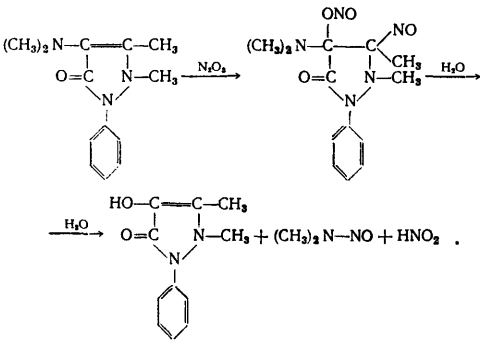


Tetra-alkylammonium compounds can be nitrosated in heterogeneous media on treatment with gaseous nitrogen oxides.[89,90] This reaction leads to pollution with nitrosamines of the products in which high concentrations of trimethylamine, acetylcholine chloride, choline chloride, etc. have been detected.[84,89,90,96,97]

## 2. Nitrosation of Other Nitrogen-containing Substances

The nitrosation of a series of nitrogen-containing substances with formation of NA can occur in two ways: the insertion of the NO-group in the molecule and the formation of complex nitroso-compounds or the cleavage of the nitrogen-carbon bonds and the interaction of the amino-groups with nitrosating agents. Reactions of the first type have been described in a number of studies.[4,12,27,95,98,99] We shall consider processes which result in the formation of volatile NA—the "nitrosative deamination" reactions.[21,24,100-102]

Certain nitrogen-containing substances are capable of reacting with nitrosating agents at unexpectedly high rates. Thus aminopyrine (2,3-dimethyl-4-dimethylamino-1-phenyl-3-pyrazolin-5-one) is rapidly nitrosated in homogeneous and heterogeneous media during manufacture and storage and also in the organism:[14,58,100,102-109]

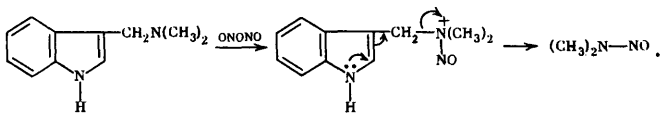


It is assumed that the high rate of this reaction is caused by the presence in the molecule of the "active" dimethylamino-group.[21,15,104] In the medicinal forms of amidopyrine, especially in tablets, the distribution of NDMA is not homogeneous: the greatest amounts are contained on the surface, which can be explained by the action of nitrogen oxide present in air within industrial and storage buildings.[58,104,110] Even the introduction of nitrosation inhibitors does not fully prevent the formation of NDMA.[58,104,106]

Tetracycline, penicillin, etc. react vigorously with nitrosating agents.[86,102,103,109] Other medicinal drugs and metabolities, for example, phenacetine, imizine, and aminazin, are also capable of "nitrosative deamination".[14,100,102,106-111]

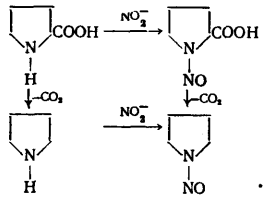
Alkaloids containing the dimethylamino-group can be a source for the formation of NA. This process has been studied in detail in relation to gramine and hordenine—the alkaloids present in barley malt used in the manufacture of beer.[23,27,101] The rate of reaction, which proceeds in accordance with the mechanism proposed by the authors, is close to the rate of nitrosation of dimethylamine, which

can be explained by the presence of the active dimethylamino-group in the gramine molecule:[27,101]



Hordenine is nitrosated much more slowly.[27,110]

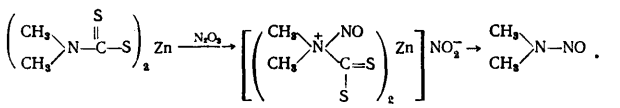
It is also possible to obtain NA from certain aminoacids and the reaction can occur via two main pathways:



In the nitrosation and decarboxylation of aminoacids such as arginine and ornithine, NPyr and NPip have been detected. The ease of formation of NA from aminoacids is attributed to the capacity of the latter for decarboxylation; for example, proline and its derivatives are effectively nitrosated.[24,93,112]

NA are formed also from certain insecticides, pesticides, and herbicides. The dimethylammonium salts of various acids, for example, 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DB (2,4-dichlorophenoxybutyric acid), 2,3,6-TCBA (trichlorobenzoic acid), MCP (4-chlorotolyloxyacetic acid), MCPP [2-(4-chloro-2-methylphenoxy)propionic acid], etc., are especially readily nitrosated.[96,103,107,113-115]

The "nitrosative deamination" of a series of pesticides and herbicides containing dialkylamino-group leads to the formation of NA:[95]



NDMA has been isolated from the products of the nitrosation of thiram(tetramethylthiuram disulphide) and ferbam [tris(dimethylcarbamodithioato-*SS'*)iron], NDPA has been isolated from trifluralin (ααα-trifluoro-2,6-dinitro-*NN*-dipropyl-*p*-toluidine), isopropalin (2,6-dinitro-4,*NN*-tripropylaniline), and nitrosobutylethylamine has been obtained from benefin (*N*-butyl-*N*-ethyl-ααα-trifluoro-2,6-dinitro-*p*-toluidine).[95,96,103,113,115,116] The pesticides which are quaternary ammonium compounds and derivatives of morpholine afford NA on interaction with nitrites.[96,103,107,114,117]

Certain products, widely used in the rubber industry, for example, vulcanisation accelerators and stabilisers, are capable of "nitrosative deamination". This applies mainly to substances containing the dialkylamino-group and to derivatives of morpholine.[38,71,99,118-121]

## 3. Catalysis and Inhibition of the Nitrosation Reaction

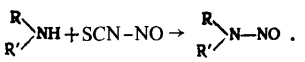
The rate of nitrosation depends on a number of factors among which the structure, basicity, and concentration of the nitrogen-containing compounds to be nitrosated, the properties and concentration of the nitrosating agent, the pH of the medium, temperature, the homogeneity of the reaction mixture, the nature of the solvent, and also the presence of catalysts or reaction inhibitors are important. The rate of reaction of various aliphatic amines with nitrites in weakly acid aqueous solutions is proportional to the concentration of the amine and the square of the concentration of nitrous acid. The kinetics of such reactions have been examined in detail.[21,32,34] The concentrations of the non-protonated form of the amine and of free nitrous acid are of decisive importance for the rapid occurrence of the reaction. The maximum rate of nitrosation of secondary amines with a high or moderate basicity, from which carcinogenic NA are usually formed, has been noted at pH close to 3.4, which corresponds to the dissociation constant of nitrous acid.[21,34,122-124] With increase in pH, the rate of reaction falls (approximately by an order of magnitude for each pH unit), but the NA are formed also at pH 5–6, while in the presence of promoting agents and certain micro-organisms, they are formed at pH > 7.[22,29,34,47-49,123] At low pH, a decrease in the rate of nitrosation was observed as a consequence of the fall in the concentration of the non-protonated form of the amine. For weakly basic amines, a decrease in pH affects less markedly the rate of their nitrosation since under these conditions the concentration of the non-protonated form of the amine is also considerable.[21,34,102]

The rate of formation of NA from amines decreases in the following sequence: morpholine > pyrrolidine ⩾ piperidine > dimethylamine > diethylamine > di-n-propylamine ⩾ di-isopropylamine.[23,33,34,102]

In real objects and in the organism, the conditions for the occurrence of nitrosation are less favourable than in solution but prolonged interaction of the reactants can lead to the accumulation of significant amounts of the NA. In this case the presence of a catalyst or inhibitors of the nitrosation reaction in the reaction medium acquires decisive importance.[5,6,10,81,125-127] The accelerating or retarding effect depends on the reaction medium; certain compounds, for example, phenols, can exhibit the opposite influence under different conditions.

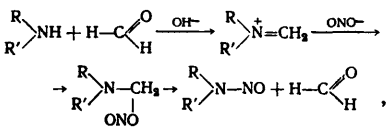
*(a) Catalysts.* A considerable number of compounds catalysing nitrosation are known.[4,5,10,47,59,115,125,126,128,129] Nucleophilic anions such as thiocyanates and halides are capable of accelerating this reaction significantly.[47,125,126,129-131] In terms of their activities, they can be arranged in the sequence $SCN^- > I^- \geqslant Br^- > Cl^-$.[21,126,131] In the case of $SCN^-$, the reaction proceeds via the formation of the fairly stable nitrosyl thiocyanate:[55,59,69,126,129,130,132]

$$HONO + SCN^- + H^+ \rightleftarrows SCN{-}NO + H_2O,$$



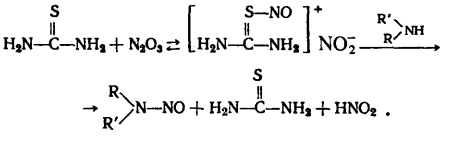
The thiocyanate accelerates this reaction at low pH in solution and also in various objects and in the organism.[47,126,130,132] The $I^-$ ion is an effective nitrosation catalyst; its activity is manifested under different conditions, including heterogeneous nitrosation with nitric oxide.[42,47,126,131] In the presence of $I^-$ and also $Br^-$ and $Cl^-$, nitrosation proceeds via the formation of nitrosyl halides.[31,47,131]

A number of carbonyl compounds accelerate the synthesis of NA. Formaldehyde shows the highest efficiency; chloral, benzaldehyde derivatives, pyridoxal, mabnodialdehyde, hexachloro- and hexafluoro-acetones, etc. are also active.[28,47,52,125,133] The proposed mechanism of the reactions occurring with participation of carbonyl compounds at pH ⩾ 7 includes the formation of iminium ions:[4,47,52,133]

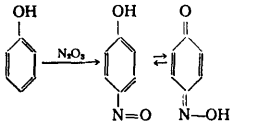


Other substances evolving formaldehyde or reacting analogously probably also catalyse the nitrosation reaction.

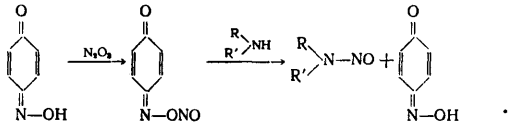
The compounds capable of accelerating this reaction include thiourea and certain thiols.[47,66,130,134] In the case of thiourea, which catalyses the nitrosation of secondary amines at a pH of about 4.0, the reaction proceeds via the *S*-nitroso-adduct:[47,134]



The situation is more complicated with phenols and certain other OH-containing substances which are capable of exerting both catalytic and inhibiting influences on the nitrosation reaction depending on the structure, the pH of the medium, etc.[42,47,66,69,80,135-138] Phenol reacts rapidly with nitrosating agents, for example, with $HNO_2$, at pH < 4:[47,135]



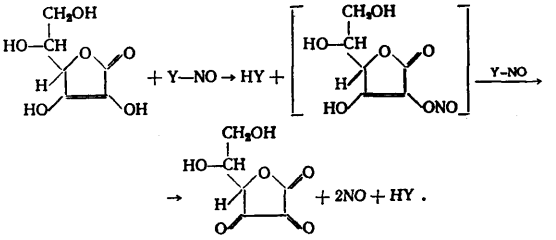
Quinone monoxime is rapidly nitrosated, probably affording the *O*-nitro-derivative, which reacts with the amine:[47,66,135-137]



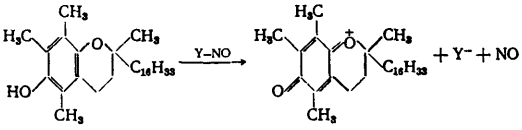
The catalytic action of resorcinol can be explained analogously.[80,135,139] In principle, under certain coditions catechol and hydroquinone can exhibit an accelerating effect;[47,138] however, the probability of their nitrosation is low, since they show a greater tendency to form the corresponding benzoquinones under these conditions.[80,135,138] Other compounds containing the OH group, for example gallic and chlorogenic acids, camphorol, and quercetin, can likewise catalyse the formation of NA.[32,135]

The ability to accelerate the nitrosation reaction has also been discovered in certain unsaturated compounds, surfactants, conjugate bases of bile acids, etc.[32,140] We may note that certain species of micro-organisms are able to accelerate significantly the formation of NA.[47,74]

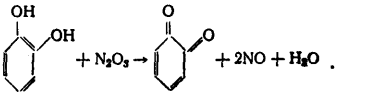
*(b) Inhibitors.* A large number of compounds able to inhibit the formation of NA have been described.[4,5,12,21,47,106,115,125,126] Naturally, the retardation of the reaction is achieved most simply by altering the conditions and in the first place the pH. This is difficult to achieve in various objects and in the organism. For this reason, much attention has been devoted to the search for effective inhibitors and the determination of the mechanism of their action. Virtually all the compounds proposed for this purpose show an inhibiting influence on the reaction as a result of the inactivation of nitrosating agents, but this can be achieved in different ways. Many substances inhibiting the formation of NA react with nitrosating agents, reducing them to the relatively inactive nitric oxide. Most studies have probably been devoted to ascorbic acid and its derivatives, which are able to inhibit effectively in this way the nitrosation reaction over a wide pH range:[6,46,47,55,68,81,106,115,140-144]



Ascorbic acid is especially active in hydrophilic media. The use of α-tocopherol in hydrophobic media has been recommended:[47,81,115,140,142,143,145]



Together with α-tocopherol, one can also use ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) and dihydroethoxyquin.[146] The oxidation of catechol, hydroquinone, and derivatives of gallic acid and polyphenols, which have been proposed as inhibitors, capable for forming *o*- and *p*-benzoquinones, proceeds via the same pathway:[47,55,69,80,120,135,138,139]



Sulphur dioxide and the bisulphite ion reduce the nitrosating agents to nitric or nitrous oxide:[32,115]

$$SO_2+N_2O_3+H_2O\rightarrow 2NO+H_2SO_4,$$
$$SO_2+2NO+H_2O\rightarrow N_2O+H_2SO_4.$$

Hydroxylamine, various hydrazines, sodium azide, and certain other reductants retard analogously:[33,47,115,147,148]

$$NH_2OH+Y{-}NO\rightarrow N_2O+H_2O+HY,$$
$$NH_2NH_2+Y{-}NO\xrightarrow{H^+}N_2O+NH_4^++HY,$$
$$N_3^-+Y{-}NO\rightarrow N_2O+N_2+Y^-.$$

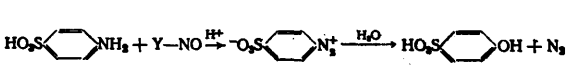
Certain alcohols, for example, ethanol and ethylene glycol, carbohydrates (glucose, sucrose), and other compounds containing the OH group inhibit the formation of NA, being converted into the corresponding alkyl nitrites:[42,78,144,149-152]

$$ROH+Y{-}NO\rightarrow RONO+HY.$$

Thiols react analogously but more vigorously:[57,144,149,150]

$$RSH+Y{-}NO\rightarrow RSNO+HY.$$

The substances examined inactivate nitrosating agents, converting them into the less reactive esters (thioesters) of nitrous acid or nitric (nitrous) oxide. In order to prevent the reactivation of nitric and nitrous oxides, it is essential to use high concentrations of the inhibitors, in particular ascorbic acid and tocopherol.[141,145,153] It is more useful to seek inhibitors converting active nitrosating agents into substances incapable of reacting with amines. The possibility of using derivatives of primary aromatic amines, for example, sulphanilic acid and sulphanilamide, which give rise to diazo-compounds on reaction with nitrosating agents, has been established:[118,141,154,155]



There is no doubt that new effective nitrosation inhibitors can be found among other readily diazotising compounds.

Urea and sulphamic acid, proposed for the retardation of the formation of NA, also lead to the decomposition of nitrosating agents;[47,81,106,115,144,147]

$$O{=}C(NH_2)_2+2Y{-}NO\rightarrow 2N_2+CO_2+H_2O+2HY,$$
$$NH_2SO_3H+Y{-}NO\rightarrow N_2+H_2SO_4+HY.$$

The use of ammonia, tannin, vanillin, thymol, squalene, pyrrole, glutathione, indole, etc. as inhibitors has also been indicated.[6,21,47,69,126,139,153]

It is useful to employ inhibitors to prevent the pollution of various objects by nitrosamines and the formation of these carcinogens in the organism.

## III. PROPERTIES OF *N*-NITROSAMINES

### 1. Physical Properties

The NA considered are oily liquids or solids which are moderately soluble in water and readily soluble in many organic solvents: methylene chloride, chloroform, carbon tetrachloride, ether, alcohols, acetone, dimethyl sulphoxide, dimethylformamide, etc.; they are highly volatile and can be steam-distilled.[1,3,8,32]

The IR spectra of NA have characteristic absorption bands. Thus $\nu(N{=}O)$ for a series of dialkylnitrosamines is in the range 1425 to 1470 $cm^{-1}$, while $\nu(N{-}N)$ is in the range 1010–1150 $cm^{-1}$.[3,32,50]

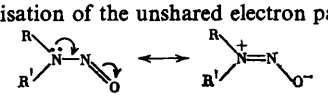
Two maxima, which in the case of aqueous solutions are in the ranges 230–235 nm ($\varepsilon \cong 7000{-}8100$) and 330–350 nm ($\varepsilon \cong 100$), have been found in the UV spectra of NA.[1,3,8,50] It has been noted that the positions of the bands in the IR and UV spectra of NA depend significantly on the nature of the solvent.[32,50]

Fairly intense ions have been observed in the mass spectra of NA obtained on electron impact: the molecular $M^+$ ion and the fragment ions $M^+ - 17$ (OH split off), $M^+ - 30$ and $M^+ - 31$ (NO or NOH split off), and also an ion with $m/z$ 30 ($NO^+$). In the mass spectra obtained on chemical ionisation, the maximum content is that of the $(M+1)^+$ ion.[9,156-158]

The bond lengths in aliphatic NA have been determined. Thus in NDMA the length of the N–O bond is 123 nm, while N–N bond length is 134 nm instead of 145 nm, which is the N–N in compounds where both nitrogen atoms are linked to carbon.[31,32] The N–N bond dissociation energy in NDMA is about 55 kcal $mole^{-1}$, which is higher than in nitrodimethylamine.[3,32,50]

The stability of NA is fairly high, exceeding that of nitrodialkylamines. They are not cleaved with solutions of alkalies and dilute acids and are hardly decomposed by scattered light.[3,22,32] These properties of NA predetermine the duration of their presence in the environment.[7,10,13]

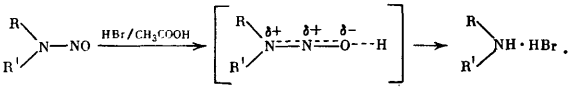
The dipole moments of many aliphatic NA are 3.9–4.4 D, which indicates the polarity of their molecules, determined by the possibility of the delocalisation of the unshared electron pair:[32,50,159]



### 2. Chemical Properties

The structure and the comparatively high mobility of the π electrons in the molecules are responsible for the fairly high reactivity of NA. They can be protonated, can form hydrogen bonds with alcohols and phenol, can combine with certain substances, for example, alkyl halides, can form complexes with various salts, etc.[22,32,50,159] Many of these processes are of not only theoretical but also practical interest. In principle, all the reactions of NA can be divided into two large groups—occurring with and without the cleavage of the N–N bond. In many cases the interaction of NA with a particular reactant can proceed via both these pathways depending on the conditions.

*(a) Denitrosation of NA by inorganic acids.* This reaction has attracted much attention by investigators, since the products of the denitrosation of NA are non-toxic and the amines formed have been used to develop methods for the analysis of NA. As already mentioned, dilute solutions of inorganic acids interact with NA very slowly. Thus NA distil from 0.1–0.2 M sulphuric acid almost without decomposition.[9,22,141,155,160,161] With increase in acid concentration cleavage of the N–N bond is accelerated; its completion requires heating of the reaction mixture or the use of catalysts, for example, $Cl^-$, $Br^-$, and $SCN^-$.[22,162,163] NA are most readily denitrosated by HBr solutions in glacial acetic acid; at room temperature, a virtually quantitative yield of the corresponding secondary amines is attained after several minutes:[12,163-166]

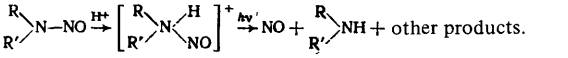


In the presence of small amounts of water or alcohol, the rate of denitrosation decreases significantly.[166,167] An excess of glacial acetic acid with phosphoric (or sulphuric) acid, containing $I^-$ or $Br^-$, and also HBr solutions in acetic anhydride can denitrosate NA in the presence of water.[167,168]

Various inorganic acids as well as their derivatives, for example, acetyl, oxalyl, and thionyl chlorides are less effective when HBr is the denitrosating agent.[163,164]

*(b) The effect of UV light, γ-radiation, and heat.* It is possible to cleave to cleave the N–N bond on treatment with UV light or γ-radiation. These reactions proceed via a fairly complex mechanism and not all the intermediates have yet been identified reliably.[124,169-174] It has been noted that the reaction takes place comparatively slowly.[43,171]

Acids and oxidants have an accelerating effect on the cleavage of NA by irradiation with UV light. Several possible pathways leading to the denitrosation of NA on treatment with UV light and acid have been proposed,[165,170,171,175-177] for example:



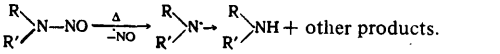
Under certain conditions, this reaction can afford high yields of secondary amines and NO ($NO_2^-$).[172,175] Account must be taken of the fact that the presence of high $NO_2^-$ or $NO_3^-$ concentrations in the reaction mixture creates favourable conditions for the denitrosation of secondary amines.[170,171]

The action of UV light on NA solutions in the presence of bases also leads to the dissociation of the N–N bond.[165,169]

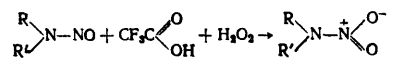
When aqueous solutions of NPip are exposed to the action of γ-radiation in the presence of added acids or alkalies, the cleavage is hindered in the presence of high concentrations of nitrates or nitrites.[42,44,45,178,179]

γ-Irradiation of food products leads to the partial decomposition of the NA contained in them.[45,178,179]

NA are hardly decomposed on heating to 200–250 °C. However, their pyrolysis takes place at 450–500 °C:[124,180-182]

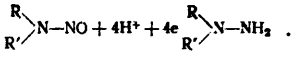


*(c) Oxidation and reduction.* The interaction of NA with oxidants or reductants can lead to the formation of the corresponding nitramines and asymmetric hydrazines or to the cleavage of the N–N bond. The oxidation of NA without dissociation of the N–N bond is achieved mainly with the aid of peracids, among which $CF_3COOH$ is the most convenient. It is usually obtained directly in the reaction mixture from trifluoroacetic acid and hydrogen peroxide:[165,166,180,181,183]

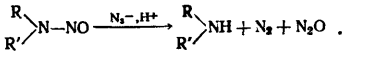


Treatment with pentafluorobenzoic acid and with a mixture of ammonium persulphate and nitric acid, as well as electrochemical oxidation, etc. lead to analogous results.[22,184,185] On treatment of NA with $K_2Cr_2O_7$ or $KMnO_4$ in concentrated sulphuric acid, ozone, etc., the N–N bond is cleaved.[165,166,176]

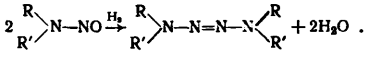
The reduction of NA with zinc in acetic acid to the corresponding dialkylhydrazines has been known for a long time,[50] the same compound being obtained on treatment with $LiAlH_4$, KOH + Al, titanium trichloride, sodium amalgam, etc.[50,186-188] and on electrochemical reduction:[165,186,187]



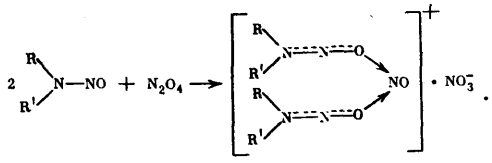
When other reductants are used and the conditions are altered, NA can be converted into mixtures of the corresponding asymmetric hydrazines and secondary amines or mainly into secondary amines. The latter are formed in fairly high yields in the presence of Raney nickel, in an acid solution of copper monochloride or iron dichloride, in the presence of a mixture of nickel, aluminium, and potassium hydroxide, aluminium amalgam, azide, etc.:[165,187]



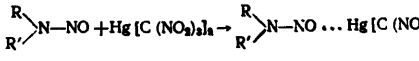
The reduction of NA results in the formation of tetra-alkyltetrazenes in addition to hydrazines and secondary amines:[165]



*(d) Complex formation and addition reactions.* The delocalisation of the electron pair $>N{=}N{=}O$ characteristic of NA predetermines their tendency towards reactions involving the formation of *O*-complexes on treatment with $BF_3$, $PCl_5$, $SbCl_5$, $AlCl_3$, etc.[32,50,159] The interaction of NA with nitrogen tetroxide probably proceeds via the mechanism[50,87]

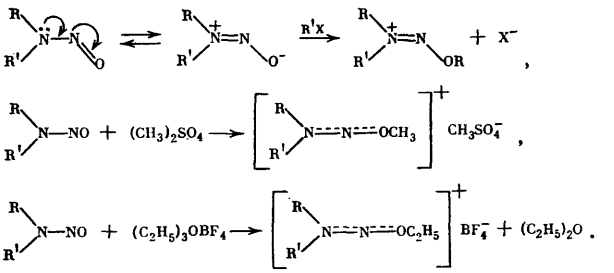


NA can combine with various metal salts, for example $CuCl_2$, $ZnBr_2$, $PdCl_2$, etc. A stable complex is formed as a result of the reaction of NA with bis(trinitromethyl)mercury:[32,50]



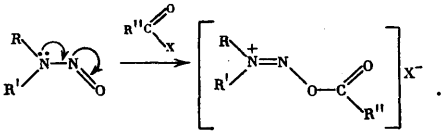
Complexes of NA with bromoplatinic acid and certain other compounds have been described.[32,50]

NA react with many alkylating agents—alkyl halides, dimethyl sulphamate, triethyloxonium fluoroborate, etc.:[159,165,166]

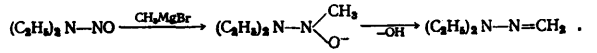


The last reaction proceeds very readily; the resulting salt is involatile and is decomposed by potassium hydroxide, which is used to decompose NA.[165]

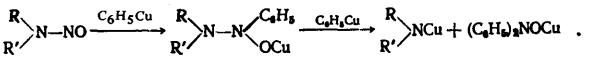
NA react with acylating agents:[32]



Certain reactions of NA with organometallic compounds—a Grignard reagent or $CH_3LI$—have been described:[22,32,165]



The interaction of NA with phenylcopper takes place with dissociation at the N–N bond:[22,32]



## 3. Effect on the Organism

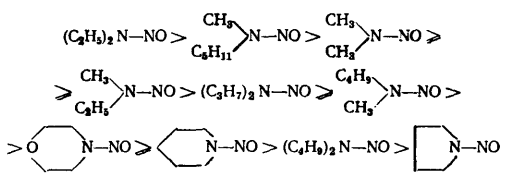
NA exhibit a pronounced toxicity, in the first place in relation to the liver and kidneys, and can give rise to haemorrhagic centrilobular necroses accompanied by blood effusions, the proliferation of bile ducts, disturbance of the renal function, etc.[1-5,8,15,17]

NDMA is the most toxic, its $LD_{50}$ value amounting to about 30 mg $kg^{-1}$ of body weight after single administration to rats. Other NA are less toxic; thus the $LD_{50}$ for NPip, NDEA, and NDPA are respectively 200, 220, and 480 mg $kg^{-1}$.[1-4,8] Many NA exhibit pronounced mutagenic and carcinogenic activities.[1-4,8,10,14,102,189-192]

According to Shabad's classification,[193] NA are powerful carcinogens. The blastomogenic properties of NA have been investigated extremely widely and have been demonstrated on 40 different animal species: monkeys, dogs, rabbits, pigs, hamsters, rats, mice, guinea pigs, etc. and also on various birds, amphibia, and fish.[1-4,8,10,16] Despite the considerable species differences, the character of the tumours induced by the corresponding NA is similar as regards location and morphological structure. For example, NDMA and NDEA induce liver tumours—cholangio- and haepato-cellular carcinomas. The species-specific blastomogenic activity of NA is much wider than that of all other known chemical carcinogens, which is important indirect evidence for the carcinogenic activity of NA in relation to man.[2-6,8,10,14,102,189,194-198]

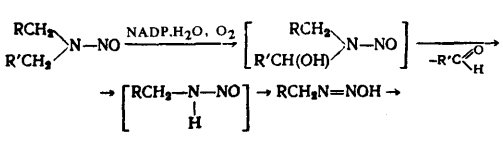
NA exhibit a broad spectrum of carcinogenic activity and can induce the formation of tumours in the liver, kidneys, stomach, alimentary tract, lungs, bladder, trachaea, throat, nasal cavity, etc.[1-5,8,10,17,20,102,199] It is significant that tumours arise both on treatment with NA and when their non-carcinogenic precursors—amines and many nitrogen-containing substances—enter the organism together with nitrosating agents.[3-6,8,10,13,14,16,102,197,200] The formation of NA from precursors has been demonstrated by chemical analysis.[76-79,100,102,105,106,114,200] Micro-organisms capable of converting nitrates and various nitrogen-containing substances into amines and of promoting the formation of NA over a wide pH range including pH $\geqslant$ 7 play an appreciable role in the endogenic synthesis of NA.[3-5,8,10,16,47,199]

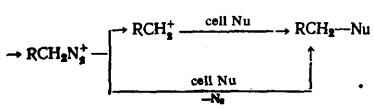
A direct dependence of the blastomogenic effect on the size of the dose and on the duration of its action on the organism has been discovered for NA.[1-5,20,201] It has been suggested that the minimum carcinogenic dose of NDMA for rats is about 0.1 mg per kilogram of body weight per day.[1,10,181] Relatively small frequently non-toxic doses of NA can induce the formation of tumours in various organs in animals and the majority of NA have a carcinogenic effect after a single administration.[1,3,16,20] The relative carcinogenic activities of NA have been calculated from determinations of the NA dose used in the formation of tumours in 50% of animals:[32,194]



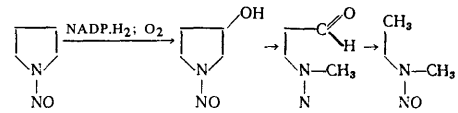
It has been shown that carcinogenic activity is exhibited not by the NA themselves but by the products of their metabolic activation under the influence of enzymes—non-specific microsomal demethylases and oxidases located in the membrane of the endoplasmic reticulum of the cell and catalysing the conversions of NA in the organism, especially in the liver.[1-3,10,16,82,192,202-208]

The metabolism of NA, in the first place NDMA and NDEA, has been investigated and the relation between the products of the biotransformation and the carcinogenic activity has been discovered. These are usually multistage processes which lead to the formation of a number of intermediate and final carcinogens.[2-4,10,14,82,195,196,202,207,209] Although the nature of all the products has not been fully elucidated, it has been suggested that the carcinogenic activity is due to the presence of active centres capable of acting on DNA, RNA, protein, etc. macromolecules in the cell.[4,10,14,16,195,196,204,205,207,209] From the chemical point of view, the reactions of NA in the organism are processes involving oxidative hydroxylation and the cleavage of the hydroxyalkyl derivatives formed under these conditions.[2,3,204,205] It is assumed that the hydroxy-group can be incorporated in various positions, for example, in the α-, β-, and ω-positions in the alkyl groups of the NA. The following mechanism has been proposed for the primary activation of NA by α-hydroxylation:[4,192,202-205]

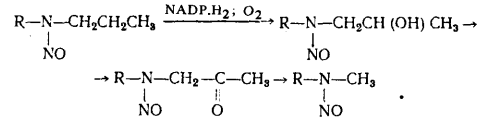




The electrophilic compounds $RCH_2N_2^+$ and $RCH_2^+$ interact actively with various nucleophiles, in particular with DNA. It has been suggested that the enzymic activation of cyclic NA begins with the cleavage of the ring and the formation of the dialkyl-NA:[205,210,211]



Enzymic β-hydroxylation can proceed as follows:[203-205,210]



Together with processes involving the metabolic activation of the NA, their inactivation reactions also takes place in the organism.[4,16,202,205] The action of the NA on the organism is determined mainly by the alkylation of DNA by the products of their metabolism, which can lead to the breakdown of the functioning of the cell genome. The *N*(7)-, *N*(3)-, and *O*(6)-positions in guanine, and the *N*(7)-, *N*(3)-, and *N*(1)-positions in adenine are most often alkylated.[3,4,14,189,195,196,203-208,212,213] It is believed that the alkylation of the DNA guanine in the *O*(6)-position is significant for the appearance of tumours and the mutagenic effect. Such alkylation damages DNA and disturbs the transfer of genetic information.[4,82,189,195,196,21

Data demonstrating the accelerating or retarding effect of a number of substances, for example, ethanol, hydroxybutylanisoles, and luminal on the NA metabolism have been obtained.[152,201,206,213,214]

It has been shown that there is a clear-cut correlation between the carcinogenic and mutagenic activities of the products of the NA metabolism, but a relation between toxicity and carcinogenic activity has not been established.[14,102,189-192] It has been suggested that the formation of NA from precursors directly in the organism is important in the genesis of cancer.[4,10,14,153,197,215] It is therefore essential to take into account the circulation in the environment and the possibility of the entry into the organism of not only the NA themselves but also of the precursors.[7,216]

## IV. PRINCIPAL METHODS OF MICROANALYSIS

The determination of carcinogenic NA in objects within the environment at the microgram and nanogram level is an extremely complex analytical problem, because such objects can contain an extremely large number of substances. For example, thousands of main and hundreds of thousands of minor components have been detected in food products.[198,217]

The main requirements which must be met by the analytical methods for NA are high sensitivity, satisfactory selectivity, and reliability of the results. The determination of NA in objects usually includes all or the majority of the following stages: isolation, preliminary purification, concentration, additional purification, preparation of derivatives, analysis and confirmation of the results. The isolation and purification are the decisive stages ensuring to a large extent the success of the analysis. The methods used to carry out these stages have been examined in a number of studies.[124,141,160,161,180,181,196,218-221]

The methods for the determination of NA makes it possible to analyse them directly or on the basis of their rection products. Spectrophotometric, fluorimetric, polarographic, chromatographic, chromato-spectrometric, and certain other methods have been

proposed for the analysis of NA in solutions and extracts from various objects.[5,12,180,181,186,218] As a rule, methods involving chromatographic separation are used nowadays.

Gas–liquid chromatography (GLC) with different columns, phases, and detectors is frequently employed to identify and determine quantitatively NA. Although NA can be analysed with the aid of flame-ionisation detectors, it is more desirable to use nitrogen-sensitive detectors—thermoionic, conductimetric, and microcoulometric.[12,160,161,180,186,218,222,223] Packed and capillary columns filled with various stationary phases, for example, 10–15% Carbowax 20 M on Chromosorb G or W, 5% Carbowax 20 MTPA on Supelcoport, 2–3% OV-1 on Chromosorb G or W, 5% SE-20 on Chromosorb G or W, and UCON-HB 5100 CPWAX57CB are used for the separation. Chromatography under isothermal conditions is rarely used, the temperature being usually programmed in the range 50–200 °C at a rate of 2–10 K $min^{-1}$. The quantitative determination is carried out as a rule with an intenal standard; the detection limit is at the level of several nanograms.[9,180,186,218,219,221,224-227]

The retention times of NA, especially NDMA and NDEA are short, and their peaks are distributed in the region of the chromatogram with a high content of peaks due to various volatile substances from the object analysed, which hinders their identification. The NA isolated from the object must therefore be carefully purified before the GLC. In a number of cases, even under the conditions of thorough purification, the agreement involving the retention times does not yet guarantee the reliability of the results of the determination.[9,180,186,218,225]

A novel chemiluminescence method for the analysis of NA was proposed in 1975 and a special apparatus—a thermoenergetic analyser (TEA)—was constructed.[55,99,114,117,124,181,182,196,219,220,228-231] Its operation is based on the decomposition of the NA in a catalytic pyrolyser at a temperature in excess of 450 °C. The NO· radical formed under these conditions interacts with ozone. The reaction results in the appearance of an excited $NO_2^*$, which passes to the ground state with emission of the characteristic radiation in the IR part of the spectrum, after which it is recorded with a photoelectron multiplier:

$$\mathrm{R_2N{-}NO \xrightarrow{\Delta} R_2N^{\cdot} + NO^{\cdot}},$$

$$\mathrm{NO + O_3 \rightarrow NO_2^* + O_2},$$

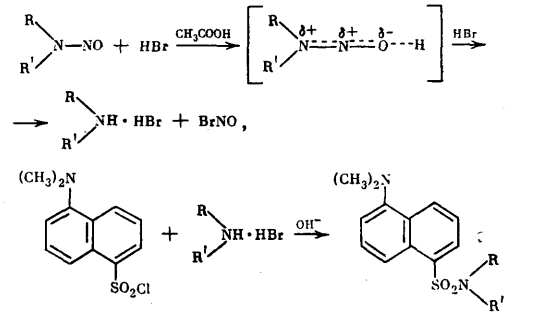
$$\mathrm{NO_2^* \rightarrow NO_2 + h\nu}.$$

In the main TEA is used as a specific detector for NA in combination with various chromatographs. In terms of its analytical parameters, the TEA method is greatly superior to other detection methods in GLC; the detection limit on the TEA is less than 1 ng.[38,55,99,124,180-182,196,219,220,226,232-235] This has ensured a wide-scale application of GLC with TEA for the determination of NA in various objects within the environment despite the fact that certain other NO- and $NO_2$-containing substances, for example, *C*-nitroso- and *C*-nitro-compounds as well as *O*-nitroso- and *O*-nitro-compounds, can give rise to false positive results.[124,180,181,196,218-220,225,228,235,236] NA have been determined most reliably in different objects by the chromato-mass-spectrometric method.[9,12,158,172,173,186,224,235,237-239] The application of a high-resolution mass-spectrometer with electron impact ionisation makes it possible to record one, most specific ion. In NA analysis, this ion is as a rule $M^+$ or $NO^+$.[157,181,183,219,235,237] It is somewhat more difficult to identify NA on the basis of low-resolution mass spectra. In this case selected ions are monitored. The presence of NA is regarded as established if the mass spectrum of the chromatographic peak with the corresponding retention times contains all the selected ions characteristic of the given NA and their intensity ratios are close to the ratios in the mass spectrum of the standard.[9,157,180,181,183,219,224,235,237] Chromato-mass-spectrometric methods of determination, especially at low resolution, require careful purification of the extracts, since the selected ions are in the region of low masses mixed with fragments of other compounds present in the extract.[9,141,218,219,224] The use of mass spectrometry with chemical ionisation is effective in the determination of NA.[9,156,157,240]

A series of GLC and chromato-mass-spectrometric methods have been proposed for the analysis of NA on the basis of their reaction products. The determination of NA after their oxidation to the corresponding nitramines, having longer retention times than the NA with detection based on electron capture or mass spectrometrically with the aid of TEA has been described.[12,180,181,183,186,219,225] The method is used rarely because of its inadequate reproducibility. Chromatographs with an electron impact detector and chromato-mass-spectrometers are used to determine NA after their conversion into halogeno-derivatives, which are frequently obtained on treatment with heptafluorobutyryl chloride or heptafluorobutyric anhydride:[12,122,180,181,183,184,186,219,223,225,241]

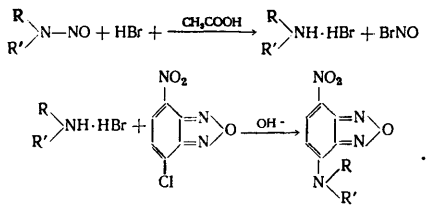
$$\mathrm{R_2N{-}NO + \left(C_3F_7C(=O)\right)_2O \xrightarrow{\text{pyridine}} R_2N{-}C(=O){-}C_3F_7}.$$

A method based on the use of dansylamides, obtained from NA by treatment with 1-dimethylaminonaphthalene-5-sulphonyl chloride, is effective for the analysis of NA in various objects:[9,155,225,237,242]



The mass spectra of the dansylamides obtained from various NA contain intense molecular and fragment ions which are either common to all the derivatives, for example the $(NH_3)_2NC_{10}H_7^+$ ion with *m/z* 171 or are characteristic of individual compounds (formed on dissociation of the S–N bond), which makes it possible to identify and determine the NA.[9,155,237,242] The monitoring of selected ions has proved optimal for the detection of dansylamides. The recording of a chromatogram based on several such ions, the retention times as well as the ratios of the intensities of the mass-spectral lines and the areas of the corresponding peaks ensure a reliable identification of the compound analysed; the detection limit is less than 1 ng.[9,155,237,242] The selected ions in the mass spectra of dansylamides are in the region relatively free from ions of impurities present in the NA extract, which makes it possible to employ low-resolution mass-spectrometers and does not necessitate a careful purification of the extract.[9,141,237,242] The adequacy of the standards obtained from the NA and the corresponding secondary amines has been established; the use of the latter simplifies the analysis and makes it safer.[141,155,237,242-244]

The chromato-mass-spectrometric method based on the preparation of NBD-amines by treatment with 7-chloro-4-nitrobenzo-2,1,3-oxadizole is entirely suitable for the analysis of NA:[155,225,243,245]



The mass spectra of NBD-amines contain intense molecular and characteristic fragment ions which can be effectively determined by monitoring the selected ions. The possibilities of this method are analogous to the method involving the use of dansylamides.[155,225,245] The chromato-mass-spectrometric methods for the determination of NA on the basis of their derivatives—dansylamides and NBD amines—can be used also to confirm the results obtained by other methods.[9,141,155,225,237,242,245]

High performance liquid chromatography is used more rarely for the analysis of volatile NA than GLC. The analysis is performed on sorbents: Lichrosorb Si 60, Microporasil, Spherisorb 3SW, Sorbax CN, etc.: the elution is carried out with mixtures of organic solvents, for example, chloroform–cyclohexane–methanol, isopropyl alcohol–hexane, and chloroform–hexane.[124,220,225,229,246] The reversed-phase version on Microbondapak $C_{18}$, Sorbax ODS, silica gel $C_{18}$, etc. with mobile phases containing acetonitrile, methanol or ethanol, water, or buffer systems is also used.[173,177,225,227] TEA, photoconductimetric, electrochemical, and UV detectors are used to detect the separated NA; the NA are frequently subjected to preliminary cleavage and are treated with the Griess reagent.[227-229,243,246,247] The detection limit is about 1 ng.[124,173,177,181,220,225] There are data concerning the analysis of NA by the high performance liquid chromatographic method in the form of derivatives.[225,243,247]

A series of simple methods for the determination of NA using thin layer chromatography (TLC) have been proposed. The methods described earlier—involving irradiation with UV light and detection on the basis of the $NO(NO_2^-)$ or $R_2NH$ formed in the form of coloured compounds, are inadequately sensitive and selective.[12,180,186,218,223,225] The methods based on the denitrosation of the NA with HBr and the preparation of fluorescent derivatives of secondary amines have much better characteristics. In principle, different reagents are suitable for this purpose[243,248] but dansyl chloride and NBD chloride are mainly used for the analysis of NA in various objects.[9,141,155,168,218,225,243,244,249] The analysis is performed directly on a plate, visually and densitometrically, or the corresponding spots are eluted and the fluorescence intensity is measured. The detection limit is 1–10 ng in the spot.[141,155,168,244,248,249] Compared with dansylamides, the determination of NA in the form of NBD-derivatives is more effective, since the latter not only fluoresce but are also coloured yellow, orange, or pink, while NBD chloride itself does not fluoresce and does not form fluorescent compounds with phenols and alcohols.[141,155,218,241,243,244,248,250] An analogous method has been proposed for the determination of NA in the form of fluorescent derivatives, obtained by the interaction of secondary amines with *N*-(8-methoxyqinoline-5-sulphonyl)aziridine,[251] but in terms of a number of characteristics it is inferior (inadequate separation of derivatives, complexity of the chromatography, etc.) to the method with NBD chloride.

The usefulness of carrying out the TLC in the presence of a control sample from part of the extract after the removal of the NA has been demonstrated. The use of such control samples, which hardly differ in terms of the composition of the impurities from the experimental sample, ensures the reliability of the analysis.[141,155,225,250] The use of comparison samples, prepared by introducing the corresponding standards into the control samples, is even more effective.[252] This procedure simplifies significantly the identification of NA, accelerates the analysis by eliminating the additional purification, and increases the reliability of the determination by reducing the probability of obtaining false results.[252]

It is thought essential to confirm the results of the determination of nanogram and microgram amounts of NA in objects within the environment. Chromato-mass-spectrometric methods are used for this purpose or the analysis is carried out by two different methods.[124,155,172,180,181,184,186,218,219,224,225,233,239,253]

## V. THE CONTENT OF *N*-NITROSAMINES IN OBJECTS IN THE ENVIRONMENT

### 1. Air

Extremely complex processes involving the formation and decomposition of NA take place in the atmosphere. The concentration of nitrogen oxides plays a significant role in the formation of NA. It is believed that the NA content in air is a problem associated with the waste from industrial undertakings, transport, and the pollution of the atmosphere by nitrogen-containing substances.[7,10,54-57,114,216,230] This is confirmed to some extent by the finding that, under conditions remote from large industrial and agricultural manufacturing undertakings, the air contains hardly any NA ($\leqslant 0.01$ μg litre$^{-1}$).[56,103,114,254] Up to 0.05 μg litre$^{-1}$ of NDMA has been found in the atmosphere over non-industral towns, while the content over industrial towns is 0.76–1 μg litre$^{-1}$.[56,103,114,255] Higher NA concentrations have been observed in chemical, leather, and rubber manufacturing plants: up to 36, 47, and 140 μg litre$^{-1}$ of NDMA and up to 46,20, and 250 μg litre$^{-1}$ of NMor respectively.[55,56,103,114,119,230,254,256] This can be explained by the presence in the above undertakings of a series of readily nitrosated substances, for example, amines and vulcanisation accelerators.[71,99,114,119,121,230,254-257] Other NA may be present in the air within these factories at lower concentrations.[56,99,254,256,258]

The air in showrooms for new automobiles contains the following amounts of NA (in μg kg$^{-1}$): NDMA $\geqslant$ 1.0, NDEA $\geqslant$ 0.4, and NMor $\geqslant$ 2.5, which can be explained by their evolution from rubber components.[119,121,254,256,259] A series of NA have been found in air within smoking rooms and kitchens, which has been attributed to their presence in tobacco smoke and their evolution in the frying and cooking of food.[55,98,114,183,200]

It is noteworthy that in the atmospheres of towns domestic and tobacco smoke contains significant amounts of nitrogen oxides.[56,78,98,161,255,260]

### 2. Water

Comparatively little attention has been devoted to the determination of NA in water possibly because they are either absent from purified drinking water or their concentration is extremely low ($\leqslant 0.01$ μg litre$^{-1}$).[8,13,114,117,241,261,262] Relatively larger amounts are present in chlorinated water (NDMA $\leqslant$ 1.0 μg litre$^{-1}$) and deionised water (NDMA $\leqslant$ 0.5 μg litre$^{-1}$ and NDEA $\leqslant$ 0.8 μg litre$^{-1}$).[35,103,114,117,261] NA have been detected in various types of waste water. Thus up to 2 μg litre$^{-1}$ of NDMA has been found in sea water in the vicinity of waste discarding points in a fish processing plant, the discarded water containing NDMA ($\geqslant$3 μg litre$^{-1}$) and NDEA ($\geqslant$0.2 μg litre$^{-1}$).[5,114,117,255,261] The waste water from various factories contains up to 10 μg litre$^{-1}$ of NDMA, 5.5 μg litre$^{-1}$ of NDEA,

and certain other NA,[103,114,183,223] while in individual types of water containing slime the content of NA is up to 940 μg litre$^{-1}$.[128,223] The concentration of NA in waste water containing amines is usually enhanced.[182,252]

In order to prevent the pollution of the environment by nitrosamines, it is essential to purify the waste water, since these carcinogens can pass from water into plants.[7,10,216,222,223,262] Nitrites and nitrates have been detected in natural waters, the concentration of the latter being high.[10,55,255,261]

### 3. Soil

The turnover of NA in the soil has been inadequately investigated. NA and their precursors can enter the soil from the atmosphere, together with fertilisers and pesticides, with waste water, etc. Although many different amines, other nitrogen-containing bases, nitrates, and nitrites are present in the soil, NA do not usually accumulate in the soil.[5,10,216,262] This can be explained by the occurrence of NA degradation processes under the influence of light, temperature, and the microflora, evaporation into the atmosphere, transfer to water, sorption by plants, etc.[7,10,13,75,216,222,255,262]

### 4. Inorganic Nitrogen-containing Fertilisers and Pesticides

High concentrations of NA, sometimes exceeding 1000 μg kg$^{-1}$ have been found in a number of pesticides.[8,12,39,96,103,113,114,116,117,183] There is a high content of NDMA in dimethylammonium salts of 2,4-D, 2,4-DB, MCPP, MCP, and TCBA, alkylbenzyldimethylammonium chlorides, cetylethyldimethylammonium bromide, thiram, etc.[96,103,107,115] NMor is present at high concentrations in *N*-alkyl-*N*-ethylmorpholinium ethyl sulphate and NDPA is present in trifluralin (treflan), isopropalin, etc.[96,103,107,114,117]

The presence of NA in inorganic nitrogen-containing fertilisers has not been reported but they are a most important source from which nitrates enter the soil, natural water basins, and plants.[7,10,75,216,263]

### 5. Products of the Chemical and Rubber Industry

NA have been detected in products produced on a large scale such as amines and solvents. The amines contain the corresponding NA; thus dimethylamine contains up to 17300 μg kg$^{-1}$ of NDMA, pyrrolidine contains up to 53000 μg kg$^{-1}$ of NPyr, and dipropylamine contains up to 13000 μg of NDPA; the concentrations of NA are somewhat lower in other secondary and tertiary amines.[12,183,257]

NMor has been detected in chloroform and methylene chloride at concentrations reaching 376 and 32 μg kg$^{-1}$ respectively. Small amounts of NDMA and NDEA have been found in a number of other solvents. NA are as a rule not detected in these products after purification.[58,264] NDMA, NDEA, and other NA have been found in certain types of synthetic detergents.[14]

Rubber vulcanisation accelerators contain considerable amounts of NA, for example, NDMA (4–800 μg kg$^{-1}$) in tetramethylthiuram disulphide, (NDEA 10–100 μg kg$^{-1}$) in tetraethylthiuram disulphide, and zinc diethyldithiocarbamate, NMor (60–3500 μg kg$^{-1}$) in various morpholine derivatives, etc.[38,71,99,118,119,121,232,253] NA have been discovered also in various rubber articles, including those designed for medical and food related purposes. The contents of NDMA, NDEA, and NMor are 50.3, 2.2, and 120 μg kg$^{-1}$ respectively in medical tourniquets, rubber hose for the milk industry, and certain rubber articles for the food industry prepared by the continuous vulcanisation method.[38,40,118,154,253,259,265] In the rubber teats for babies' bottles, babies' dummies, stoppers for bottles with babies' food, rubber toys, and rubber gloves, the maximum concentrations of NDMA, NDEA, NDBA, NPip, and NMor are 200, 100, 2796, 180, and 86 μg kg$^{-1}$ respectively.[38,40,71,99,118,121,229,232,253,265,266] NA are capable of diffusing from rubber into liquids in contact with it—milk, milk product, fruit juices, etc.[11,38,99,232,253,266] This made it necessary to pay significant attention to reducing the NA content in rubber for food and medical purposes. The introduction of inhibitors into rubber mixtures and the improvement of the technology made it possible to solve this problem.[118,154,174,265]

### 6. Medicinal Preparations

NA, usually NDMA, have been found in a number of medicinal preparations. For example, amidopyrine contains 1–371 μg kg$^{-1}$ of NDMA, while the content in individual specimens is up to 980 μg kg$^{-1}$ of this NA; there is up to 68 μg kg$^{-1}$ in imizine, up to 40 μg in nitrofurantoin, and up to 7 μg kg$^{-1}$ in oxytetracycline.[12,58,86,102,104,110,183,230] NA have been detected in medicines which incorporate amidopyrine, piperazine, phenacetin, ephedrine, imizine, pipolphen, aminazin, tetracycline, pencillin, analgin, and many others.[14,102,104-106,109-111,230]

It has been shown that many medicinal preparations can be nitrosated directly in the human stomach.[102,106,108]

### 7. Animal Feed

NA and their precursors are encountered in various types of animal feed. Nitrates, sometimes nitrites, and (only in individual specimens) small amounts of NA have been detected in meadow grass.[231,263,267] Up to 150 mg kg$^{-1}$ of NDMA together with precursors have been found in mixtures of juicy feed together with concentrates and in feed for birds and rabbits.[5,8,102,268]

Various concentrates, including fish meal, in which the NDMA and NDEA concentrations can reach 417–2000 and 36 μg kg$^{-1}$ respectively are used as additives to feeds.[8,128,268,269] NDMA (2 and 85 μg kg$^{-1}$), NDEA (24 and 512 μg kg$^{-1}$), and certain other NA have been found in grass and maize silos.[270-272] NA have been discovered in the milk of cows and goats and the eggs of birds receiving feed with a high content of NA and their precursors.[263,268,271] Significant amounts of NDMA, NDEA, NPip, and NPir (up to 79, 65, 300, and 26 μg kg$^{-1}$ respectively) have been detected in the diet of laboratory animals.[8,58,127,231,269]

### 8. Food Products

The most important food products, prepared dishes and drinks, have now been analysed and in a number of countries systematic monitoring of the NA content in such products has been introduced. NA precursors, in the first place nitrates and nitrites, are determined in many products;[5,7,10,19,40,122,218,273] account must be taken of the fact that the technology of the preparation of many meat products and cheeses provides for the introduction into them of definite amounts of nitrates and nitrites.[11,107,122,127,198,218,274] Much attention has been devoted to the study of the content of amines in food products, although the presence of a broad spectrum of these substances and the necessity for their determination together with nitrates and nitrites was demonstrated as early as 1976.[275] Data

characterising the degree of contamination of food products and drinks by NA and their precursors have been published in a series of monographs and reviews.[3,5,8,10,12,19,23,39,75,122,125,128,148,183,200,215,218,220,276]

As a rule, NA are encountered much more rarely at relatively lower concentrations in raw and cooked products then in fried, smoked, salted, and preserved products. Thus the overall content of NDMA and NDEA can reach 5 μg $kg^{-1}$ only in individual specimens of fresh, freshly frozen, and cooked meat, while the content in cooked sausages can be 10 μg $kg^{-1}$.[9,122,125,127,160,161,217,233,260,277,278] NA are frequently detected in various types of bacon, large smoked sausages, small smoked sausages, smoked pork products, and preserves, the maximum NDMA, NDEA, NDBA, NPyr, and NPip concentrations amounting to 84, 16, 4, 207, and 50 μg $kg^{-1}$ respectively.[9,40,114,122,125,127,148,160,233,236,265] The amount of NPip increases sharply, sometimes up to 1600 μg $kg^{-1}$, in smoked products prepared with an excess of spice.[125,127,183,220,279] Nitrosothiazolidine (in an amount of the order of 20 μg $kg^{-1}$) has been found in bacon and in fried, smoked, and preserved meat. NMor is encountered extremely rarely in food products.[11,236,276,278,280,281] There is a fairly high content of nitrates and nitrites—up to 145 and 750 μg $kg^{-1}$—in meat products, especially smoked, salted, and preserved products.[40,122,127,148,160,215,233,236,273]

Fish products and sea food are less often found to contain NDMA—up to 9 μg $kg^{-1}$ in individual fresh species. The maximum NDMA, NDEA, NPyr, and NPip concentrations in salt-dried, pickled, smoked, and fried fish can reach 84, 51, 37, and 19 μg $kg^{-1}$ respectively, the frequency of their detection being very high.[3,125,148,160,161,220,234,253,260,265,282] Up to 400 μg $kg^{-1}$ of NDMA [125,128] has been found in salted and salt-dried fish prepared using technical common salt containing nitrates, while hot-smoked fish, obtained using the smoke from wood chips, may contain up to 206.5 μg $kg^{-1}$ of NDMA.[234] Much NDMA (up to 12 600 μg $kg^{-1}$) has been found in sauces made of sea food (prawns etc.) especially those containing nitrates and nitrites.[183] Apart from nitrites and nitrates, whose concentrations are 30 and 100 mg $kg^{-1}$, fish and fish products contain amines.[148,160,260]

Milk and plant products are much less contaminated by NA and they contain as a rule only NDMA. NA are almost absent from milk, acid milk products, and curd cheese. However, NDMA and NDEA have been found in dried milk and individual varieties of cheese, for example, Gouda, Cheddar, and wine cheese—up to 68 and 20 μg $kg^{-1}$ respectively.[5,58,122,161,183,215,218,238,250,272,274,277,279] It is noteworthy that the content of nitrites and nitrates in milk and cheeses can reach 1.6 and 76 μg $kg^{-1}$.[122,218,238,274]

NA are absent from most cereals, vegetables, and fruit; NDMA (2–5 μg $kg^{-1}$) has been found in stored vegetable and fruit preserves, but the levels of NDMA and NPyr in salt-pickled vegetables reach 63 and 32 μg $kg^{-1}$ respectively.[125,141,148,244,282] As a rule, fruit and vegetables contain a fairly large amount of nitrates, for example, lettuce and beetroot contain up to 8000 mg $kg^{-1}$.[55,75,148,200,244,282]

NDMA have been found in beer and alcoholic drinks (up to 68 μg $litre^{-1}$ in dark varieties of beer).[6,39,122,148,200,246,277,279,282] It is believed that the NA concentration in beer depends on the quality of the malt.[40,101,233,246,260,277]

Like water, drinks contain nitrates.[55,200] It has been noted that the content of NA, nitrites, and nitrates in the same type of product vary within considerable limits depending on the conditions of their manufacture and storage.[125,127,183,218]

The amount of NA entering daily the human organism together with the diet has been calculated. Naturally, it is different for different countries but it does not usually exceed 2.3 μg per day for man.[3,5,6,10,55,122,148,200,220,277] Significant amounts of nitrates and nitrites enter the human organism together with food products.[6,10,39,55,122,148,200]

Hygienic norms, according to which the limiting permissible NDMA and NDEA concentrations (overall) in various food products must not exceed 2–4 μg $kg^{-1}$ have been specified.†

One of the ways in which food products are contaminated by NA is circulation in food chains. The circulation of NA is a complex process in which account must be taken not only of the transfer of the NA themselves but also of their precursors, capable of forming these carcinogens readily and directly in the given objects. The total NA and their precursors passing from the environment into plants and food products, the formation of NA, and the various reactions of these compounds and their denitrosation determine ultimately their concentration in the given object.[7,126]

Circulation in food chains begins with the contamination of plants by NA and their precursors.[7,10,193,216] It has been shown that NA, nitrites, and nitrates are capable of passing from water and soil and also from residues of inorganic nitrogen-containing fertilisers, pesticides, and industrial and agricultural waste contained in them into plant products and accumulating there.[7,10,216,222,263,267]

Together with various types of feed and water, NA, nitrites, and nitrates enter the organisms of animals and pass into the products of animal husbandry—milk, meat, and eggs.[7,10,216,263,268,271] When various products, especially those with high concentrations of NA precursors, are stored, NA are formed in them.[122,125,128,244,284] Together with food, drinks, and water, NA and their precursors enter the human organism; NA are formed from their precursors in the gastrointestinal tract.[3-5,10,102] It has also been shown that NA can pass into food products from packing materials.[11,281]

The second way in which food products are contaminated is technological treatment: smoking, frying, salting, drying, dry-curing, etc.[40,127,216,233,234,260,284] Much evidence has been obtained showing that high NA concentrations are then observed in products containing considerable amounts of nitrites and nitrates.[10,40,122,127,233,260,273] Smoking using smoke containing nitrogen oxides results in a significant increase of NA levels.[10,160,234,260] It has been shown that a decrease in the concentration of nitrates, nitrites, and nitrogen oxides and also the introduction of inhibitors and the improvement of technology make it possible to obtain smoked, salted, fried, etc. products with a low NA content.[5,40,122,127,198,233,234,273,277]

A decrease in the amount of NA in various nitrogen-containing substances entering the environment as a result of the effective purification of industrial and transport waste and strict regulation of the use of nitrogen-containing inorganic fertilisers, pesticides, etc. is important for the reduction of the degree of contamination of food products by NA.[7,10,216]

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Thus carcinogenic NA are capable of being formed from a wide range of nitrogen-containing substances and nitrosating agents directly in various objects and living organisms. The ease of formation and the fairly high reactivity and stability of NA are responsible for their wide distribution in the environment and the possibility of their undesirable influence on man.

The development of effective and reliable chromatographic methods for the identification and quantitative determination of these compounds in various objects in microgram and nanogram amounts has played an important role in the study of NA. Systematic analyses made it possible to discover objects with a high NA content and the principal causes of their accumulation and to propose certain measures in order to prevent or sharply reduce the contamination of such objects by these carcinogens.

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