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Nitrates, Nitrites, and Nitrosamines

Extensive research is needed to establish how great a food hazard these nitrogenous substances present.

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The potential hazards of nitrosamines as toxicants formed in, or as a result of eating, certain foodstuffs have been described in newspapers (*1*), in testimony before congressional committees (*2*), in technical articles (*3*), and in consumer publications (*4*). We believe it appropriate therefore to review the status of knowledge of these substances to gain perspective and to distinguish between actual and potential occurrence of these compounds or their formation in vivo (or both). Some authors have carried out test tube reactions between nitrite and secondary amines and have indicated that these precursor substances are components of a number of foods we eat or drugs we take (*5*). However, it does not necessarily follow that by either in vitro or in vivo mechanisms we are being exposed to nitrosamines from a variety of sources. Evidence for the presence or formation of nitrosamines in foods is limited and some of the earlier reports of nitrosamines in human nutrients may have been based on inadequate analytical procedures (*6*). Furthermore, because pharmacological data on the action of various nitrosamines are incomplete at this time, we believe that it is not yet possible scientifically to ascertain the true danger from nitrosamines in our environment. The demonstrated carcinogenicity of many of these compounds, however, indicates that well-planned, long-range research is mandatory to obtain the needed answers.

Not only may nitrites conceivably serve as reactants with amines or amides to form toxic nitroso compounds, but the nitrites—and their precursor nitrates—have themselves been implicated in causing toxicity in animals and humans, and particularly in children. Ecologists have expressed concern regarding nitrate concentrations in the environment and the effect on the food and water supply.

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Nitrates

Our major intake of nitrates in foodstuffs comes primarily from vegetables or water supplies that are high in nitrate content, or from nitrates used as additives in the meat-curing process.

Nitrates are natural constituents of plants. They are present in large quantities in many vegetables, but they occur in only minor amounts in fruit (*7*). Spinach, beets, radishes, eggplant, celery, lettuce, collards, and turnip greens are among the vegetables that generally contain very high concentrations of nitrates (*7*). The nitrate content of some samples may be more than 3000 parts per million. However, the absolute values reported vary extremely because of genetic, environmental, sampling, and maturity factors.

The most important factors that favor large accumulation of nitrate in vegetables include (i) a nitrate-rich environment such as may be caused by high levels of fertilization, especially during the ripening period (*8*); (ii) species that are prone to accumulate nitrate (*9*); (iii) plant nutrient deficiencies (such as that caused by a lack of molybdenum) (*10*); (iv) conditions of reduced light intensity during maturation (*11*); (v) lack of water (*12*); and (vi) plant damage from chemical treatments (*13*). Interactions among these factors complicate the picture and account for the wide ranges of values reported for nitrate of the same vegetables.

A person is likely to consume as much or more nitrates from his vegetable intake as from the cured meat products he eats (*14*). Sodium or potassium nitrate is permitted as an additive to meat products in the United States at levels of 2¾ ounces to 3½ ounces per 100 pounds of meat (*15*). In some countries, not including the United States, addition of small amounts of nitrate is permitted in the manufacture of some varieties of cheese (*7*).

Concern has frequently been expressed about the high nitrate content of some water supplies, particularly those from wells. This nitrate comes from many sources, including precipitation, soil and rock, agricultural use of fertilizers, nitrogen fixation by microorganisms and plants, and especially decomposition of plant and sewage wastes followed by the leaching of nitrates into groundwater. Health agencies have issued reports that, for water to be safe for domestic use, the concentration of nitrate should not exceed 10 parts per million expressed as nitrate-nitrogen (*16*). Numerous examples may be found in which the nitrate content of well water is in excess of this amount (*17*). This situation presents some hazard of potential toxicity. However,

the usual nitrate intake of an adult may be less from the water supply than from either the vegetables or meat products ordinarily consumed (*7*).

In the quantities normally occurring in food or feed, nitrates become toxic only under conditions in which they are, or may be, reduced to nitrites. Otherwise, at reasonable concentrations, nitrate ions are rapidly excreted in the urine. The nitrites, then, constitute the principal toxic agent, and high intake of nitrates constitutes a hazard primarily under conditions that are favorable for their reduction to nitrite. Four such situations in which this may occur are well documented.

1) The microbial environment in the rumen of cattle causes reduction of nitrate to nitrite. Subsequent absorption of the nitrite ion may result in toxicity to cattle. Hence a danger is present when feeds or water of high nitrate content are consumed (*18*). In the horse, the enlarged cecum and colon also provide a location for microbial reduction of nitrate, and subsequent toxic effects (*19*).

2) The lesser stomach acidity of infants under about 4 months of age may permit the growth of microorganisms that can reduce nitrate to nitrite. As a result, providing water of high nitrate content to infants is a real hazard. Numerous deaths from this cause have been recorded (*20*).

3) When spinach, whether processed or unprocessed, is stored under conditions that permit the growth of microorganisms, nitrate may be reduced to nitrite. A number of cases of toxicity in infants have been reported from spinach left at room temperature for some time after cooking or after a jar of baby food was opened. Conceivably, such nitrite toxicity may also develop in other vegetables or in prepared foods of high nitrate content, but most of the cases reported deal particularly with spinach (*21*).

4) Reduction of nitrate to nitrite has occurred in damp forage materials that were high in nitrate content. Ingestion by livestock proved toxic (*22*). Also, release of oxides of nitrogen from ensiled forages may be hazardous to man and animals (*23*).

In the curing of meat, some of the added nitrate is usually reduced to nitrite but authentic cases of toxic effects from added nitrate only were not found.

Thus, nitrates are not toxic per se, but may under some circumstances be the starting point for a chain of reactions that result in the conversion to toxic substances. Hence, prudence dictates that we monitor the nitrate content of our foods, feeds, and water supplies, divert any samples of particularly high content into channels where they will do no harm, and be aware of actions that can lead to decreasing the use and amount of nitrate in foods when such decrease becomes warranted.

Nitrites

The proved toxicity of nitrites is due primarily to their interaction with blood pigment to produce methemoglobinemia, and their presumptive toxicity relates to their possible reaction, under normally encountered situations, with amines or amides to form toxic nitroso compounds.

Hemoglobin (Hb), the respiratory pigment containing Fe(II), normally transports oxygen to the tissues as a loose complex—oxyhemoglobin ($Hb \cdot O_2$). After the iron is oxidized to the ferric state, the pigment loses its ability to transport oxygen and forms a brown compound, methemoglobin (MetHb). The presence of nitrite in the blood results in MetHb formation. When the methemoglobin concentration exceeds 70 percent, asphyxia occurs, although at lower levels the reaction is reversible (*24*). The nitrite is very strongly bound to the heme of methemoglobin (*25*).

Although there are a number of instances in cattle of nitrite poisoning from water or forage, or in infants from nitrite-containing spinach, the incidence of toxicity is really quite low and the intakes required for serious toxic effects are usually large. As was mentioned in the previous section, the nitrites are in these instances derived from reduction of nitrates. Poisoning of adult humans by nitrite apparently has not been a problem. However, accidental addition of excessive amounts of nitrite to foods has led to instances of poisoning of both adults (*26*) and children (*27*).

Nitrites are more toxic than nitrates, and restriction of the daily intake for man to 0.4 milligram per kilogram of body weight is recommended (*28*). However, nitrites have been used therapeutically as medication for vasodilation and as an antidote for cyanide poisoning in doses of 30 to 300 milligrams without severe toxic effects (*29*). Consumption levels that may cause long-term hazards in man have not been established.

Nitrates and Nitrites in Cured Meat and Fish

The principal source of nitrite in our diets is processed (cured) meat or fish, and nitrites are considered a potential reactant precursor for nitrosamines.

Originally meat was cured in brine containing potassium nitrate as one of the ingredients. However, the actual curing agent was found to be the nitrite produced by the bacterial reduction of the nitrate salt. When it was shown that nitrite could be substituted for nitrate in the cure solution with the production of a more uniform and completely satisfactory product in a shorter period of time, a legal limit of no more than 200 milligrams of residual nitrite (calculated as $NaNO_2$) per kilogram of meat was established (*30*). The action of nitrite in the cure process is threefold: (i) formation of characteristic color, (ii) production of cured flavor, and (iii) antibacterial activity.

The role of nitrite in color formation is to furnish nitric oxide which reacts with myoglobin (Mb) to give nitrosylmyoglobin (MbNO) the red-pink pigment of cured meat. Although the fate of nitrite after addition to meat is not fully known, the following reactions have been postulated to occur in the presence of added reductants (AH_2) (*31*).

$$NaNO_2 + H^+ \longrightarrow HNO_2 + Na^+$$
$$2HNO_2 \longrightarrow N_2O_3 + H_2O$$
$$N_2O_3 + AH_2 \longrightarrow AHNO + HNO_2$$
$$AHNO \longrightarrow AH\cdot + NO$$
$$NO + MetMb \longrightarrow MetMbNO$$
$$MetMbNO + AH_2 \longrightarrow MbNO$$

In the presence of ascorbic acid, cysteine, quinones, or other reductants, nitrous acid, formed from the nitrite ion, is reduced to nitric oxide, which forms a complex with metmyoglobin (MetMb). The nitrosylmetmyoglobin (MetMbNO) is reduced by ascorbic acid to nitrosylmyoglobin. Nitrosylhemochrome, the cure pigment, results when the product is heated.

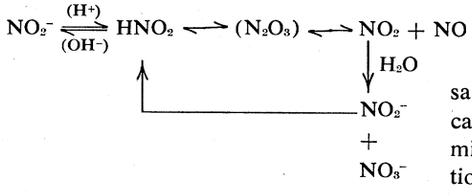
Another mechanism for the production of nitric oxide (NO) proposes (*32*) the reduction of nitrite mediated by reduced cytochrome c [Fe(II)Cyto c] and reduced nicotinamide adenine dinucleotide (NADH):

$$No_2^- + Fe(II)Cyto\ c \longrightarrow NOFe(III)Cyto\ c$$
$$NOFe(III)Cyto\ c + NADH \longrightarrow NO + Fe(II)Cyto\ c$$

Cured meat flavor is a function of the activity of nitrite on meat components. While emphasis has been placed on the reaction of nitrite with meat pigments, the so-called "cosmetic effect," and on the antibacterial action of the compound, little attention has been paid to the development of the characteristic cured flavor. However, there have been several reports on the effect of nitrite on the flavor of bacon, ham, and frankfurters (*33*). Initial studies indicate that flavor can be obtained at concentrations of nitrite lower than the legal limits. There is no information available at this time, however, about the reactions or the meat components involved.

The antibacterial effects of nitrite appear to be necessary to maintain the stability of cured canned meat products exposed to less-than-sterilizing heat treatment. The growth of *Clostridium botulinum* and toxin production are inhibited. While the mode of action of nitrite is still unknown, it has been reported that division of vegetative cells does not occur in the presence of this ion (*34*). Growth of surviving organisms is also inhibited by the action of residual nitrite.

Inhibition of the growth of *C. botulinum* in these meat products is a complex phenomenon involving interaction of the number of spores present, the amount of heat applied, and the concentrations of sodium chloride and sodium nitrite used (*35*). The interdependence of these factors is so great that minor changes in the conditions of one may require balancing modifications in all the others. The *p*H of the product may also play an important role in the bactericidal effect of nitrite, although it is difficult to adjust the *p*H of meat. It has been shown in model systems that there is approximately a tenfold increase in antibacterial effect with a decline of one *p*H unit in the range of *p*H 7.5 to 6.0 (*36*). Under the more acid conditions, larger concentrations of undissociated nitrous acid are available and, according to Shank *et al.* (*37*), this is the molecular species responsible for inhibition of the growth of clostridia. The dynamics of nitrous acid formation may be shown as a cyclic reaction:



Concomitant oxidation-reduction reactions lead to the formation of nitric oxide and nitrogen dioxide. The latter reacts with water forming nitrate ion and nitrite ions, which reenters the cycle. At the *p*H of greatest antibacterial activity (*p*H 4.5 to 5.5) the amount of nitrous acid present is at a maximum.

A number of episodes of botulism in recent years were caused by consumption of improperly handled or improperly processed fish or fish products (*38*). Control of *C. botulinum* in these products is attained by the use of sodium nitrite cure preparations. The preservation of fresh fish fillets or lightly smoked saltwater fish—such as salmon, tuna, halibut, and cod (*39*)—as well as freshwater fish—chub, for example (*38*)—has been described. Federal regulations limiting the residual nitrite in smoked, cured tuna to 10 mg/kg and in sable, salmon, and shad to no more than 200 mg/kg have been established. The nitrite content of smoked chub must be not less than 100 mg/kg (*30*).

The use of nitrite in the preservation of fish may result in the formation of nitrosamines. Certain species of fish, particularly of saltwater fish (*40*), contain large quantities of trimethylamine, trimethylamine oxide, and dimethylamine which may react with nitrite to form *N*-nitrosodimethylamine.

N-Nitroso Compounds

The reaction of nitrite with some classes of amines is a matter of public health interest at this time. Nitrosamines have been used as intermediates in a number of industrial processes (*41*); hence exposure to these compounds may be more widespread than assumed. Animal studies have shown that *N*-nitrosamines and the related *N*-nitrosamides are carcinogenic (*42*), and they may be mutagenic and teratogenic (*43*) as well. Nitrosamines having a wide variety of molecular structures are carcinogenic; of the approximately 100 compounds tested, about 75 percent produced lesions in test animals. Various tissues respond to the action of these compounds, some of them specifically to a certain nitrosamine or to groups of nitrosamines. The effects of nitrosamines can be elicited by several routes of administration—oral, intravenous, inhalation, subcutaneous, intraperitoneal, and topical. Local sarcomas have been observed rarely at the site of injection; the carcinogenic activity usually occurs elsewhere. It would seem, therefore, that the nitrosamines themselves are not carcinogens but may behave as carcinogen precursors (*44*). Studies with radioactive tracers suggest metabolic degradation of the nitroso compound to form an alkylating radical or ion that attacks the 7-position of guanine in nucleic acids. Although no cancer in man has yet been traced to nitrosamines as causative agents, the experimental results in animals suggest that these compounds also would be carcinogenic to man.

The acute toxicity or eventual carcinogenicity of the nitrosamines may, depending on the compound and the circumstances, show itself at very low dosages (*43*). Results of feeding studies are not yet decisive as to precise dose-response relationships. In a study with rats given a single dose, concentrations of *N*-nitrosodimethylamine greater than 5 ppm induced tumors in more than 70 percent of the animals, whereas continuous feeding of 1 ppm in the diet has been suggested as a threshold dose (*45*).

N-Nitrosamines, principally *N*-nitrosodimethylamine, have been reported in a number of foods. However, improved analytical procedures and recognition of artifacts in the preparations have now led to questions concerning the validity of these reports.

Nitrosation has in the past been considered to occur with secondary amines only. Recent studies have indicated that nitrosamines are also formed from tertiary amines and quaternary ammonium compounds that occur naturally in foods (*46*) and drugs (*47*).

The nitrosation reaction may proceed chemically or it may occur as a result of the metabolic activity of microorganisms. Ease of nitrosation, chemically, may be influenced by many factors, including the basicity of the amine (*48*), *p*H (*49*), substrate concentration (*49*), and the presence of some inorganic ions (*50*). The thiocyanate ion is of particular interest. This ion is normally present in human saliva in amounts ranging from 12 to 33 mg/100 ml (higher amounts are found in the saliva of smokers) and is capable of increasing the rate of nitrosation of morpholine (*51*).

In addition to the potential of formation of nitrosamine in processed foods, there may be nitrosamine forma-

tion in the gastrointestinal tract from ingested nitrite and secondary amines or their precursors (*52*). In several in vitro studies nitrosamine was formed from amines and nitrite in media in which intestinal microorganisms were growing (*53, 54*). In other tests, with human patients exhibiting conditions in which there was no, or only low, stomach acidity, nitrosation of diphenylamine, a nontoxic easily nitrosated amine, occurred (*55*). The appearance of tumors in various organs or the methylation of the 7-position in the nucleic acid guanine in rats that had ingested amines or amides and nitrite was considered presumptive evidence for the formation of nitroso compounds (*56*). The role of the intestinal bacteria in nitrosation under these conditions, however, is still not clear. Under healthy conditions the human stomach and upper gastrointestinal tract contain very few organisms (*57*), but when the acidity decreases, conditions may be favorable for the presence of nitrate-reducing, nitrosating bacteria. Nitrates and nitrites ingested with food or water are normally absorbed very rapidly from the stomach and upper gastrointestinal tract, appearing eventually in the urine. Thus, though the residence time of the nitrates and nitrites may be too short for gastric involvement, the potential exists for bladder involvement. It has been shown that rats with experimentally induced bladder infections of *Escherichia coli* excreted nitrosated piperidine after they had ingested the amine and a water solution of nitrate (*53*).

Correlation between many preformed nitrosamines and tumor induction in animals has been demonstrated. However, the relation between nitrite (nitrate) and amines (or their precursors), the end formation of nitrosamines in foods or in vivo, and their carcinogenic effect is still tenuous. While nitrosamine formation occurs more or less readily in vitro, in the normal, healthy human gastric conditions do not seem to be favorable for nitrosamine formation. At this time there is not enough information concerning the naturally occurring amines and their precursors, the *p*H changes in the stomach during digestion, the rate of absorption of the reactants, or the role of the intestinal flora.

The number of reports of nitrosamines in foods confirmed by mass spectrometry, the only procedure currently recognized as definitive, is very limited. Important gaps in our information exist. In addition to lack of information about all the compositional factors of the foods, we are also ignorant of the processing factors that could lead to nitrosamine formation.

Other environmental factors have been mentioned as potential sources of nitrosamines. Tobacco smoke has been implicated in the development of lung cancer. The tobacco plant is rich in amines, and an even larger number of these compounds has been identified in tobacco smoke as a result of the pyrolysis of the nitrogenous constituents of the plant (*58*). Nitrate is also present either as a plant constituent or it may have been added during processing. Under these conditions the presence of nitrosamines has been reported in tobacco (*59*) and in tobacco smoke (*58, 60*). However, reports of nitrosamines in tobacco smoke should be evaluated for reliability of analytical methods used, and the possibility that they may be formed (artifacts) in the collecting traps should be considered (*58*).

A number of commonly used drugs that are taken either in large doses or for long periods of time contain secondary amine groups or structures that may be amine precursors. There is some interest in the potential internal nitrosation of such drugs if ingested with nitrite or water containing high levels of nitrate. Model studies (*47*) reacting nitrite with oxytetracycline and antipyrine yielded *N*-nitrosodimethylamine, and *N*-nitrosodiethylamine was obtained when the drug disulfiram was used. Although no nitrosamine could be isolated from the system containing nitrite and tolbutamide, nitrosohexamethyleneimine was formed from tolazamide. Extended studies with more drugs and investigation of the applicability of model systems to the human experience are needed.

Summary

We are faced with evaluating the potential hazard of nitrate, nitrite, and nitrosamines in our environment. The extent of real danger is not yet known, but deliberate consideration of the available information would suggest that the hazard is not sufficiently great to cause alarm. There may be some who advocate immediate elimination of or drastic reduction in amounts of nitrite or nitrate in cured meat and fish. Before actions such as these would be taken, we should be very sure that we are not foregoing the needed preservative effects of nitrite, which protects us against serious outbreaks of food poisoning. We could be replacing one hazard by another, more serious one.

There is under way in the scientific community a commendable amount of research on many aspects of this important problem. This effort is necessary because so little is known of the possible in vivo synthesis of nitrosamines.

Thus we should continue to regard nitrites and nitrosamines as possible important toxicants but should be cautious about taking any action relative to modification of our food supply until we are sure the action is needed, justified, and proper.

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Gypsy Moth Control with the Sex Attractant Pheromone

Mass trapping or permeation of the air with pheromone can prevent male gypsy moths from finding mates.

Morton Beroza and E. F. Knipling

The gypsy moth [*Porthetria dispar* (L.)], a serious defoliator of forest, shade, and orchard trees in northeastern United States, is spreading rapidly to the South and gradually to the West and threatens to become a national problem.

There is deep division among scientists, administrators, environmentalists, and public officials about whether its spread can be stopped or should be stopped. On some occasions we read of citizens and township officials begging for relief from the moth's depredations; at other times it is claimed that after the initial flareup damage can be small, and that we should learn to live with the insect and find ways to minimize the damage rather than attempt to halt expansion of the present infestation. The way in which the gypsy moth problem has been handled has been criticized (*1*), but the critics have not come up with practical and ecologically acceptable solutions.

In this article we describe the problem and discuss the possibilities of using the recently identified sex pheromone of the gypsy moth (*2*) to combat this insect.

History

The gypsy moth, a native of Europe, Asia, and North Africa, was brought to Medford, Massachusetts, in 1869 for the purpose of producing silk for local industry; unfortunately, some insects accidentally escaped. The moth became established, but was largely unnoticed until 20 years later when there was a devastating population explosion. The following comment of a local resident is typical (*3*): "In 1889 the walks, trees and fences in my yard and the sides of the house were covered with caterpillars. I used to sweep them off with a broom and burn them with kerosene, and in half an hour they were

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