A MONOGRAPH

on

# NITRATES AND NITRITES
(Including Nitrosamines)

to

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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TABLE OF CONTENTS

SUMMARY . . . . . i

CHEMICAL INFORMATION. . . . . 1

Nomenclature . . . . . 1

Empirical Formula. . . . . 1

Molecular Weights. . . . . 1

Description. . . . . 2

Analytical Method. . . . . 5

Occurance and Levels . . . . . 10

BIOLOGICAL DATA . . . . . 20

Acute Toxicity . . . . . 20

Short-Term Studies . . . . . 28

Long-Term Studies. . . . . 52

Special Studies. . . . . 62

BIOCHEMICAL ASPECTS. . . . . 78

Breakdown. . . . . 78

Absorption-Distribution. . . . . 81

Metabolism and Excretion . . . . . 85

Effects of Enzymes and Other Biochemical Parameters. . 88

Drug Interaction . . . . . .104

CONSUMER EXPOSURE INFORMATION . . . . . .106

BIBLIOGRAPHY. . . . . .

## SUMMARY

Sodium nitrate ($NaNO_3$) potassium nitrate ($KNO_3$), sodium nitrite ($NaNO_2$) and potassium nitrite ($KNO_2$) are water soluble inorganic salts. They are stable in dry form at room temperature.

Nitrates can be determined in foods by the Official AOAC m-xylenol method(932), by the brucine procedure(1209), or by reduction of nitrate to nitrite and calculation of the nitrate content by difference between the nitrate determined before and after reduction(619).

Sodium and potassium nitrate are permitted to be added to cured meat and meat products and cured poultry products at 500 ppm. No more than 200 ppm residual nitrite can be present in the final product from the use of nitrates either alone or in combination with nitrites(69).

The acute oral toxicity of sodium nitrate to male rats determined as $LD_{50}$ ranges from 3100 to 7100 mg/Kg depending upon body weight(1540). No acute oral toxicity data on potassium nitrate in rats were available at the time of preparation of this report. However, the reported lethal doses of potassium nitrate for cattle(269), Sheep (269), and chickens(781) are 75-140, 70, and 2000 mg/Kg respectively.

The fatal dose of potassium nitrate for adult humans is in the range of 35 to 30 g or 18-68 mg/Kg nitrate nitrogen(269). Lower doses of sodium or potassium nitrate have caused acute methemoglobinemia, particularly in infants resulting from the conversion of nitrate to nitrite *in vivo*(1221,1992). The Public Health Service Drinking Water Standards establish a limit of 10 mg nitrate-nitrogen (45 mg nitrate) per liter in water(1221).

No reports of either short or long-term studies on the toxicity of nitrates to laboratory animals have appeared to date. However, in cattle methemoglobinemia, decreased serum vitamin A contents and degenerative damages in the coronary arterioles and renal glomeruli, have been observed at doses of 300 to 600 mg/Kg over 40-240 day periods[429, 2409].

In chickens and laying hens, doses of sodium nitrate up to 300 ppm nitrate nitrogen in the drinking water show no observable effects on blood hemoglobin, liver Vitamin A, weight gain, or mortality[12,1337]. In turkeys, potassium nitrate in drinking water over the range 250-1000 ppm resulted in poor growth and high mortality with low vitamin A rations [1337].

Reproductive performance was not affected adversely, nor were teratologic effects observed in mice, rats, hamsters, or rabbits with doses of sodium nitrate of 400, 250, 400, and 250 mg/Kg, respectively [625] or with doses of potassium nitrate of 400, 180, 280, and 206 mg/Kg, respectively [626].

No evidence of mutagenic effects of sodium nitrate were observed in host mediated or cytogenetic assays or in the dominant lethal gene test in rats. [1540] There is no evidence in the literature on the carcinogenicity of nitrate <u>per se</u>.

Nitrates can be reduced to nitrites by microorganisms in foods [516, 2183], in the animal or human gastrointestinal tract [1221], or by enzymes in animal tissues [165]. Nitrate may also be reduced in meats by strictly chemical reactions[516].

Sodium nitrate is absorbed relatively slowly from the gastrointestinal tract of dogs[757] and rabbits [1106]. In both cases it is excreted primarily in the urine rather than in the feces.

Potassium nitrate is a more effective diuretic agent than sodium nitrate. However, both are less effective than ammonium nitrate or organic diuretic drugs [896].

Dietary potassium nitrate exerts an adverse effect on thyroid function in rats and sheep[194] but this effect can be overcome by rats over a two-week period[195]. Oral Administration of potassium nitrate to rats as the sole drinking fluid causes significant elevation of systolic blood pressure [643].

Sodium nitrate potentiated the contraction of frog santomius muscle[1042]. However, muscular tetanic tension is reduced by this compound [1781].

Sodium nitrate does not inhibit the proliferation of mouse liver cells but does retard growth of L and HeLa cells in tissue culture[1617]. However, nitrates do not exert any significant antimicrobial effect against food spoilage and food poisoning microorganisms [267, 753, 2000, 2183].

Methods for determining nitrites in foods are based on diazotization of nitrite with 4-aminobenzenesulfonic acid or amide followed by coupling with 1-naphthylamine or N, N-dimethyl-1-naphthylamine and spectrophotometric determination of the azo dye formed [932, 1052, 1053, 1207, 1208, 1209].

Sodium and potassium nitrite are permitted in meat, meat products and poultry products at a maximum residual nitrite content of 200 ppm from nitrite, or from nitrite in combination with nitrate.

The acute oral toxicity of sodium nitrite for the male rat expressed as $LD_{50}$ is in the range 104 to 276 mg/Kg depending upon body weight [1541]. Reported lethal doses of sodium nitrite for the cat [2188], dog [269], rabbit [1592], and pig [2407], are 73, 30.6, 80-90, and 105-124 mg/Kg, respectively.

Acute toxcity data on potassium nitrite were not available at the time of preparation of this report. However, a lethal dose of 200-400 mg/Kg has been reported for sheep [2014].

The human oral lethal dose for sodium nitrite is in the range 22-33 mg/Kg[269]. Lower doses of sodium or potassium nitrite have caused acute methemoglobinemia, especially in infants [778, 1221, 1992, 2339].

The results of short term toxicological studies in the rat (up to 200 days) with a dose of 20 milliequivalents per liter of sodium nitrite in the drinking water showed increased methemoglobin levels, and increased weights of liver, spleen, kidneys and myocardium[1494]. There were significant pathological changes in the liver, spleen, kidneys, adrenals, brain and heart[1499]. In the cat, feeding a total dose of 4.1 g sodium nitrite over a 104 day period did not result in adverse effects on weight gain or organ weights [2188].

In cattle, at doses as high as 319-375 mg/Kg/day in silage, high levels of methemoglobin were observed without significant gross pathological lesions or effects on serum calcium, magnesium phosphorous or β-carotene[2409].

Administration of 200 ppm of sodium nitrite in the drinking water over the four weeks results in growth depression and lowered Vitamin A levels in chickens and turkeys but does not effect egg production by laying hens[12]. Similar effects are noted when potassium nitrite was administered in the diet to chickens or turkeys[1337].

In a long term study in which sodium nitrite was fed in the drinking water to three generations of rats at a dose of 100 mg/Kg/day (LD25), a shortened life span was observed in all groups as compared to controls [490]. There were no significant changes in hemoglobin levels nor were any carcinogenic or teratologic effects observed.

When rats were fed a diet of 40 percent canned meat treated with 0.5 or 0.02 percent sodium nitrite over a period of 29 months, significantly lower weight gains were noted at the 0.5 percent $NaNO_2$ level after 78 weeks [2280]. However, there were no effects on mortality, on hematology, or on serum enzymes, nor were there any significant gross pathological changes or increases in tumor formation as compared to controls.

There were no adverse effects on reproduction of mice, rats, hamsters, and rabbits by sodium nitrite of doses of 23, 10, 23, and 23 mg/Kg, respectively[627] or by potassium nitrite at doses of 32, 10, 32, and 23 mg/Kg, respectively [628]. Delayed skeletal maturation was observed in hamsters at sodium and potassium nitrite doses of 23 and 32 mg/Kg, respectively [627, 628].

No evidence of mutagenic effects of sodium nitrite was found in either host mediated assays or with the dominant lethal gene test in rats[1541]. However, there was a sharp increase in the number of aberrant cells in the cytogenetic assay [1541].

Nitrite (in the form of nitrous acid) combines with hemoglobin to form methemoglobin[1221, 1992] and with the myoglobin pigment in meat to give the typical pink color of cured meat [633, 1760, 1761, 2418]. Nitrite reacts with ascorbic acid[268, 1424] with quinones [633] and with phenols such tyrosine [1674]. It also reacts with pepsin and reduces its activity[1675]. In nitrite toxicosis in the guinea pig both lactic and succinic dehydrogenase activities in the myocardium are decreased [2269].

Nitrites are absorbed from the gastro intestinal tract more rapidly than nitrates [269, 1513, 1221, 1992]. Dietary nitrite significantly decreases the assimilation of fats but not carbohydrates [1697] and delays gastric emptying time[2036].

A hypotensive effect is exerted by nitrites through vasodilation[937]. With human erythocytes, sodium nitrite caused destruction of hemoglobin and loss of osmotic integrity[825].

Sodium nitrite inhibited the proliferation of mouse liver, L, and HeLa cells in tissue culture[1617]. It is bacteriostatic toward a wide range of food spoilage and food poisoning microorganisms within the limits permitted in food[320, 2180, 2182].

Clostridium botulinum is inhibited by nitrite under the conditions used in meat curing; the inhibitory concentration depends upon type and strain, number of vegetative cells or spores present; pH, temperature, prior heating of nitrite, and sodium chloride concentration[82,547,753, 1023,1024,1653,1678, 1679,1680]. Similar effects are observed with Clostridium perfringens[737,1199,1653] and Clostridium sporogenes[82,500,501,502,1654,1972, 2172, 2182].

The growth of Staphylococcus aureus is inhibited by nitrite at concentrations used in meat curing under acid pH conditions[259,320,1219,2182]. However, the production of staphylococcal enterotoxin A[1334] or enterotoxin B[1369] is not inhibited by nitrite.

Nitrite reacts with secondary and tertiary amines to form N-nitrosamines[603,1256,1257,2418]. Analytical methods for detecting nitrosamines in foods are primarily based on gas-liquid chromatography coupled with mass spectroscopy[258,405,588,892,894,2204]. However, this technique is only semiquantitative at best at the low levels of N-nitrosamines detected in foods. There is some question of the validity of many of the reports in the literature on detection of nitrosamines in food because of lack of confirmation by such methods as mass spectroscopy.

N-nitrosamines are known carcinogens [2366] and have been formed experimentally in the gastrointestinal tracts of mice [755,1871], rats [19,20,1258,1872], rabbits [754], and human subjects [1874] by simultaneous administration of nitrite and secondary amines.

However, the results of feeding studies reported in the literature using meats containing high levels of nitrite do not show any evidence of carcinogenesis [2280]. Further long-term studies in a variety of animal species are needed to resolve the differences between the results of short-term *in vivo* studies conducted under laboratory conditions and of long-term feeding studies.

# CHEMICAL INFORMATION

## Nomenclature

### Common Names

Sodium Nitrate - Soda niter, nitrate of soda, chile saltpeter
Potassium Nitrate - Saltpeter, nitrate of potash
Sodium Nitrite - nitrite of soda
Potassium Nitrite - nitrite of potash

### Chemical Names

Sodium Nitrate
Potassium Nitrate
Sodium Nitrite
Potassium Nitrite

### Trade Names

See Common Names

### Chemical Abstracts Service Unique Registry Number

| | |
|---|---|
| Sodium nitrate | 7631-99-4 |
| Potassium nitrate | 7757-72-1 |
| Sodium nitrite | 7632-00-0 |
| Potassium nitrite | 7758-09-0 |

## Empirical Formula

Sodium Nitrate - $NaNO_3$
Potassium Nitrate - $KNO_3$
Sodium Nitrite - $NaNO_2$
Potassium Nitrite - $KNO_2$

### Structural Formula

See empirical formulas

## Molecular Weights

Sodium Nitrate - $NaNO_3$ - 85.01
Potassium Nitrate - $KNO_3$ - 101.10
Sodium Nitrite - $NaNO_2$ - 69.01
Potassium Nitrite - $KNO_2$ - 85.10

## Description[621-624]

### General Characteristics

Specifications for sodium and potassium nitrates and nitrites are given in Table 1.

Sodium Nitrate. Sodium nitrate occurs as colorless, odorless crystals or crystalline granules. It is moderately deliquescent in moist air.

Potassium Nitrate. Potassium nitrate occurs as odorless, colorless, transparent prisms, or white granules or crystalline powder; it has a salty taste and produces a cooling sensation in the mouth and is slightly hygroscopic in moist air.

Sodium Nitrite. Sodium nitrite occurs as a white to slightly yellow, granular powder or as white, nearly opaque fused masses or sticks. It has a mild saline taste and is deliquescent in air.

Potassium nitrite. Potassium nitrite occurs as small, white yellowish granules or cylindrical sticks. It is deliquescent.

### Physical Properties

Physical properties of sodium and potassium nitrates and nitrites are presented in Table 2.

### Stability

Sodium and potassium nitrates and nitrites are stable in dry form at room temperature. Decomposition temperatures are given in Table 2. According to the Food Chemicals Codex[621-624] these compounds should be stored in tightly sealed containers.

TABLE 1. FOOD CHEMICALS CODEX SPECIFICATIONS FOR SODIUM AND POTASSIUM NITRATES AND NITRITES[621-624]

| Impurities | Limits, parts per million (and percent) | | | |
|---|---|---|---|---|
| | $NaNO_3$ | $KNO_3$ | $NaNO_2$ | $KNO_2$ |
| Arsenic | 3 ppm (0.0003%) | 3 ppm (0.0003%) | 3 ppm (0.0003%) | 3 ppm (0.0003%) |
| Chlorate | - | Passes test | - | - |
| Heavy metals (as Pb) | 10 ppm (0.001%) | 20 ppm (0.002%) | 20 ppm (0.002%) | 20 ppm (0.002%) |
| Lead | - | 10 ppm (0.001%) | 10 ppm (0.001%) | 10 ppm (0.001%) |
| Loss on drying | - | 1% | 0.25% | - |
| Total chlorine | Passes test (0.2%) | - | - | - |
| say (not less than after drying | 99.0% | 99.0% | 97.0% | 90.0% |

TABLE 2. SUMMARY OF PHYSICAL PROPERTIES OF SODIUM AND POTASSIUM NITRITES AND NITRATES[2358]

| Physical Property | $NaNO_3$ | $KNO_3$ | $NaNO_2$ | $KNO_2$ |
|---|---|---|---|---|
| Density | 2.261 | 2.109 (16 C) | 2.168 (0 C) | 1.915 |
| Melting point | 306.8 C | 334 C | 271 C | 440 C |
| Boiling point | (d) 380 C | (d) 400 C | (d) 320 C | (d) |
| Solubility (g/100 ml) | | | | |
| Water | 92.1 (25 C) | 13.3 (0 C) | 81.5 (15 C) | 281 (0 C) |
| Water - 100 C | 180 | 247 | 163 | 413 |
| Ethyl alcohol | Slightly soluble | Insoluble | 0.3 (20 C) | Slightly soluble |

(d) Decomposes

## Analytical Methods

### Nitrates and Nitrites

Schuller and Veen[1928] reviewed the various methods that have been used for determining nitrates and nitrites in foods. Nitrates are more difficult to determine directly in foods than nitrites because carbohydrates, chloride, and nitrite interfere with the nitration of such reagents as 2,4 phenol disulfonic acid, brucine, and 2,4 or 3,4 xylenol.[619, 1207-1210]

Dhont[448] described a polarographic method for determining nitrate in meat and meat curing brines based on nitration of phenoldisulfonic acid to yield 6-nitro-2,4 disulfonic acid. This nitro compound was then reduced at a dropping mercury electrode. Nitrite interfered with the polarogram and could be removed by treatment with urea at pH 3 for 30 minutes. Amino acids did not interfere and chloride interference could be decreased by increasing the amount of water in the phenoldisulfonic acid reagent. The minimum level of $KNO_3$ that could be detected was 0.015 percent with a relative error of 25 percent at this low concentration.

The Association of Official Analytical Chemists (AOAC) have adopted a m-xylenol procedure for determining nitrate in food after suitable preparation of the sample.[932]

Methods used for determining nitrite in foods are based on diazotization of nitrite with reagents such as 4-aminobenzenesulfonic acid, 4-aminobenzesulfonamide followed by coupling with 1-naphthylamine or N, N-dimethyl-1-naphthylamine. The absorbance of the azo dye formed is determined spectrophotometrically.[1207, 1208, 1210, 1928]

Kamm, et al[1252] used 1-naphthylamine as both diazotization and coupling reagent to determine nitrite in baby foods at concentrations as low as 1 ppm nitrite nitrogen. The absorbance of the 4-(1-naphthylazo)-1-naphthylamine formed was read spectrophotometrically at 555 nM.

Both nitrite and nitrate can be determined in foods, particularly cured meat by diazotization coupling methods. Nitrate is reduced to nitrite by cadmium and is calculated by difference between the nitrite determined before and after reduction.[619, 1252]

Kamm, et al[1253] reported that 100 percent reduction of nitrate to nitrite can be achieved with a suitably designed cadmium column. The diazo product from the reaction of 1-naphthylamine with nitrite was stable up to 4 hours in $CHCl_3$. These results were confirmed by Manning, et al[1330] in the determination of nitrate and nitrite in milk and dry milk products.

The official AOAC method for determining nitrite in cured meat utilizes the Griess reagent (1-naphthylamine hydrochloride in acetic acid). The color developed is read spectrophotometrically at 520 nM.[932]

Nitrosamines

Secondary and tertiary amines react with nitrous acid (and nitrite in acid solution) to yield N-nitrosamines.[603, 2418] The N-nitrosamines are carcinogenic and the detection of low concentrations of these compounds in meat and fish cured with nitrate or nitrite has led to the development of a number of analytical methods for detecting N-nitrosamines in foods. In general, these procedures involve initial extraction of N-nitrosamines from foods using suitable solvents, isolation by steam[1959, 1961] or vacuum

distillation[2204] concentration, followed by further cleanup using ion exchange or column chromatographic methods,[1961] and then detection and estimation by thin layer chromatography,[531, 935, 1961] gas-liquid chromatography,[560, 629, 1652, 1959] differential polarography[2334] colorimetric methods,[416, 578, 578a] microcoulometry[1442] or by gas-liquid chromatography combined with mass spectrometry.[258, 405, 588, 892, 894, 2204] It will be shown subsequently that all of these procedures are subject to some limitations resulting from interfering materials and that gas chromatography coupled with mass spectrometry gives the most reliable results of any of the methods available at the present time.

Extraction and Separation Methods. Extensive cleanup procedures must be used prior to determining N-nitrosamines in food samples because of the many interfering substances present. Furthermore, N-nitrosamines are present in such low concentrations in foods that the sample must be concentrated prior to analysis. Steam distillation separates only volatile nitrosamines; nonvolatile nitrosamines may also be present.

According to Walters, et al[2334] nonvolatile nitrosamines can be adsorbed from aqueous solutions onto activated carbon. Lipids interfere with this adsorption. N-nitrosamines with the exception of these containing phenyl groups and N-nitroso dipentylamine can be eluted by refluxing the activated carbon with methanol. However, the extent of elution of various nitrosamines by the procedure was somewhat lower than desirable; only N-nitrosodiethylamine was eluted to greater than 90 percent (94%) while the elution of other N-nitrosamines ranged from 26 to 84 percent.

A variety of solvents, including water methanol, ethylene dichloride, and ether has been used for initial extraction of N-nitrosamines from foods. In general, the pH should be adjusted to neutral or alkaline values prior to distillation to prevent decomposition of the N-nitrosamines and to prevent formation of nitrosamines by reaction of nitrite in the samples with amines produced by degradation of amino acids.

Sen, et al[1961] used ether and ethylene dichloride as solvents for extraction of N-nitrosamines from wheat flour, and cheese or fish, respectively. The extracts were desolventized and steam distilled to separate the volatile N-nitrosamines from nonvolatile materials.

Newell and Sisken[1442] described a procedure for separating N-nitrosodiethylamine (NDMA) from nonvolatile fractions of apple homogenates and milk by vacuum distillation at 1 mm Hg to minimize losses from hydrolysis and volatility. The NDMA in the distillate removed from water by percolation through a polymer bead column followed by heating to remove the NDMA from the polymer beads prior to gas chromatographic analysis. Recoveries of NDMA obtained by this method were 70 percent or higher.

In a study of the separation of carcinogenic nitrosamines by gel chromatography, Eisenbrand, et al[530] found that a mixture of symmetrical di-n-alkyl nitrosamines could be separated on Sephadex LH-20 and determined by the ultraviolet absorption at 230-235 nM. However, when a similar mixture of nitrosamines was added to wheat flour, ultraviolet absorbing contaminants in the extract interfered with the determination of the nitrosamines by ultraviolet spectrophotometry.

Determination of Nitrosamines

In general, polarographic methods for determining nitrosamines are sensitive to less than 1 ppm but are not specific because unsaturated aldehydes and ketones present in foods also reduce in the same -0.8 to 1.0 volt range as nitrosamines.[274] Differential polarography of samples before and after photolysis of the NO group of nitrosamines by ultraviolet light gives an improved estimate of nitrosamines in the presence of light-stable contaminants.[2334] However, various N-nitrosamines degrade photolytically at different rates. For this reason Burns and Alliston[274] caution against the fixed irradiation periods used by Walters, et al[2334] and instead recommend the use of half lives of those nitrosamines that follow first order decomposition kinetics as a guide to irradiation conditions. In this connection, determination of N-nitrosamines by colorometric determination of nitrite by diazotization with the Griess reagent after ultraviolet irradiation is not completely specific, particularly in the presence of nitrates or C-nitro compounds at an irradiation wave length of 250 nM.[2334]

Fan and Tannenbaum[578] describe an automatic colorimetric determination involving ultraviolet irradiation at 360 nM. This procedure provides a linear response to N-nitroso compounds at concentrations of less than 1 ppm and eliminates nitrate interference. However, interferences by nitramines and C-nitro structures could be a problem.

Thin layer chromatography (TLC) can be used to separate N-nitrosamines after extensive cleanup of the sample,[1961] but even then interfering compounds may not be removed completely. A relatively large sample is required which makes TLC unsuitable for determining small concentrations of N-nitrosamines in tissue samples and body fluids taken from laboratory animals.[1959]

Various gas-liquid chromatographic methods for determining N-nitrosamines in foods have been reported in the literature.[560, 629, 935, 1542, 1959, 1961] However, an extensive sample cleanup is also required prior to GLC analysis to minimize interferences.[893] Furthermore, GLC is limited to the determination of volatile N-nitrosamines. However, the use of GLC in combination with mass spectrometry allows the separation and identification of volatile N-nitrosamines with a high degree of specificity not obtainable by other methods.[258, 405, 588, 892, 894, 2204] Table 3 summarizes the detection limits of N-nitrosamines obtained by various investigators using GLC-mass spectrometry.

Bryce and Telling[258] found that the percent recoveries of various N-nitrosamines added to luncheon meat varied widely at the 1, 2, and 10 μg/Kg levels. They concluded that the GLC-mass spectrometry technique provides only a semiquantitative analysis of N-nitrosamines in the 1-5 μg/Kg range. Crosby, et al[405] reached similar conclusions. At these low levels the error in the analysis may be ± 50 percent.

## Occurrence and Levels

### Plants

Nitrates. Vegetables accumulate nitrate from the soil readily. Lee[1221, 1222] presents data on nitrate contents of vegetables obtained by various investigators over the years. This information is summarized in Table 4. It will be noted that nitrate contents of vegetables vary widely.

A recent report of the National Academy of Sciences[377] points out that the nitrate contents of vegetables at the present time are not markedly different from that of vegetables produced fifty years ago.

TABLE 3. SUMMARY OF LIMITS OF DETECTION OF N-NITROSAMINES IN FOODS OBTAINED BY VARIOUS GAS-LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY PROCEDURES

| Investigator | Food | Lower Limit μg/Kg | Method |
|---|---|---|---|
| Telling, et al[2204] | Pork, luncheon meat, ham | 25-65 | Monitoring of $NO^+$ |
| Gough and Webb[738] | | 2 | Monitoring nitrosamine ions lowered to 0.2 μg/Kg by peak-cutting |
| Bryce and Telling[258] | Pork, luncheon meat | 5-10 | Monitoring of $NO^+$ |
| | | 1 | Monitoring of nitrosamine ions |
| Fazio, et al[587] | Smoked fish | 4 | Monitoring of the m/e 74 ion |
| Crosby, et al[405] | Bacon, fish, luncheon meat, pork, salami, cheese | | Procedure of Gough and Webb[738] |

TABLE 4. NITRATE CONTENT OF VEGETABLES[1222]

| Authors | Year | Type, etc. | No. of Samples | ppm $NO_3$ High | Mean | Low |
|---|---|---|---|---|---|---|
| | | **Radish** | | | | |
| Richardson, W. D. | 1907 | Market | 6 | 3056 | 1804 | 531 |
| Wilson, J. K. | 1949 | Market | | 1176 | | 303 |
| Smith, G. E. | 1966 | Grown | | 9000 | | 2250 |
| Smith, G. E. | 1966 | Market | | 6500 | | 850 |
| Jackson, W.A., et al | 1967 | Fresh | 2 | 1734 | 1492 | 1250 |
| | | **Beets** | | | | |
| Richardson, W. D. | 1907 | Market | 7 | 8062 | 2552 | 930 |
| Wilson, J. K. | 1949 | Grown | | | 1333 | |
| Smith, G. F. | 1966 | Grown | | 4500 | | 600 |
| Jackson, W. A., et al | 1967 | Fresh and Canned | 3 | 1654 | 1197 | 671 |
| | | **Egg Plant** | | | | |
| Richardson, W. D. | 1907 | Market | 4 | 4430 | 1474 | 354 |
| Smith, G. E. | 1966 | Grown | | 4750 | | 650 |
| Jackson, W. A., et al | 1967 | Fresh | 1 | | 1857 | |
| Jackson, W. A., et al | 1967 | Canned | 2 | 2174 | | 1542 |
| | | **Broccoli** | | | | |
| Wilson, J. K. | 1949 | Grown | | 3846 | | 785 |
| Smith, G. E. | 1966 | Market | | 850 | | |
| Jackson, W. A., et al | 1967 | Frozen | 2 | 594 | | 506 |
| | | **Spinach** | | | | |
| Richardson, W. D. | 1907 | Market | 5 | 3809 | 1892 | 310 |
| Richardson, W. D. | 1907 | Canned | 5 | 1949 | 1100 | 266 |
| Wilson, J. K. | 1949 | Market | 3 | 2353 | | 1600 |
| Wilson, J. K. | 1949 | Quick Frozen | | | 3636 | |
| Jackson, W. A., et al | 1967 | Fresh and Frozen | 3 | 739 | 524 | 238 |
| Jackson, W. A., et al | 1967 | Canned | 2 | 550 | 473 | 396 |
| Phillips, W. E. J. | 1968 | Canned | 3 | 1435 | 886 | 505 |
| Phillips, W. E. J. | 1968 | Frozen | 3 | 2463 | | 429 |
| Phillips, W. E. J. | 1968 | Fresh | 3 | 2330 | | 1501 |
| | | **Lettuce** | | | | |
| Richardson, W. D. | 1907 | Market | 5 | 3544 | | 399 |
| Wilson, J. K. | 1949 | Grown (Head) | | 1250 | | 870 |
| Wilson, J. K. | 1949 | Market (Curly) | | 1818 | | 400 |

TABLE 4. (CONTINUED)

| Author | Year | Type, etc. | No. of Samples | ppm $MO_3$ High | Mean | Low |
|---|---|---|---|---|---|---|
| | | **Lettuce** (Continued) | | | | |
| Smith, G. E. | 1966 | Grown | | 2250 | | 150 |
| Smith, G. E. | 1966 | Market | | 6000 | | 300 |
| Jackson, W. A., et al | 1967 | Fresh | 5 | 893 | 664 | 488 |
| | | **Celery** | | | | |
| Richardson, W. D. | 1907 | Market | 5 | 2879 | 1496 | 797 |
| Richardson, W. D. | 1807 | Market | 2 | | 190 | |
| Wilson, J. K. | 1949 | Grown | | | 1212 | |
| Wilson, J. K. | 1949 | Market | | | 1240 | |
| Wilson, J. K. | 1949 | Market | | | 3232 | |
| Smith, G. E. | 1966 | Market | | 3250 | | 500 |
| Jackson, W. A., et al | 1967 | Fresh | 2 | 2957 | 2786 | 2614 |
| | | **Turnip** | | | | |
| Richardson, W. D. | 1907 | Market | 5 | 2879 | 1012 | 88 |
| Smith, G. E. | 1966 | Grown | | 4500 | | 250 |
| Smith, G. E. | 1966 | Market | | 3500 | | 50 |
| Jackson, W. A., et al | 1967 | Frozen (Greens) | 1 | | 1619 | |
| Jackson, W. A., et al | 1967 | Canned (Greens) | 3 | 2168 | | 1199 |
| | | **Parsley** | | | | |
| Richardson, W. D. | 1907 | Market | 2 | 1417 | 1056 | 753 |
| Jackson, W. A., et al | 1967 | Fresh | 1 | | 1698 | |
| Smith, G. E. | 1966 | Market | | 2250 | | 400 |
| Jackson, W. A., et al | 1967 | Fresh | 1 | | 299 | |
| | | **Cabbage (Green)** | | | | |
| Richardson, W. D. | 1907 | Market | 5 | 487 | 176 | 35 |
| Wilson, J. K. | 1949 | Grown | | | 2222 | |
| Wilson, J. K. | 1949 | Market | | 1200 | | 186 |
| Smith, G. E. | 1966 | Market | | 1650 | | 150 |
| Jackson, W. A., et al | 1967 | Fresh | 2 | 471 | 315 | 158 |
| | | **Cauliflower** | | | | |
| Richardson, W. D. | 1907 | Market | 3 | 398 | 132 | 26 |
| Wilson, J. K. | 1949 | Grown | | | 2000 | |
| Smith, G. E. | 1966 | Market | | 1250 | | 100 |
| Jackson, W. A. | 1967 | Fresh | 1 | | 53 | |

TABLE 4. (CONTINUED)

| Author | Year | Type, etc. | No. of Samples | ppm $MO_3$ High | Mean | Low |
|---|---|---|---|---|---|---|
| | | **Carrots** | | | | |
| Richardson, W. D. | 1907 | Market | | 89 | 66 | 40 |
| Wilson, J. K. | 1949 | Grown | | | 442 | |
| Wilson, J. K. | 1949 | Market | | | 325 | |
| Wilson, J. K. | 1949 | Quick Frozen | | | 181 | |
| Smith, G. E. | 1966 | Grown | | 500 | | 100 |
| Smith, G. E. | 1966 | Market | | 500 | | 50 |
| Jackson, W. A., et al | 1967 | Fresh, Frozen, and Canned | 4 | 194 | 102 | 18 |
| | | **Ham** | | | | |
| Richardson, W. D. | 1907 | | 20 | | 845 | |
| | | **Bacon** | | | | |
| Richardson, W. D. | 1907 | | 19 | | 383 | |
| | | **Dried Beef** | | | | |
| Richardson, W. D. | 1907 | | 12 | | 968 | |

The nitrate contents of spinach and beets have received special attention because of the use of these vegetables in processed baby foods. Table 5 shows nitrate contents of baby foods as determined by various investigators.

Nitrite. The nitrite contents of vegetables are low. According to Phillips,[1671] nitrite accumulated in fresh spinach stored under refrigeration over a 28-day period, and in frozen spinach if it was allowed to thaw out at room temperature for prolonged periods. However, nitrite did not accumulate in canned, baby food, or frozen spinach.

Tables 6 and 7 summarize nitrite and nitrate nitrogen contents of prepared infant foods as determined by Kamm, et al.[1052] All of the nitrite nitrogen values of vegetables and fruits fall in the narrow range of 0-2 ppm.

Nitrosamines. N-nitrosamines have been reported to be present in the fruit of Solanum incanum, a solanaceous bush found in South Africa[503] and in fresh and cooked apples.[1542] However, Sen, et al[1961] failed to detect N-nitrosamines in wheat flour and Crosby, et al[405] did not detect them in chopped spinach leaf in brine. Keybets, et al[1096] could not detect nitrosamines at the 0.1-0.5 ppm level in nitrite-containing spinach. They concluded that nitrosamine production in spinach would occur only in the presence of high concentrations of secondary amines and nitrite.

## Animal Products

Nitrates. Nitrates have long been used for curing meats such as ham, bacon, corned beef, etc. Nitrate can be reduced to nitrite either by-nitrate reducing bacteria[1265, 2180] or by enzymes present in tissues.[165]

TABLE 5. NITRATE CONTENT OF BABY FOODS[1222]

| Authors | Year | Type, etc. | No. of Samples | ppm $NO_3$ High | Mean | Low |
|---|---|---|---|---|---|---|
| Wilson, J. K. | 1949 | Beets | | 750 | | 333 |
| Kamm, L., et al | 1965 | Beets | 6 | 2165 | 977 | 634 |
| Commoner, B. | 1968 | Beets | 5 | | 370 | |
| NIEHS | 1969 | Beets | | | 245±97 | |
| Wilson, J. K. | 1949 | Spinach | | 833 | | 616 |
| Kamm, L., et al | 1965 | Spinach | 5 | 1668 | 1373 | 1074 |
| Commoner, B. | 1968 | Spinach | 4 | | 56 | |
| NIEHS | 1969 | Spinach | | | 66±21 | |
| Wilson, J. K. | 1949 | Green beans | | | 37 | |
| Kamm, L., et al | 1965 | Wax beans | 2 | 568 | 444 | 321 |
| Kamm, L., et al | 1965 | Green beans | 3 | | 163 | |
| Commoner, B. | 1968 | Beans | 8 | | 48 | |
| NIEHS | 1969 | Green beans | | | 43±10 | |
| Wilson, J. K. | 1949 | Squash | | | 0 | |
| Kamm, L., et al | 1965 | Squash | 5 | 409 | 282 | 44 |
| Commoner, B. | 1968 | Squash | 3 | | 126 | |
| NIEHS | 1969 | Squash | | | 84±28 | |
| Wilson, J. K. | 1949 | Carrots | | 3 | | 0 |
| Kamm, L., et al | 1965 | Carrots | 8 | 167 | 101 | 66 |
| Commoner, B. | 1968 | Carrots | 7 | | 26 | |
| NIEHS | 1969 | Carrots | | | 38±10 | |
| Kamm, L., et al | 1965 | Mixed vegetables | 2 | 106 | | 92 |
| Kamm, L., et al | 1965 | Garden vegetables | 5 | | 180 | |
| Wilson, J. K. | 1949 | Peas | | | 0 | |
| Commoner, B. | 1968 | Peas | 3 | | 2 | |
| Kamm, L., et al | 1965 | Meats | | 48 | | 4 |
| NIEHS | 1969 | Meats | | | 5.4±3.4 | |
| Commoner, B. | 1968 | Corn | 2 | | 2 | |
| Commoner, B. | 1968 | Sweet potatoes | 1 | | 16 | |
| Kamm, L., et al | 1965 | Graham crackers | 1 | | 211 | |
| Kamm, L., et al | 1965 | Pre-cooked cereals | | 211 | | 18 |
| Kamm, L., et al | 1965 | Dairy products | | 31 | | 0 |
| Kamm, L., et al | 1965 | Fruits | | 31 | | 0 |

TABLE 6. SURVEY OF THE NITRITE AND NITRATE NITROGEN CONTENT OF 194 PREPARED INFANT FOODS (1052)

| Food | No. of Samples | Nitrite, ppm | | Nitrate, ppm | |
|---|---|---|---|---|---|
| | | Range | Average | Range | Average |
| Fruits | 22 | 0-2 | 0.5 | 0-7 | 2 |
| Dairy products | 9 | 0.4-4 | 2 | 0-7 | 3 |
| Puddings | 5 | 0-2 | 0.6 | 0.8-11 | 4 |
| Egg products (including custards) | 13 | 0-2 | 0-9 | 0.9-11 | 4 |
| Meats | 15 | 0-0.6 | 0.3 | 1-11 | 4 |
| Meat dinners | 20 | 0-2 | 0.5 | 0.9-16 | 7 |
| Dry and concentrated food supplements | 27 | 1-10 | 3 | 4-49 | 17 |
| Precooked cereal products | 22 | 2-8 | 4 | 0.4-48 | 13 |
| Vegetables | 61 | 0-2 | 0.5 | 0.2-492 | 68 |

TABLE 7. PREPARED INFANT FOODS CONTAINING MORE THAN 20 PPM OF NITRATE NITROGEN (1052)

| Food | No. of Samples | Nitrite N, ppm | | Nitrate N, ppm | |
|---|---|---|---|---|---|
| | | Range | Average | Range | Average |
| Mixed vegetables | 2 | 0-2 | 0-8 | 21-24 | 22 |
| Carrots | 8 | 0-2 | 0.7 | 15-38 | 23 |
| Green beans | 3 | 0-1 | 0.8 | 16-17 | 37 |
| Garden vegetables | 5 | 0-2 | 0.7 | 19-62 | 41 |
| Graham crackers | 1 | | 2 | | 48 |
| Squash | 5 | 0-1 | 0.7 | 10-93 | 64 |
| Wax beans | 2 | 1-2 | 2 | 73-129 | 101 |
| Beets | 6 | 0-2 | 0.8 | 144-192 | 222 |
| Spinach | 5 | 0.3-1 | 0.5 | 244-379 | 312 |

The nitrite formed reacts with heme pigments, particularly myoglobin to give the typical red color of cured meats.[633, 1760, 1761] Typical nitrate contents of meats and baby foods containing meat are given in Tables 4, 5, and 6.

Nitrite. Nitrite is the active ingredient in the curing of meat. It has the functions of color fixation, cured meat flavor development, and bacterial inhibition.[2350, 2418] The role of nitrite as an inhibitor of growth and toxin production by selected bacteria of public health significance will be presented in a subsequent section. The Food and Drug Administration has established a legal limit of 200 ppm of residual nitrite (calculated as $NaNO_2$) in cured meats and poultry products regardless of whether the original source of nitrite added to meat is nitrate, nitrite, or a combination of both.[69] Typical nitrite contents of baby foods that contain meat products are shown in Table 6.

Nitrite may also be found in low levels in milk products dried in direct gas fired spray driers. Manning, et al[1330] reported that natural gas burners used in direct fired spray driers produce $NO_2$ levels in the range of 10-100 ppm in the drying air. They found nitrite nitrogen levels in nonfat dry milk produced in natural gas fired driers ranging from less than 0.5 to as high as 2.0 μg/g of milk solids (nonfat). However, volatile nitrosamines could not be detected in either freshly prepared or stored dry milk produced by direct gas fired driers.[1756]

Nitrosamines. The quantitation of N-nitrosamines in cured meat and fish has received considerable attention as mentioned in a previous section.

Table 8 presents N-nitrosamine contents of selected meat and fishery products that have been reported in the literature.

TABLE 8. N-NITROSAMINE CONTENTS OF SELECTED FOODS OF ANIMAL ORIGIN

| Food | N-Nitrosamine Content, μg/g | Method | Reference |
|---|---|---|---|
| Meat | | | |
| Bacon, back, pale Danish | 1-4 | Gas-liquid chromatography mass spectroscopy | Crosby, et al[405] |
| Smoked sausage | 0.8-2.4 | Hydrazine-colorimetric | Ender and Ceh[550] |
| Frankfurters | 8 | Gas-liquid chromatography mass spectrometry | Fiddler, et al[604] |
| Luncheon meat | 1-4 | Ditto | Crosby, et al[405] |
| Country ham (Smithfield) | 3 | " | Fazio, et al[589] |
| Fish | | | |
| Cod, raw | 1-4 | " | Crosby, et al[405] |
| Cod, salted | 1-4 | " | Ditto |
| Sable, raw | 4 | " | Fazio, et al [588] |
| Sable, smoked | 9 | " | Ditto |
| Ditto + nitrate | 13, +4 | " | " |
| " + nitrite | 8,9 | " | " |
| " + nitrate | | " | " |
| " + nitrite | 20,26 | " | " |
| Salmon, smoked | 5 | " | " |
| Salmon, ditto + nitrate | 16,17 | " | " |
| " + nitrite | 4,6 | " | " |

Natural Inorganic Sources

Nitrogen in the environment moves from the atmosphere to the soil and then is ultimately returned to the atmosphere through the nitrogen cycle. Nitrogen ($N_2$) is fixed symbiotically by leguminous plants and their associated root nodule bacteria. The plants are eaten by animals and man and the proteins, amino acids and nonprotein nitrogen compounds in these tissues are ultimately degraded by microorganisms to ammonia and to nitrogen.

Nitrate and nitrite are intermediates in the nitrogen cycle. Nitrate may be added to soil in the form of fertilizers containing ammonium nitrate manufactured by the synthetic fixation of nitrogen. Nitrate can be formed in soil by the action of bacteria on ammonium ion formed in soil from the addition of anhydrous ammonia as a nitrogen fertilizer.

For further information on the accumulation of nitrate in soils and water, consult the recent report of the Committee on Nitrate Accumulation of the National Research Council.(377)

## BIOLOGICAL DATA

### Acute Toxicity

Nitrate and Nitrite

Nitrate becomes toxic in food or feed through reduction to nitrite. Nitrite reacts rapidly with hemoglobin in an acid medium, and more slowly (several hours for completion) in neutral or alkaline media to form methemoglobin.(751) Nitrite binds strongly and apparently irreversibly to either the apoprotein or porphyrin group of methemoglobin.(2264) Methemoglobin cannot bind molecular oxygen.

Table 9 shows acute toxicity data for sodium and potassium nitrates and nitrites. Newell and Maxwell[1540, 1541] present data showing that the acute toxicity of both sodium nitrate and sodium nitrite in the rat expressed as $LD_{50}$, increases with increasing body weight. Comparable acute toxicity data for potassium nitrate and potassium nitrite were not available at the time of preparation of this report.

Naidu and Venkatrao[1513] reported that a dose of 275 mg of sodium nitrite administered orally to a 2 Kg dog resulted in staggering and paralytic symptoms within four minutes and respiratory and heart stoppage and death 25 minutes after administration. In both cases only small amounts of nitrite were found in the vomit or in the viscera after death which indicated that the major portion was either absorbed or destroyed.

Acute nitrate poisoning may occur in domestic livestock[585] including cattle,[224, 1000, 1001, 1363, 1467, 2011, 2341] sheep,[920, 1003, 1004, 1245, 2014] swine,[2407] and poultry[1337] from consumption of plants or feedstuffs containing high levels of nitrate. Nitrate is reduced to nitrite by bacteria in the rumen or in the intestinal tract and the nitrate so produced is absorbed rapidly ultimately resulting in methemoglobenemia and in severe cases, death.

In cattle, acute nitrate poisoning occurs when the nitrate content of forage exceeds 0.5 percent (dry weight basis) or 500 ppm in drinking water. Clinical symptoms (anoxia, dyspnea, cyanosis, brown or chocolate blood) are observed when methemoglobin levels reach 30 to 40 percent, and death occurs at methemoglobin levels of 80-90 percent.[262]

A single dose of 20 g potassium nitrite was lethal to 50 Kg sheep but a 50 g dose was not lethal to heavier sheep according to Sinclair and

TABLE 9. ACUTE TOXICITY OF SODIUM POTASSIUM NITRATES AND NITRITES

| Substance | Animal | Sex | No. | Route | Dosage mg/Kg body weight[a] | Measurement | Reference |
|---|---|---|---|---|---|---|---|
| Sodium nitrate $NaNO_3$ | Rat | Male | - | Oral | 5,200-7,100 (110-140 g rat) (6,100) | $LD_{50}$ (constant concentration) | (1540) |
| | Ditto | Ditto | | Ditto | 4,000-6,700 (210-225 g rat) (5,100) | $LD_{50}$ (50%) | Ditto |
| | " | " | | " | 3,100-5,200 (410-510 g rat) (5,200) | $LD_{50}$ (50%) | " |
| | " | " | | " | 4,300-6,000 (115-150 g rat) (4,000) | $LD_{50}$ (constant volume 10 ml/Kg) | " |
| Potassium nitrate $KNO_3$ | Cattle | Female | | " | 75-140[b] | Lethal dose | (269) |
| | Sheep | - | | " | 70[b] | Lethal dose | Ditto |
| | Chicken | - | | | 2000 | Lethal dose | (781) |
| Sodium nitrite | Rat | Male | | " | 200-276 (110-140 g rat) | $LD_{50}$ (constant volume 10 ml/Kg) | (1541) |
| | Ditto | Ditto | | " | 140-208 (140-208 g rat) (171) | $LD_{50}$ (constant volume 10 ml/Kg) | Ditto |
| | " | " | | " | 104-173 (430-510 g rat) (134) | $LD_{50}$ | " |
| | " | Male & female | - | " | 77-130 | $LD_{50}$ | (490) |
| | " | - | | " | 180 | $LD_{50}$ | (2046) |
| | " | Male | 11 | " | 1,100-2,000 | Lethal dose | (2188) |
| | " | Female | 9 | " | 460-1,200 | Lethal dose | Ditto |

TABLE 9. (Continued)

| Substance | Animal | Sex | No. | Route | Dosage mg/Kg body weight | Measurement | Reference |
|---|---|---|---|---|---|---|---|
| Sodium nitrite $NaNO_2$ | Cat | Female | 1 | " | 73 | Lethal dose | (2188) |
| | Pig | - | 3 | " | 105-124 | Lethal dose | (2407) |
| | Rabbit | - | - | " | 80-90 | Lethal dose | (1592) |
| | Dog | - | - | " | 6.4[b] | Lethal dose | (269) |
| Potassium nitrite $KNO_2$ | Sheep | - | 2 | " | 200-400 | Lethal dose | (2014) |

(a) Mean values in parentheses
(b) Nitrogen basis

Jones.[2015] They found that 20 g of potassium nitrite could be incorporated into daily feed of sheep without ill effects because of rapid breakdown of the nitrite in the numen.

Heavy mortalities of swine have been observed when water containing 1740 to 2970 ppm nitrates were used to make rations which ultimately contained 393.6 and 1127 ppm nitrite respectively.[2407] For chickens, nitrite levels above 0.2 percent in the feed result in high mortalities.[1337] Turkey poults are more susceptible to nitrate-nitrite poisoning than are chicks, but vitamin A addition at a level of 10,000 International Units (I.U.) to the ration overcame nitrate-nitrite toxicity.[1337] This effect of vitamin A will be discussed subsequently under the topic "Biochemical Aspects".

Kilgore, et al[1106] studied the effects of dietary nitrate in rabbits to compare levels of methemoglobin with those of nitrate added to or present in feed. Sodium nitrate intakes ranged from 0.004 g (in oats) to 3.380 g (rabbit chow plus 1 gm $NaNO_3$/Kg body weight). The highest methemoglobin value was 3.2 g per 100 ml at a dietary $NaNO_3$ intake of 3 g/Kg body weight. In general, blook methemoglobin levels rose significantly with increased levels of nitrates ingested either as $NaNO_2$ or as present naturally in plant feedstuffs.

According to Smith and Simpson,[2045] the fatal dose of potassium nitrate for an adult human is 15 to 30 g and of sodium nitrite, 10 g. Lee[1221, 1222] gives the value of 20 mg/Kg as the lethal dose of nitrite for man. Naidu and Venkatrao[1513] determined a lethal dose for man of 31.4 mg/Kg for sodium nitrite and 40.8 mg/Kg for potassium nitrite (2.0 and 2.6 g of

$NaNO_2$ and $KNO_2$, respectively for a 140 lb (63.6 Kg) man) based on lethal doses determined for dogs. They pointed out that the size of the fatal dose of nitrite could not be determined from analyses of the nitrite contents of organs and tissues.

In addition to acute poisoning incidents from ingestion of nitrites, considerable attention has been directed toward the problem of methemoglobinemia in infants and children that may result from consumption of nitrate-contaminated water(778, 1221, 1992, 2339) or a lesser extent from meat or fish containing excessive nitrite.(1221) In particular, infants less than four months old who have a gastric juice pH of 4 or higher and who have nitrate-reducing bacteria in their upper gastrointestinal tracts are susceptible to nitrate-nitrite methemoglobinemia.(378, 1125)

Typical case reports of acute methemoglobinemia in infants caused by high nitrate contents in water supplies are given by Walliker and Baxter(2330) and Vigil, et al.(2308)

Table 10 summarizes selected clinical reports of nitrite poisoning from food sources. Both cured meats and fish have been implicated in these cases.

Nitrosamines

Barnes and Magee(116) report the following single oral acute lethal doses of N-nitrosodimethylamine: rats, 50 mg/Kg; rabbits, 15 mg/Kg; mice, 20 mg/Kg; guinea pigs, 25-50 mg/Kg; dogs, 20-50 mg/Kg. In all cases, administration of this compound produced extensive centrilobular liver necrosis.

TABLE 10. SUMMARY OF SELECTED CLINICAL CASES OF ACUTE NITRATE AND NITRITE POISONING FROM FOOD SOURCES

| Country & Year | Source of Nitrate or Nitrite | Number of Persons Affected | Number of Fatalities | References |
|---|---|---|---|---|
| Linthorp, England 1936 | Yorkshire pudding and cabbage contaminated with sodium nitrite | 3 | 3 | (1373) |
| St. Louis, Missouri 1938 | Beef stew contaminated with sodium nitrite | 3 | 3 | (1618) |
| Germany 1940 | Soup contaminated with nitrite from meat curing | 8 | 1 | (1925) |
| Germany 1940 | Sausage containing excess nitrite | 1 | 0 | Ditto |
| Germany 1940 | Meat curing brine containing potassium nitrate | 1 | 1 | " |
| India 1935-40 | Food contaminated with excess nitrite | 4 | 4 | (1512,1513) |
| | Miscellaneous poisoning with nitrite | 8 | 8 | Ditto |
| New York, New York 1944 | Substitution of sodium nitrite for sodium chloride in cooking | 11 | 1 | (752) |
| Syracuse, New York 1950 | Meat curing extract substituted for maple syrup | 7 | 0 | (2206) |
| New Orleans, La. 1955 | Excess nitrite in bologna and wieners | 10 | 0 | (1605) |
| Yugoslavia 1954-55 | Water contaminated with meat curing brine | 6 | 0 | (163) |
| Florida 1956 | Uncooked wieners | 2 | 1 | (1605) |

TABLE 10. (Continued)

| Country & Year | Source of Nitrate or Nitrite | Number of Persons Affected | Number of Fatalities | References |
|---|---|---|---|---|
| Camden, New Jersey 1959 | Fish adulterated with sodium nitrite | 3 | 1 | (2026) |
| Johannesburg, South Africa 1961 | Sausage | 7 | 0 | (641) |
| Buffalo, New York 1966 | Polish sausage | 1 | 0 | (97) |

## Short-Term Studies

For the purposes of this report, short-term studies are defined as those conducted over less than half the normal life span of the animal. This section covers short-term studies involving the administration of nitrates and nitrites, and those involving the administration of nitrites in combination with secondary amines.

### Nitrate

A report of unpublished Food and Drug Administration data[46] states that no adverse effects and no disturbances in the blood were noted when two dogs were fed 2 percent sodium nitrate in their diets for 105 and 125 days, respectively. No information was given on pathological manifestations resulting from feeding nitrate over this period of time.

The adverse effects of dietary nitrate have been studied in domestic livestock in short-term "chronic" studies, including methemoglobinemia in cattle,[1363, 1467] sheep,[1103, 1104, 1245, 2014] and poultry;[12, 1337] abortion, reproduction, growth, lactation in cattle[429, 1000, 1001, 2011, 2409] and vitamin A nutrition in cattle[800, 1026] and in sheep.[731, 904] A summary of pertinent short-term studies of nitrate in domestic livestock is given in Table 11. Winter, et al[2409] concluded that heifers fed an adequate ration can tolerate ingestion of nitrate or nitrite at levels sufficient to convert 40 to 50 percent of the total hemoglobin to methemoglobin without adverse effects on pregnancy.

TABLE 11. SUMMARY OF SELECTED SHORT-TERM STUDIES ON THE EFFECTS OF NITRATE ON DOMESTIC LIVESTOCK

| Species | No. of Animals | Source of Nitrate | Concentration or Dose | Duration | Observations | References |
|---|---|---|---|---|---|---|
| Cattle | 14 | $KNO_3$ in water | 8.4 ppm $NO_3^-$-N | 5 weeks | Increased methemoglobin, increased carotene and decreased Vitamin A in blood - no gross toxicosis observed | (1363) |
| Cattle | 3 | $KNO_3$ in rumen | 100 g/day | 13 days | Abortion after 3, 5, & 13 doses | (2011) |
| | 3 | Ditto | 70 g/day | 47 days | Normal time of calving | |
| Cattle | 10 | $KNO_3$ in feed | 440 mg/Kg<br>660 mg/Kg | 3 estrous cycles before breeding<br>40, 150, or 240 days of pregnancy | Conception rate lower in in heifers fed 660 mg/Kg $NO_3$-1 abortion in 440 mg/Kg group, 2 abortions, 2 deaths in 660 mg/Kg group | (429) |
| Cattle | 15 | $NaNO_3$ in silage | Adjusted to give 40-50% methemoglobin level (range 1314-692 mg/Kg body weight per day) | From 2nd month of pregnancy to calving or abortion | No significant gross pathological lesions; degenerative changes in coronary artericles and necrosis of renal glomeruli; low serum Vitamin A<br>No effect on serum, calcium, magnesium, phosphorus or carotene | (2409) |
| Sheep | 3 | Grass | 0.5% $NO_3^-$-N | 6 weeks | Nitrate and nitrite not detected in blood, no methemoglobin-increase in rumen pH and ammonia content | (2014,2015) |

TABLE 11. (Continued)

| Species | No. of Animals | Source of Nitrate | Concentration or Dose | Duration | Observations | References |
|---|---|---|---|---|---|---|
| Sheep | 1 | Grass | 0.18-9.28 $KNO_3$ per 100 g (dry wt) | 15 weeks | Decrease in rumen volatile fatty acids and pH | (1003) |
| Chickens | 20 | $NaNO_3$ in water | 0,75,150,& 300 ppm $NO_3^-$-N | 28 days | No reduction in weight at 300 ppm $NO_3$-N. No effect on blood methemoglobin | (1337) |
| Laying Hens | 15 | $NaNO_3$ in water | 0,75,150,& 300 ppm $NO_3^-$-N | 28 days | No consistent effect on rate of laying, daily water consumption, mortality, egg quality, or liver Vitamin A and β-carotene at 300 ppm $NO_3$-N | (12) |
| Turkeys | 20 | $KNO_3$ in diet | 0,250,500,750,& 1000 ppm $KNO_3$ | 5 weeks | Poor growth and high mortality in low Vitamin A rations; addition of Vitamin A overcame nitrate toxicity | (1337) |

Nitrite

According to a report of unpublished Food and Drug Administration studies,[46] sodium nitrite was fed to rats over a 168-day period without adverse effects on growth or organ weights. In a typical study, one rat ingested 0.167 g of sodium nitrate in 121 days or 93 ppm in the daily diet. With cats, a total of 4.1 g of sodium nitrite was administered over 105 days without adverse effects on growth or organ weights. No histopathological investigation was done in either of these studies.

Some short-term studies on the effects of nitrite on domestic livestock are summarized in Table 12. Ruminants can detoxify relatively large quantities of nitrite if fed in increments over an extended period. Chickens and laying hens are considerably more resistant to nitrite poisoning than are turkey poults. Vitamin A addition to the diet overcomes nitrite toxicity in chickens and turkeys fed vitamin A deficient diets.

Heuper and Landsberg[937] conducted a short-term toxicity study (18 weeks) in rats to determine the cardiovascular effects of large doses of inorganic nitrite. Sodium nitrite was administered orally in a meat diet. Their methods and observations are summarized in Table 13. Histopathological examination revealed degenerative vascular and parenchymous lesions in the heart, lung, brain, kidney, and testis. They attributed these changes to a stagnant hypoxemia as a result of the slowing of the blood flow from hypotensive vasodilation by nitrite. However, the small number of animals used does not permit statistical evaluation of the results.

The possible adverse effects of ingesting nitrite in cured meat and fish was investigated by Tarr and Carter.[2188] Sodium nitrite was incorporated into the diets of rats and cats. Pink salmon was used as the vehicle in the rat studies and meat in the cat studies. The dosage was

TABLE 12. SUMMARY OF SELECTED SHORT-TERM STUDIES ON THE EFFECTS OF NITRITE ON DOMESTIC LIVESTOCK

| Species | No. of Animals | Source of Nitrite | Concentration or Dose | Duration | Observations | References |
|---|---|---|---|---|---|---|
| Cattle | 2 heifers | $NaNO_2$ in rumen | 20 g/day | 3 weeks | After 2 weeks blood of 1 heifer had a methemoglobin value increase from 12.0 to 18.0%. Normal calf born 2 weeks after termination of experiment-lesions of fetal membrane typical of lowland abortion | (2011) |
| Cattle | 4 heifers | $NaNO_2$ in silage | 319-375 mg/Kg body weight per day | From 2nd month of pregnancy to calving or abortion | No significant gross pathological lesions; typical methemoglobin levels-31.8-43.2%. No effects on serum calcium, magnesium, phosphorus or carotene | (2409) |
| Sheep | 2 ewes | $NaNO_2$, intraperitoneally | 0.71 mM $KNO_2$/Kg body weight/day | 35 days | Methemoglobin levels ranged from 22-40% with one ewe, from 43-58% with second ewe-no gross toxic effects | (463) |
| Chickens | 20 | $NaNO_2$ in water | 0.25,50,100,& 200 ppm $NO_2^-$-N | 28 days | Significant reduction in weight at 200 ppm $NO_2$-N; significantly lower Vitamin A level at 200 ppm $NO_2^-$-N | (12) |
| Chickens | 20 | $KNO_2$ + $KNO_3$ in diet | 400 ppm $KNO_2$ + 1000 ppm $KNO_3$ | 5 weeks | Growth depression at 5 weeks overcome by Vitamin A addition | (1337) |

TABLE 12. (Continued)

| Species | No. of Animals | Source of Nitrite | Concentration or Dose | Duration | Observations | References |
|---|---|---|---|---|---|---|
| Laying Hens | 15 | $NaNO_2$ in water | 0,50,100,& 200 ppm $NO_2^-$-N | 28 days | No effect on egg production or egg quality or on Vitamin A or β-carotene | (12) |
| Turkeys | 20 | $NaNO_2$ in water | 0,50,100,& 200 ppm $NO_2^-$-N | 28 days | Growth rate of poults decreased with increasing $NO_2^-$-N; also decrease in water and feed consumption-significant decrease in liver Vitamin A at 200 ppm $NO_2^-$-N | (12) |
| Turkeys | 20 | $KNO_2$ in diet | 0,100,200,400,& 800 ppm $NO_2^-$- | 4-5 weeks | Toxicity symptoms-poor growth, anorexia, incoordination, small tremors, rapid and labored breathing, frothing. Other 50% mortality at 400 ppm $NO_2$ on low Vitamin A diet; overcome by Vitamin A addition | (1337) |

TABLE 13. SUMMARY OF SHORT TERM TOXICITY STUDY OF SODIUM NITRITE IN THE RAT[937]

Method:

Species: Rat
Strain: Not given
Sex: Male
Age at Start of the Experiment: Not given (weight range 160-236 g)
Duration of Study: 18 weeks
Vehicle: Meat diet, ad libitum
Dose Schedule: 0.4 g $NaNO_2$ per rat daily for 2 weeks; 0.2 g/day for 1 week; 0.4 g/day for 3 weeks; 0.5 g/day for remainder of study
Number of Animals: 6

Observations Made:

Symptomatology: Listlessness, cynotic color of tails, ears and feet
Weight Gain or Loss: Marked weight loss-amount not given
Survival: 2 died in first two weeks, 2 were killed after 4 weeks
Clinical Chemistry - Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology: Meningeal vessels congested with brownish blood, flattened cerebral gyri, collapsed brownish lungs, soft brown liver, dark red kidneys; small testes, coffee ground-colored miscosal erosions in the stomach containing similarly colored material, intestine congested and filled with hemorrhagic material.
Microscopic Pathology

Brain - Meningeal membranes edematous containing engorged vessels with occasional intermeningeal hemorrhages and local or diffuse lymphocytic infiltration of the meninges
- Congested intercerebral vessels surrounded by perivascular edema or small hemorrhages
- Frequent perivascular glia cell accumulations and fibroblastic proliferations with diffuse glial
- Cell infiltrations in nervous substance involving region of brain stem and cerebellum

Heart - Degenerative myocardial and coronary vascular lesions
- Myocardial edema with increase in intertitial histiocytic cells
- Coronary myocardial vessel lesions included swollen and completely or partially hyalinized media, enlarged nucleii which were reduced in number. Lumens of some vessels were narrowed or compressed

Lung - Polypous, plaquelike calcifications in suboendothelial spaces and media of large and small branches of the pulmonary artery and calcified portion in some small arterioles occluded part of the lumen

TABLE 13. (Continued)

| | |
|---|---|
| | - Nucleii of smooth muscle cells in the stumps of the media irregularly arranged in small clusters; fibroblastic cells interspersed with muscle cells |
| | - Normal pulmonary parenchyma |
| Stomach | - Plastic mucosa congested containing small hemorrhagic defects |
| Liver | - Fine granular or vacuolar degeneration of the cytoplasm of liver cells |
| Spleen | - Markedly congested pulp containing moderate to large amounts of intracellular and extracellular amorphous brown pigment |
| Kidney | - Hyaline, granular degeneration of the tubular epithelium, thickened and locally hyalinized wall of large renal artery branches |
| | - Walls of renal arterioles markedly thickened, containing vacuolated and swollen smooth muscle cells, lumens narrowed |
| | - Wedge shaped areas in cortex consisting of thickened interstitial tissue infiltrated with lymphocytes, atrophic, and cystic tubules |
| Testes | - Calcified plates involving the media of testicular arteries |
| | - Degenerative lesions of spermatogenic epithelium of tubules |
| Bone Marrow | - Congested marrow in sternum and femur composed of dense myeloid tissue |
| | - Marked erythropoiesis |
| Other Organs | - Normal except for varying degrees of congestion. |

based on an average-sized man consuming 454 g (1 lb) of fish containing 0.2 percent (908 mg) of sodium nitrite per day six days a week. Tables 14 and 15 present the results obtained in the rat studies and Tables 16 and 17, the results of the cat studies.

In neither animal was the growth rate nor the weight of the thyroid, heart, lungs, spleen, liver, kidneys or adrenals affected adversely by the doses of sodium nitrite administered. Also, the reproductive performance of the rats was not affected at a total intake of an average rat of 0.167 g sodium nitrite over 121 days. Again, no statistical analysis was performed on the data obtained in this study.

Musil[1494-1499] also investigated the effect of chronic intake of sodium nitrite in the rat to determine possible adverse effects from long-term ingestion of methemoglobin-forming substances. The intake of 20 milliequivalents of sodium nitrite in the drinking water over a 200-day period produced no change in the hemoglobin level, but elevated the methemoglobin level. Statistically significant increases in the heart weights were observed. Spleen weights increased only in females. A summary of these studies is presented in Table 18. In general, female rats tended to be more sensitive to chronic nitrite ingestion than males.

## Nitrosamines

The toxic and carcinogenic properties in a variety of animal species of the N-nitrosamines, RR'N·NO, where R and R' are alkyl, hydroxyalkyl or one may be an aryl group, and where the amino nitrogen may be incorporated into a morpholine or piperidine ring have been studied extensively.[55, 61, 63, 68, 76, 116, 490-493, 850, 853, 1304-1313, 2163, 2366]

TABLE 14. SUMMARY OF SHORT-TERM TOXICITY STUDY OF SODIUM NITRITE IN THE RAT (2188)

Method:

Species: Rat
Strain: Albino - not cited
Sex: 10 males, 8 females
Age at Start of the Experiment: Not given
Weight Range: Males - 29-66 g; females - 26-60 g
Duration of Study: 127-168 days
Vehicle: Pink salmon, ad libitum
Dose Schedule: Daily feeding, except Sunday with pink salmon containing 0.2 percent $NaNO_2$ with following per diem intake of $NaNO_2$ for the body weight given:
30 g rat - 0.005 g; 70 g rat - 0.010 g; 110 g rat - 0.015 g; 150 g rat - 0.020 g $NaNO_2$
Typical animal received 0.167 g $NaNO_2$ in 12 days
Route of Administration: Oral
Number of Animals: 5 brother pairs, 4 sister pairs; one of each pair fed fish containing nitrite and the other, control fish without nitrite (9 fed nitrite, 9 controls)

Observations Made:

Symptomatology: Slight transient respiratory distress during first 10 days, lasting 30 minutes or less
Weight Gain: No differences between weight gain of nitrite-fed rats and controls - see Table 16
Survival: No deaths
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: See Table 16
Gross Pathology: Not reported
Microscopic Pathology: Not reported
Number of Generations and Litters: 2-3 litters - all normal - no difference between nitrite-fed animals and controls

TABLE 15. COMPARISON OF ORGAN WEIGHTS OF NITRITE-FED AND CONTROL RATS (2188)

| Data Obtained | 4 females (Nitrite-fed) | 4 females (Controls) | 5 Males (Nitrite-Fed) | 5 Males (Controls) |
|---|---|---|---|---|
| Total body weight (g) | | | | |
| Initial | 27-56 | 26-60 | 29-66 | 31-63 |
| Final | | | | |
| Extremes | 186-229 | 183-217 | 218-350 | 243-335 |
| Mean | 210 | 204 | 280 | 285 |
| Organ weight (%) | | | | |
| Kidneys | | | | |
| Extremes | 1.00-1.09 | 0.98-1.11 | 0.81-0.95 | 0.82-1.05 |
| Mean | 1.05 | 1.07 | 0.90 | 0.94 |
| Liver | | | | |
| Extremes | 7.3-8.8 | 6.5-7.3 | 4.0-5.0 | 4.1-5.0 |
| Mean | 7.7 | 6.6 | 4.7 | 4.6 |
| Heart | | | | |
| Extremes | 0.84-1.06 | 0.76-1.05 | 0.63-1.01 | 0.64-0.79 |
| Mean | 0.97 | 0.87 | 0.70 | 0.73 |
| Spleen | | | | |
| Extremes | 0.18-0.23 | 0.16-0.38 | 0.16-0.27 | 0.18-0.26 |
| Mean | 0.21 | 0.28 | 0.22 | 0.22 |
| Thyroid | | | | |
| Extremes | 0.0058-0.0081 | 0.0054-0.0078 | 0.0039-0.0064 | 0.0052-0.0069 |
| Mean | 0.0072 | 0.0069 | 0.0053 | 0.0059 |
| Lungs | | | | |
| Extremes | 0.79-1.20 | 0.77-0.93 | 0.75-1.00 | 0.74-1.04 |
| Mean | 0.94 | 0.82 | 0.81 | 0.83 |
| Adrenals | | | | |
| Extremes | 0.025-0.032 | 0.027-0.028 | 0.0086-0.013 | 0.0091-0.013 |
| Mean | 0.028 | 0.028 | 0.011 | 0.011 |

TABLE 16. SUMMARY OF SHORT-TERM TOXICITY STUDY OF SODIUM NITRITE IN THE CAT (2188)

Method:

Species: Cat
Strain: Not cited
Sex: 3 males, 2 females
Age at Start of Experiment: Kittens - age not given
Weight Range: Males, 855-1,300 g; females, 1,010-1,270 g
Duration of Study: 15 weeks
Vehicle: Fish containing nitrite
Dose Schedule: Daily - total intake up to 4.1 g $NaNO_3$ by a typical cat over the experiment
Route of Administration: Oral
Number of Animals: 2 controls - 1 male, 1 female; 3 experimentals - 2 males, 1 female

Observations Made:

Symptomatology: None
Weight Gain: No difference between treated animals and controls, See Table 21
Survival: No deaths
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: See Table 21
Gross Pathology: Not reported
Microscopic Pathology: Not reported

TABLE 17. COMPARISON OF ORGAN WEIGHTS OF NITRITE-FED AND CONTROL CATS (2188)

| Initial wt. (g) | Final wt. (g) | Body weight (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kidneys | Liver | Heart | Spleen | Thyroid | Lungs | Adrenals |
| 1300 CM | 3400 | 1.04 | 4.5 | 0.87 | 0.23 | 0.0061 | 1.3 | 0.012 |
| 1010 CF | 2340 | 0.88 | 4.5 | 0.84 | 0.18 | 0.0068 | 1.2 | 0.012 |
| 1230 NM | 3200 | 0.79 | 4.6 | 0.75 | 0.14 | 0.0055 | 1.0 | 0.010 |
| 855 NM | 2370 | 1.2 | 5.1 | 0.93 | 0.20 | 0.0089 | 1.5 | 0.015 |
| 1270 NF | 2410 | 0.89 | 0.86 | 0.86 | 0.15 | 0.0071 | 0.85 | 0.009 |
| .... F | 2550 | 1.1 | 4.4 | 0.80 | 0.20 | 0.0067 | 0.91 | 0.016 |

*This female was killed by a lethal dose of $NaNO_2$ and had not received the same treatment as the other animals.

C = control, N = nitrite-fed, M = male, F = female. Second, third and fourth animals were from the same litter.

TABLE 18. SUMMARY OF SHORT-TERM TOXICITY STUDY OF SODIUM NITRITE IN THE RAT(1494,1496,1498,1499)

Method:

Species: Rat
Strain: Wistar
Sex: Males and females
Age at Start of Experiment: Weanling
Height Range: Weanlings - 60-65 g
Duration of Study: 150 and 200 days
Vehicle: Water
Dose Schedule: 0,10, 20 millequivalents per liter of $NaNO_2$ over entire study
Route of Administration: Oral, ad libitum
Number of Animals: 10 males and 10 females in control groups, 8-10 males, and 9-13 females each in 10 meq and 20 meq experimental group

Observations Made:

Symptomatology: None
Weight Gain: Males - no significant difference between control group and experimental groups. Females - significant decrease in weight of those groups concerning nitrite as compared to controls
Survival: No deaths
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Lower erythrocyte counts in experimental groups other than controls. After 200 days, statistically significant increase in mean hematocrit values in males at both 10 and 20 meq. $NaNO_2$ levels, statistically significant increases in hematocrit in females at 200 days at 20 meq. $NaNO_2$ level. Statistically significant increase in lymphocytes in males and females at 20 meq. $NaNO_2$ level after 200 days. No significant differences in eosinophils basophils, or monocytes. Higher methemoglobin levels in all groups on $NaNO_2$.
Organ Weights:
Liver: No significant increase at 20 meq. of $NaNO_2$ over 200 days
Spleen: Statistically significant increase in spleen weights of females in 150 and 200 days at both 10 and 20 meq. levels of $NaNO_2$
Kidneys: Statistically significant increase in both males and females after 200 days at 20 meq. level of $NaNO_2$
Myocardia: Statistically significant increase in males after 150 and 200 days at the 20 meq. level of $NaNO_2$
Gross Pathology: Not reported

TABLE 18. (Continued)

| | |
|---|---|
| Microscopic Pathology: | |
| Liver: | Turbid swelling, particularly in the apical region of a necrosed lobe |
| Spleen: | Widened cords of lymphoid tissue, lowered basophilia of lymphatic ganglia, with massive ligament cover and thickened ligament septa |
| Kidneys: | Turbid swelling of epithelium of tubules, widened lumen, glomerular convolutions suffused with blood and degenerated, fine round cell infiltration into tubular field of some glomeruli |
| Adrenal: | Coarse grained protoplasma and finely diffused vacuoles under ligament envelope. Nuclei of cells have considerable basophilia. Plasma of cortical areas turbid, sinusoidal cavities of the medulla are suffused |
| Brain: | Superficial and deep vessels suffused with blood, small hemorrhages in lateral brain chambers, dilated lymphatic vessels |
| Heart: | Marked dilation of coronary vessels and of myocardial capillaries. Shifting of coloration of myocardium to basophilic side-indicating incipient hypoxia of the heart muscle. |

However, evidence for the sensitivity of man to N-nitrosamines is lacking except for epidemiological studies.[1307]

The mode of action of N-nitrosamines has been attributed to the formation of an active alkylating agent (identity unknown) from the nitrosamines in vivo. Carcinogenic response in animals can result from oral, intravenous, subcutaneous, intraperitoneal, topical, and inhalation routes of administration. Only the oral route will be discussed in this report because of its pertinence to the possible hazards from N-nitrosamines ingested in foods or from N-nitrosamines formed from the reaction of nitrite and amines in vivo.

The distribution of tumors induced by N-nitrosamines in various organs tends to be a characteristic of the particular compound.[1307] For N-nitrosodimethylamine (NDMA), the liver, kidney, and lungs are the primary sites for tumors in the rat.[76, 1305, 1311, 1312, 2166, 2211] In particular, there is evidence that the endoplasmic reticulum may be theprimary site of action of hepatocarcinogens such as NDMA.[149]

Table 19 presents the results of a chronic feeding study of NDMA in rats.[116] Significant liver damage was noted at 100 ppm and 200 ppm levels. However, the kidneys were normal. In subsequent studies[1309-1310] short-term feeding of NDMA at 100 ppm and 200 ppm levels in the diet for 1 to 4 weeks resulted in liver damage that healed, but tumors were observed in the kidneys (Table 20).

The production of nitrosamines from nitrite and secondary amines has been studied in vivo[19, 20, 77, 490, 655, 754, 1124, 1258, 1457, 1847, 1872, 1874] and in simulated in vivo systems using gastric juice.[1868, 1869, 1962]

TABLE 19. SHORT-TERM "CHRONIC" TOXICITY STUDY OF N-NITROSODIMETHYLAMINE IN RATS (116)

Method:

Species: Rat
Strain: Albino
Sex: Male and female
Age at Start of Experiment: (Young, age not given)
Duration of Study: 34-110 days
Vehicle: Arachis oil (10 ml/Kg diet)
Dose Schedule: 50, 100, 200 ppm N-nitrosodimethylamine (NDMA); control
Route of Administration: Oral
Number of Animals: 6 in each group

Observations Made:

Symptomatology: 100 ppm and 200 ppm weight loss and emaciation
Weight Gain: Mean weight or 50 ppm NDMA significantly lower than control after 110 days. 100 ppm NDMA-mean weight was 65% of controls after 60 days; 200 ppm NDMA-complete absence of body fat after 34-37 days
Survival: 50 ppm NDMA-no deaths, 100 ppm NDMA - all died after 62-95 days 200 ppm all died or near death at 34-37 days
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weight: Not reported
Gross Pathology: 50, 100 ppm NDMA, no abnormalities; 200 ppm; evidence of recent hemorrhage into the gut. Retroperitoneal and retrosternal lymph glands, red; Liver, small and obvious fibrosis; pleural surfaces of lungs showed discrete brown stains
Microscopic Pathology:
Liver: Signs of acute liver injury in animals fed 100 or 200 ppm; islands of periportal tissue contained parenchymal cells with unusual nucleus forms, rarely showing mitosis; little fatty change, glycogen absent; bands of necrotic tissue showed varying proportions of degenerating hepatic cells, red cells, red cell debris, macrophages, fibroblasts, and young connective tissue; in longer term survivors, some bile duct proliferation and more fibrous tissue, often a deposit of yellow pigment staining for iron.
Lungs: Congested, sometimes with hemorrhagic areas
Kidneys: Normal
Spleen: Reduced red cell content and more prominent reticuloendothelial component
Retrosternal Lymph Glands: Contained amorphous eosinophilic debris, degenerating erythrocytes, and macrophages filled with yellow-brown pigment.

TABLE 20. SUMMARY OF SHORT-TERM TUMOR INDUCTION STUDY IN RATS FED WITH N-NITROSODIMETHYLAMINE (1308-1310)

Method:

Species: Rat
Strain: Porton
Sex: Males, female
Age at Start: Not given (weight range 100-150 g)
Duration of Study: 24 weeks
Vehicle: Arachis oil (10 ml/Kg of diet)
Dose Schedule: 50, 100, 200 ppm in diet (50 ppm for 4, 8, 12, and 24 weeks; 100 ppm for 1, 2, 3, and 4 weeks; 200 ppm for 1 week)
Route of Administration: Oral
Number of Animals: 6 in each group

Observations Made:

Symptomatology: Not given
Weight Gain: Not reported
Survival: Not reported
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology: (including carcinogenic observations) kidney tumors of 2 types
(1) Well differentiated tumors - appear as pinkish grey lobulated masses arising from kidney. May adhere to adjacent organs
(2) Anaplastic - pale pinkish white of fleshy gelatinous and friable consistency; may occupy much of the abdominal cavity and weigh 100 g or more

Liver tumors - whitish mass occupying and replacing large portion - numerous nodules of similar consistency throughout peritoneal cavity

Microscopic Pathology: (including carcinogenic observations)
Kidney: Well differentiated type tumors extend from renal capsule throughout width of cortex to medula. Structure of tumor mimics that of renal tubules. Tumor cell has small round vesicular nucleus with scanty chromatin and central basophilic nucleolus. Anaplastic tumors grow by infiltration rather than by expansion, tissue slightly vascular, cells occur in masses with no definite arrangement, closely packed together with oval, elongated or spindle-shaped nuclei. Nucleus membrane well stained and sharp, chromatin scanty or scattered; cytoplasm, basophilic, mitotic figures are frequent

TABLE 20. (Continued)

Liver: Hepatocellular carcinomas; liver mass composed of neoplastic glandular structures, varying amount of fibrous stroma, sometimes scanty, but predominantly dense. Liver tumors occurred only in rats fed 50 ppm NDMA but not at 100 or 200 ppm levels

Lung: Primary pulmonary adenomas and secondary tumors from liver and kidney. In severe cases, greater part of lung tissue replaced by anaplastic tumor similar to that in liver.

Summary of Tumor Incidence

| Level of NDMA ppm | No. of Rats | Sex | Duration of NDMA Administration, weeks | Incidence in Rats Surviving 30 Weeks or More | |
|---|---|---|---|---|---|
| | | | | Liver | Kidney |
| 50 | 6 | F | 4 | 0/6 | 0/6 |
| 50 | 6 | F | 8 | 4/5 | 1/5 |
| 50 | 6 | F | 12 | 4/5 | 1/5 |
| 50 | 6 | F | 24 | 1/1 | 0/1 |
| 100 | 6 | M | 1 | 0/6 | 0/6 |
| 100 | 6 | M | 2 | 0/6 | 2/6 |
| 100 | 6 | M | 3 | 0/6 | 3/6 |
| 100 | 6 | M | 4 | 0/5 | 4/5 |
| 200 | 6 | M | 1 | 0/4 | 4/4 |

Druckery, et al[490] did not observe tumors in rats fed diethylamine or nitrite in the diet or in drinking water. Sander and Burkle[1872] attribute this lack of tumor formation to the highly basic reaction of dimethylamine which is probably not nitrosated at the pH of the stomach to any appreciable extent.

Morpholine ($pK_B$=5.6) and N-methylbenzylamine ($pK_B$=7.1) which are less basic than dimethylamine induced tumors in rats when these compounds were fed with nitrite[1872] (Table 21). Hepatic tumors were the cause of death of rats fed 0.5 percent morpholine plus 0.5 percent nitrite, while esophageal tumors were the cause of death in those fed 0.5 percent N-methylbenzylamine and 0.5 percent nitrite.

In a subsequent report, Sander[1871] presented the results of carcinogenesis experiments in which rats and mice were fed diets containing various levels of N-methylbenzylamine and sodium nitrite (Tables 22 and 23). Esophageal tumors were observed in all rats fed a diet containing 0.25 percent N-methylbenzylamine and 0.32, 0.16, or 0.08 percent sodium nitrite for 152 days. No esophageal tumors were observed in groups receiving diets containing 0.25 percent N-methylbenzylamine and 0.06, 0.04, 0.02, or 0.01 percent sodium nitrite for this time period. When drinking water containing 0.6 percent amine was administered along with 0.3 percent sodium nitrite in the diet for 56 days, one nasal carcinoma but no esophageal tumors were observed.

All 10 mice in a group of 10 developed carcinomas of the forestomach and a few papillomas of the esophagas when a diet containing 0.1 percent amine and 0.15 percent sodium nitrite was fed for 16 days. With

TABLE 21. SUMMARY OF SHORT-TERM TUMOR INDUCTION STUDY IN RATS FED SIMULTANEOUSLY WITH SODIUM NITRITE AND THE AMINES MORPHOLINE AND N-METHYLBENZYLAMINE (1872)

Method:

Species: Rat
Strains: Sprague-Dawley
Sex: Female
Age at Start of Experiment: Not given - average weight 120 ± 3 g
Duration of Study: 270 days
Vehicle: Standard diet
Dose Schedules: (1) Control (2) 0.5% $NaNO_2$ (3) 0.5% morpholine (4) 0.5% morpholine + 0.5% $NaNO_2$ (5) 0.5% N-methylbenzylamine (6) 0.5% N-methylbenzylamine + 0.5% $NaNO_2$ in diets for 8 weeks (total dose 28 g of amine or nitrite per Kg of body weight over 8 weeks)
Route of Administration: Oral
Number of Animals: 6 groups, 7 rats per group

Observations Made:

Symptomatology: Moderate decrease in rate of growth of rats fed either $NaNO_2$ or secondary amine alone in diet. Considerable impairment of growth of rats fed $NaNO_2$ and secondary amine, with rough skin in particular
Weight Gain: (8 weeks) (1) Control; 110 g (2) 0.5% $NaNO_2$; 90 g (3) 0.5% morpholine + 0.5% $NaNO_2$; 30 g (5) 0.5% N-methylbenzylamine; 70 g (6) 0.5% N-methylbenzylamine + 0.5% $NaNO_2$; 20 g
Survival: No deaths in control groups: group 4 (morpholine + nitrite, 150-234 days); group 6 (methylbenzylamine + nitrite) 202-220 days
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weight: Not reported
Gross Pathology: Group 4 (morpholine alone) moderate fatty degeneration of liver; Group 5 (N-methylbenzylamine alone) - No gross changes; Group 4 (morpholine + nitrite) - peritoneal bleeding through rupture of the liver in a hemorrhagic necrotic region; numerous grey-white, partly black and pea-sized nodules on surface of livers. Multiple small tumors in the lungs of 2 rats and in the kidney of 1 rat; Group 6 (N-methylbenzylamine + nitrite); severe suppurative aspiration pneumonia cause of death; exophytically growing tumors, some the size of a lentil particularly in the esophagus

TABLE 21. (Continued)

Microscopic Pathology (including carcinogenic observations)

Groups 3 and 5 - normal

Group 4 - morpholine + nitrite:

liver - marked difference in cell and nucleus size of hepatic epithelia. Kupffer's cells and perivascular macrophages contained iron pigment; large gray-white tumor growth consisted of large polygonal cells resembling liver epithelia; also papillary, tubular and anaplastic nodules. Some tumor invasion into large portal veins

Lungs - cell structure of tumor nodules corresponded to malignant new growth detected in liver

Kidney - tumors papillary, but also some renal cortex adenomas were found

Group 6 (N-methylbenzylamine + nitrite)

Esophagus - multicentrically developed epithelium hyperplasias and epithelial growths involved. Round basal cells in the centers of tumors; in the lumen, cells covered by variously thick layers of the stratus spinosum and granulosum

Trachea and Bronchia - pavement epithelium metaplasia partly with formation of papillomatous proliferations

TABLE 22. SUMMARY OF TUMOR INDUCTION STUDY USING N-METHYLBENZYLAMINE AND NITRITE IN RATS[1871]

Method:

Species: Rats
Strain: Rats SIV-50
Sex: Female
Age at Start of Experiment: Not given, weight, 120 g
Duration of Study: 28 months (dose schedule over 56, and 52 days given below)
Vehicle: Diet or drinking water
Dose Schedule: Diet (1) 0.25% N-methylbenzylamine alone and with (2) 0.32% (3) 0.16% (4) 0.08% (5) 0.06% (6) 0.04% (7) 0.02%, and (8) 0.01% $NaNO_2$ for 152 days (total dose of methylbenzylamine, 38 g/Kg body weight/drinking water: 0.3% $NaNO_2$ in diet, 0.6% N-methylbenzylamine in water for 56 days
Route of Administration: Oral
Number of Animals: Groups 1-4 - 4 rats each; groups 5-8 - 8 rats each

Observations Made:

Symptomatology:- None, except ultimate tumor formation and death
Weight Gain or Loss: Not reported
Survival: Not reported
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology: Not reported
Microscopic Pathology: Not reported
Summary of Carcinogenesis Observations

| Group and Treatment | No. of Animals | Tumor Observations |
|---|---|---|
| (1) 0.25% N-methylbenzylamine | 4 | No esophageal tumors |
| (2) (1) + 0.32% $NaNO_2$ | 4 | Esophageal tumors in all rats in Groups 2-4 |
| (3) (1) + 0.16% $NaNO_2$ | 4 | |
| (4) (1) + 0.08% $NaNO_2$ | 4 | |
| (5) (1) + 0.06% $NaNO_2$ | 8 | No esophageal tumors in 28 months in Groups 5-8 |
| (6) (1) + 0.04% $NaNO_2$ | 8 | |
| (7) (1) + 0.02% $NaNO_2$ | 8 | |
| (8) (1) + 0.01% $NaNO_2$ | 8 | |
| (9) 0.3% $NaNO_2$ in diet + 0.6% Methylbenzylamine in water | 8 | Tumor issuing from nasal cavity |

TABLE 23. SUMMARY OF SHORT TERM TUMOR INDUCTION STUDY USING N-METHYLBENZYLAMINE AND NITRITE IN MICE[1871]

Method:

Species: Mouse
Strain: NMRI
Sex: Female
Age at Start of Experiment: Not given, weight 20 g
Duration of Study: 12 months
Vehicle: Diet
Dose Schedule: 0.1% N-methylbenzylamine + 0.15% or 0.10% $NaNO_2$, 16 days (total dose 5 g/Kg body weight)
Route of Administration: Oral
Number of Animals: 10 in each group

Observations Made:

Symptomatology: Not reported
Weight Gain: Not reported
Survival: Not reported
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology: Not reported
Microscopic Pathology: Not reported

Summary of Carcinogenic Observations

| Group and Treatment | No. of Mice | Tumor Observations |
|---|---|---|
| (1) 0.1% N-methylbenzylamine + 0.15% $NaNO_2$ in diet | 10 | All mice had stomach carcinomas within 12 months |
| (2) 0.1% N-methylbenzylamine + 0.1% $NaNO_2$ in diet | 10 | 3 mice had stomach carcinomas within 12 months |

0.1 percent amine and 0.1 percent sodium nitrite in the diet for this same period, only 3 out of 10 mice developed carcinomas of the forestomach. This investigator emphasizes that these results are only valid for the experimental design given and other results might be obtained with other species or diets.

Greenblatt, et al[755] have recently presented the results of a comprehensive study of the induction of lung adenomas in Swiss mice by concurrent administration of sodium nitrite in drinking water and the secondary amines piperazine ($pK_a$,9.8 and 5.6), morpholine ($pK_a$,8.7), dimethylamine hydrochloride ($pK_a$,10.7), and N-methylaniline ($pK_a$,4.7) in the diet (Table 24). A highly statistically significant increase in incidence of lung adenomas at the end of 40 weeks more pronounced in males than females was observed with piperazine (6.25 g/Kg of diet); morpholine (6.33 g/Kg of diet) or N-methylamiline (1.95 g/Kg) in the diet in combination with sodium nitrite (1.0 g/liter of drinking water). Dimethylamine hydrochloride (5.9 g/Kg of diet) was ineffective when fed in combination with nitrite. This result was attributed to the strongly basic nature of this amine. No effects were noted when sodium nitrite or a secondary amine were administered alone. These investigators conclude that the induction of lung adenomas in this study result from in vivo nitrosation of secondary amines with nitrite probably in the stomach, producing carcinogenic N-nitrosamines.

## Long-Term Studies

Long-term studies are defined for the purposes of this report as those in which the compound being evaluated is administered over more than half the normal lifetime of the animal species.

TABLE 24. SUMMARY OF SHORT TERM TUMOR INDUCTION STUDY USING SECONDARY AMINES AND SODIUM NITRITE IN MICE (755)

Method:

Species: Mouse
Strain: Random bred Swiss
Sex: Male and female
Age at Start of Experiment: 6-11 weeks
Duration of Experiment: 40 weeks
Vehicle: Diet for amines, drinking water for sodium nitrite
Dose Schedule: $NaNO_2$, 1.0 g/liter, 5 days per week, Dimethylamine-HCl, 5.90 g/Kg of diet, piperazine, 6.25 g/Kg of diet; morpholine 6.33 g/Kg of diet, methylaniline, 1.95 g/Kg diet, each fed alone and in combination with $NaNO_2$; positive controls; dimethylnitrosamine 0.010 g/liter; dinitroso-piperazine, 0.040 g/liter, nitrosomorpholine, 0.080 g/liter; nitrosomethylaniline 0.070 g/liter all in drinking water: control diet with no additions. All treatments for 28 weeks
Route of Administration: Oral
Number of Animals: See summary table below

Observations Made:

Symptomatology: Cyanosis observed in group receiving $NaNO_2$ + N-methylaniline
Weight Gain: No difference in weight gains between untreated control groups and those receiving amines plus $NaNO_2$ or amines or $NaNO_2$ alone. Decrease in weight in females fed nitrosomorpholine and nitrosopiperazine. Males in all groups consistently weighed 6-8 g more than females
Survival: Similar for all groups except for N-nitrosodimethylamine group most of which died by 30th week
Clinical Chemistry: pH of mouse gastric contents in untreated animals ranged from 1.9-5.8 (high values with large amounts of food in the stomach)
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology: Not reported
Microscopic Pathology: Not reported

TABLE 24. (Continued)

Summary of Carcinogenic Observations

| Treatment | Initial No. of Mice Females/Males | Effective No. of Mice Females/Males | Adenomas per Mouse |
|---|---|---|---|
| None | 80/80 | 71/73 | 0.18 ± 0.43 |
| $NaNO_2$ | 40/40 | 38/36 | 0.2 ± 0.5 |
| Dimethylamine-HCl | 20/20 | 19/19 | 0.1 ± 0.3 |
| Piperazine | 40/40 | 36/32 | 0.2 ± 0.5 |
| Morpholine | 20/20 | 19/19 | 0.1 ± 0.3 |
| Methylaniline | 20/20 | 19/17 | 0.2 ± 0.6 |
| Nitrosodimethylamine | 20/20 | 17/14 | 6.4 ± 3.9* |
| Dinitropiperazine | 40/40 | 37/38 | 4.9 ± 4.2* |
| Nitrosomorpholine | 20/20 | 14/8 | 4.5 ± 4.3* |
| Nitrosomethylaniline | 20/20 | 20/16 | 2.0 ± 2.4* |
| Dimethylamine HCl $NaNO_2$ | 20/20 | 19/19 | 0.3 ± 0.6 |
| Piperazine + $NaNO_2$ | 40/40 | 39/36 | 1.8 ± 2.2* |
| Morpholine + $NaNO_2$ | 20/20 | 16/19 | 1.4 ± 1.4* |
| Methylaniline + $NaNO_2$ | 20/20 | 19/19 | 1.5 ± 1.8* |

* Significantly greater than control groups ($P < 0.001$)

Only three long-term studies of sodium nitrite have been reported.[490, 1309, 1310, 2280] No long-term studies of potassium nitrate, or sodium or potassium nitrite were found in the comprehensive literature survey upon which this report is based.

Druckery, et al[490] fed three generations of rats with 100 mg sodium nitrite per Kg body weight daily (equivalent to $LD_{25}$) in the drinking water. They also fed diethylamine in the diet to the $F_1$ generation simultaneously with nitrite ingestion from the drinking water to determine possible carcinogenic effects from any nitrosamines that might be formed (Table 25).

A shortened life span was observed in all groups fed sodium nitrite as compared with the controls (730 days for controls; 630, 625, and 610 days, respectively for the three generations of rats fed with sodium nitrite). Hemoglobin levels and numbers of erythocytes were within normal ranges in all groups. Only a few tumors were observed during histopathological examination and these were considered to be spontaneous. They concluded that neither nitrite alone nor in combination with diethylamine had any carcinogenic or teratogenic effect in the 161 test animals and 300 offspring studied.

The results of a long-term tumor induction study by Magee and Barnes[1309-1310] in which rats were fed N-nitrosodimethylamine (NDMA) are shown in Table 26. Liver tumors but no kidney tumors were observed in the group fed 50 ppm of NDMA in the diet for 42 weeks and 20 and 10 ppm for 102 weeks. Neither liver nor kidney tumors was observed in the group fed 5 ppm NDMA for 104 weeks.

In a recent report, van Logten, et al[2280] presented the results of a long-term study in which rats were fed a diet consisting of 40 percent canned meat treated with 0.5 or 0.2 percent sodium nitrite with and without

TABLE 25. SUMMARY OF A LONG-TERM TOXICITY AND CARCINOGENICITY STUDY OF SODIUM NITRITE IN THE RAT[490]

Method:

Species: Rat
Strain: BD
Sex: Males and females
Age: 70 days for parents. $F_1$ and $F_2$ generations at weanling stage
Duration of Study: Lifetime of parents and of $F_1$ and $F_2$ generations
Vehicle: Drinking water ad libitum
Dose Schedule: Controls + (1) 100 mg/Kg $NaNO_2$ per day
(2) 100 mg/Kg $NaNO_2$ per day + 500 mg/Kg dimethylamine per day ($F_1$ and $F_2$ only)
Route of Administration: Oral
Number of Animals Per Level: Parents, nitrite alone 30; $F_1$, 30; $F_2$, 34, Nitrite + diethylamine: $F_1$, 30; $F_2$, 37

Observations Made:

Symptomatology: None observed
Weight Gain or Loss: Increase in weight of parent generation, 11 percent lower than controls
Survival: Mean survival times, days: nitrite alone - parents, 630, $F_1$, 625; $F_2$, 610; nitrite and diethylamine - $F_1$, 630; $F_2$, 625. Control rats - 730 days. Maximum life span (days) of nitrite-alone groups - parents, 940; $F_1$, 1,065; $F_2$, 970
Clinical Chemistry: Not reported
Hematology: Normal hemoglobin and erythrocyte levels in all groups in $F_1$ and $F_2$ generations
Organ Weights: Not reported
Gross Pathology: Pneumonias and pulmonary abscesses - no gross evidence of chronic toxic damage
Microscopic Pathology: No histopathological evidence of chronic toxic damage
Number of Generations and Litters: 3 generations (parents + $F_1$ and $F_2$) 15 matings, gave 94 offspring (6.3 per litter); 22 matings of $F_1$ gave 149 offspring ($F_2$) (6.7 per litter)

Summary of Carcinogenic Observations

Nitrite Alone: Parents (970 days) - Mammary fibroadenoma; $F_1$, 750 days, 2 sarcomas; 820 days, myeloic leukemia; 890 days, sarcoma of lung, $F_2$, 370 days-myeloic leukemia; 470 days, liver carcinoma; 720 days, cancer of skin, head; 910 days, 3 mammary fibroadenomas

TABLE 25. (Continued)

| | |
|---|---|
| Nitrite Plus Diethylamine: | $F_1$, 550 days, adrenal adenoma, 910 days uterus myoma, $F_2$ - 700 days, uterus myoma<br>Concluded that only spontaneous tumors occurred since there was no accumulation of myeloic leukemia and liver carcinoma while test proceeded. |
| Teratogenic Observations: | No evidence of teratogenic action (details not given) |

TABLE 26. SUMMARY OF LONG-TERM TUMOR INDUCTION STUDY IN RATS FED N-NITROSODIMETHYLAMINE (1309, 1310)

Method:

Species: Rat
Strain: Porton
Sex: Male and female
Age at Start: Not given (weight range 100-150 g)
Duration of Study: 42 to 104 weeks
Vehicle: Arachis oil (10 ml/Kg of diet)
Dose Schedule: 50, 20, 10, 5 ppm in diet for 42, 102, 102, 104 weeks, respectively
Route of Administration: Oral
Number of Animals: (1) 50 ppm, 20; (2) 20 ppm, 6; (3) 10 ppm, 6; (4) 5 ppm, 6

Observations Made:

Symptomatology: Lung infections main cause of death of rats early in study. In aged rats, bring inflammation; chronic kidney disease
Weight Gain: Not reported
Survival: Not reported
Clinical Chemistry: Not reported
Urine Analysis: Not reported
Hematology: Not reported
Organ Weights: Not reported
Gross Pathology (including carcinogenic observations)
Liver tumors - hepatocellular carcinomas; firm whitish mass occupying and replacing large part of liver; similar nodules scattered throughout peritoneal cavity
Microscopic Pathology (including carcinogenic observations)
Liver tumors - Liver mass composed of neoplastic glandular structure, fairly regular to grossly irregular, varying amounts of fibrous stroma, sometimes cavity but predominantly dense
Peritoneal nodules - similar in structure to liver tumors

Summary of Tumor Incidence

| Level of NDMA ppm | No. of Rats | Sex | Duration of NDMA Administration, weeks | Incidence in Rats Surviving 30 Weeks or More | |
|---|---|---|---|---|---|
| | | | | Liver | Kidney |
| 50 | 20 | M&F | 42 | 19/20 | 0/20 |
| 20 | 6 | F | 102 | 5/5 | 0/5 |
| 10 | 6 | F | 102 | 2/6 | 0/6 |
| 5 | 6 | F | 104 | 0/6 | 0/6 |

glucono-delta-lactone. The latter compound was added to lower pH and hence provided a favorable environment for nitrosamine formation. The object of this study was not only to determine chronic toxicity, but also to determine possible carcinogenic effects. Table 27 shows the results of this study.

Growth of the rats was inhibited in the 0.5 percent nitrite group as compared to the controls. The meat containing 0.5 percent nitrite had a residual nitrite content of 4,000 ppm after processing; the meat containing 0.02 percent nitrite and glucono-delta-lactone contained 110 ppm residual nitrite. The final concentrations of free nitrite in the diet were 1,600, 750, and 4 ppm for the 0.5 percent nitrite, 0.5 percent nitrite plus glucono-delta-lactone and 0.02 percent nitrite plus glucono-delta-lactone samples, respectively.

Hematological data indicated a decreased number of erythocytes, but no effect on hemoglobin content of the blood from consumption of any of the samples containing nitrite. Liver function tests, bromsulphthalein and serum glutamic-pyruvic transaminase determinations, were not significantly different in the nitrite treated groups from those of the controls.

Tumors were equally distributed among all groups. There was no increase in tumor incidence in the groups fed with nitrite alone or in combination with glucono-delta-lactone, as compared with control groups.

The meat heated with 0.5 percent sodium nitrite and glucono-delta-lactone contained 50 ppb nitrosamines (mass spectrometric confirmation) or about 20 ppb in the diet (daily intake, 1 μg/Kg of body weight). No nitrosamines were present in the meat containing 0.02 percent nitrite and glucono-delta-lactone. No tumors were found in other experiments using a dose of

TABLE 27. SUMMARY OF A LONG-TERM TOXICITY AND CARCINOGENICITY STUDY OF SODIUM NITRITE IN CANNED MEAT FED TO RATS (2280)

Method:

Species: Rat
Strain: SPF-derived Wistar
Sex: Male and Female
Age at Start of Experiment: Weanling
Duration of Study: 29 months
Vehicle: 40 percent meat diet, ad libitum
Dose Schedule: Standard laboratory diet control; untreated (control) meat + 0.5 percent $NaNO_2$, meat + glucono-delta lactone (GDL); meat + 0.5 percent $NaNO_2$ + GDL; meat + 0.02 percent $NaNO_2$ + GDL; ad libitum initially, 20 g/day of diet finally
Route of Administration: Oral
Number of Animals Per Level: 30 males and 30 females

Observations Made:

Symptomatology: None
Weight Gain or Loss: Males and females showed statistically lower weight gain on diets containing 0.5 percent $NaNO_2$ then on meat control diet at end of 78 weeks
Survival: Good up to 18 months - neither $NaNO_2$ nor GDL influenced mortality

| Diet: | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | Initial No. | 18 Mo. | 29 Mo. | Initial No. | 18 Mo. | 29 Mo. |
| Control | 30 | 4 | 24 | 30 | 0 | 24 |
| Meat Control | 30 | 2 | 30 | 30 | 5 | 30 |
| " " + 0.5% $NaNO_2$ | 30 | 4 | 26 | 30 | 4 | 26 |
| " " + GDL | 30 | 3 | 25 | 30 | 3 | 24 |
| " " + 0.5% $NaNO_2$ GDL | 30 | 2 | 22 | 30 | 3 | 27 |
| Meat Control + 0.02% $NaNO_2$ + GDL | 30 | 1 | 28 | 30 | 4 | 27 |

TABLE 27. (Continued)

Clinical Chemistry: Normal bromsulphthalain (BSP) and serum glutamic-pyruvic transamimase (SGPT) values in both controls and nitrite-GDL treated rats - slight tendency to higher alkaline phosphatase activity in groups given 0.5% $NaNO_2$. No evidence of inhibition of liver aniline hydroxylase, aminopyrine demethylase, and glucose-6 phosphatase on termination - no significant differences in blood urea values among groups

Urine Analysis: Not reported

Hematology: No differences among any of the groups in packed cell volume, numbers of monocytes and eosinophils, mean corpusaclar volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration or leucoytes. Lower number of erythrocytes in groups fed nitrite; decrease in lymphocytes and increase in neutrophils of older animals

Organ Weights: Not reported

Gross Pathology: No abnormalities among groups

Microscopic Pathology: Histological abnormalities were scattered through all groups; glomerulonephrosis, most common, associated sometimes with uremia and metastatic calcification, especially in the heart. Also hyperplasia of the parathyroids

Summary of Carcinogenic Observations:

Considerable incidence in spontaneous tumors in rats surviving for 18 months or more distributed over all groups: 3 tumors in control diet group; 2 in meat control; 1 in meat + 0.5% $NaNO_2$; 4 in meat + GDL, 4 in meat + 0.5% $NaNO_2$ + GDL and 2 in meat + 0.02% $NaNO_2$ + GDL. No increase in tumor incidence in groups fed meat with nitrite and/or GDL over controls.

12.5 μg of pure dimethylnitrosamine per Kg of body weight per day in rats over a 20-month exposure period.

## Special Studies

### Teratogenicity and Reproduction

Nitrate. Sleight and Atallah[2037] investigated the effect of chronic administration of potassium nitrate on reproduction in guinea pigs. The results are summarized in Table 28. Reproductive performance was poor at the highest dose level of 1130 mg nitrate nitrogen. The small number of animals used in each of the experimental groups does not allow one to draw any conclusions concerning the statistical significance of these results.

Recent reports of Food and Drug Research Laboratories[625-626] to the Food and Drug Administration give the results of a teratologic evaluation of sodium and potassium nitrates in mice, rats, hamsters, and rabbits as shown in Tables 29 and 30. Neither sodium nor potassium nitrates showed any effect on nidation or maternal or fetal survival, in any of these species. Furthermore, no differences were noted in the number of soft or skeletal tissue abnormalities in the groups treated with sodium or potassium nitrates from the number occurring spontaneously or in the controls.

Nitrite. Chronic intake of potassium nitrite at 5,000 and 10,000 ppm levels in water (daily intake of 24.4 and 57.7 mg nitrite nitrogen per Kg body weight) by guinea pigs over 135 and 118 days respectively resulted in reproductive disturbances including abortion, fetal absorption, fetal mummification, and maternal death.[2037] The results are summarized in Table 31.

TABLE 28. SUMMARY OF SHORT-TERM STUDY ON THE REPRODUCTIVE EFFECTS OF POTASSIUM NITRATE IN THE GUINEA PIG (2037)

Method:

Species: Guinea pig
Strain: Not reported
Sex: Female
Age at Start of Experiment: Not reported
Duration of Study: 166-204 days
Vehicle: Water
Dose Schedule: 0,300,2,500,10,000,30,000 ppm $KNO_3$ in water (daily intake 0, 12, 102, 507, 1,130 mg $NO_3$-N per Kg body wt, respectively
Route of Administration: Oral
Number of Animals Per Level: 0 ppm, 4; 300 ppm, 6; 2500 ppm, 3; 10,000 ppm, 3; 30,000 ppm, 3

Observations Made:

Symptomatology: No overt symptoms at any level
Weight Gain: Normal
Survival: 1 death at 30,000 ppm - survival at all other levels
Clinical Chemistry: No significant alterations in serum nitrite, blood urea nitrogen, or serum potassium at any level
Urine Analysis: Not reported
Hematology: Methemoglobin levels not affected
Organ Weights: Not reported
Gross Pathology: Four mumified fetuses *in utero*; in one female death at 30,000 ppm
Microscopic Pathology: Placental uterine, and cervical lesions in females where fetuses had died
Reproduction: Reproductive performance poor at 30,000 ppm - 2 litters produced 2 live births - 4 fetal deaths from 3 females

TABLE 29. TERATOLOGIC STUDY OF SODIUM NITRATE (FOOD AND DRUG RESEARCH LABORATORIES)[625]

| | Species | | | |
|---|---|---|---|---|
| | Mice | Rats | Hamster | Rabbit |
| **Method:** | | | | |
| Strain | Albino CD-1 | Albino Wistar derived | Golden | Dutch-belted |
| Sex | Female | Female | Female | Female |
| Age at Start of Experiment | Not given (Avg.Wt.27.1 g) | Not given (Avg.Wt.204 g) | Not given (Avg.Wt.103 g) | Not given (Avg.Wt.2.16 Kg) |
| Duration | 17 days | 20 days | 14 days | 29 days |
| Dose Schedule | 0,4,20,100, 400 mg/Kg - 10 days | 0,2.5,12,54, 250 mg/Kg - 10 days | 0.4,20,100, 400 mg/Kg - 5 days | 0,2.5,12,90, 250 mg/Kg - 13 days |
| Route of Administration | Oral intubation | Oral intubation | Oral intubation | Oral intubation |

**Observations:**

| Dose Level Indicated | 400 mg/Kg | Control(0) | 250 mg/Kg | Control(0) | 400 mg/Kg | Control(0) | 250 mg/Kg | Control(0) |
|---|---|---|---|---|---|---|---|---|
| Weight gain (g) | 25.5 | 22.9 | 103 | 118 | 43.2 | 44.2 | 170 | 16.0 |
| No. mated | 25 | 24 | 22 | 23 | 25 | 25 | 15 | 15 |
| No. pregnant | 25 | 21 | 22 | 20 | 24 | 24 | 10 | 12 |
| No. surviving at term | 23 | 23 | 22 | 23 | 25 | 25 | 13 | 15 |
| No. pregnant at term | 23 | 20 | 22 | 20 | 24 | 23 | 9 | 12 |
| Total pregnancies | 25 | 21 | 22 | 20 | 24 | 24 | 10 | 12 |
| Died or aborted | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 0 |
| To term | 23 | 20 | 22 | 20 | 23 | 23 | 9 | 12 |
| Live litters | 23 | 20 | 22 | 20 | 24 | 23 | 5 | 8 |
| Implant sites, total | 279 | 232 | 233 | 245 | 297 | 303 | 31 | 58 |
| Average/dam | 12.1 | 11.6 | 10.6 | 12.3 | 12.5 | 13.2 | 3.44 | 4.83 |
| Resorptions | | | | | | | | |
| Total no. | 17 | 8 | 6 | 18 | 22 | 24 | 8 | 9 |
| Dams with 1 or more sites resorbed | 10 | 6 | 5 | 6 | 11 | 5 | 5 | 4 |
| Dams with all sites resorbed | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| Percent partial resorptions | 43.5 | 30.0 | 22.7 | 30.0 | 45.8 | 21.7 | 55.6 | 33.3 |

TABLE 29. (Continued)

| | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mice | | Rats | | Hamster | | Rabbit | |
| | 400 mg/Kg | Control(0) | 250 mg/Kg | Control(0) | 400 mg/Kg | Control(0) | 250 mg/Kg | Control(0) |
| Percent complete resorptions | - | - | - | - | - | - | 44.4 | 33.3 |
| Live fetuses | | | | | | | | |
| Total no. | 260 | 222 | 227 | 227 | 227 | 278 | 23 | 48 |
| Average/dam | 11.3 | 11.1 | 10.3 | 11.4 | 11.5 | 12.1 | 2.56 | 4.00 |
| Dead fetuses | | | | | | | | |
| Total no. | 1 | 2 | 0 | 0 | 0 | 1 | 2 | 1 |
| Dams with 1 or more dead | 1 | 2 | - | - | - | 1 | 1 | 1 |
| Dams with all dead | 0 | 0 | - | - | - | 0 | 0 | 0 |
| Percent partial dead | 4.35 | 10.0 | - | - | - | 4.35 | 11.1 | 8.33 |
| Percent all dead | - | - | - | - | - | - | 0 | - |
| Average fetus wt., g | 1.02 | 6.91 | 3.75 | 3.73 | 1.76 | 1.81 | 45.6 | 37.0 |

TABLE 30. TERATOLOGIC STUDY OF POTASSIUM NITRATE (FOOD AND DRUG RESEARCH LABORATORIES) (626)

| | Species | | | |
|---|---|---|---|---|
| | Mice | Rats | Hamsters | Rabbits |
| **Method:** | | | | |
| Strain | Albino CD-1 | Albino Wistar derived | Golden | Dutch-belted |
| Sex | Female | Female | Female | Female |
| Age at Start of Experiment | - (Avg.Wt.26.1 g) | - (Avg.Wt. 205 g) | - (Avg.Wt.94.6 g) | - (Avg.Wt.2.24 Kg) |
| Dose Schedule | 0,4,20,100,-400 mg/Kg - 10 days | 0,2,9,40, 180 mg/Kg - 10 days | 0,3,20,70, 280 mg/Kg - 5 days | 0,2,10,50, 206 mg/Kg - 13 days |
| Route of Administration | Oral intubation | Oral intubation | Oral intubation | Oral intubation |

**Observations:**

| Dose Level Indicated | 400 mg/Kg | Control(0) | 180 mg/Kg | Control(0) | 280 mg/Kg | Control(0) | 206 mg/Kg | Control(0) |
|---|---|---|---|---|---|---|---|---|
| Weight gain (g) | 33.8 | 22.9 | 118 | 118 | 43.8 | 44.2 | 210 | 160 |
| No. mated | 25 | 24 | 22 | 22 | 29 | 25 | 15 | 15 |
| No. pregnant | 21 | 21 | 20 | 19 | 23 | 24 | 13 | 12 |
| No. surviving at term | 25 | 23 | 21 | 22 | 29 | 24 | 12 | 15 |
| No. pregnant at term | 21 | 20 | 19 | 19 | 23 | 23 | 10 | 12 |
| Total pregnancies | 21 | 21 | 20 | 20 | 23 | 24 | 13 | 12 |
| Dead or aborted | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 0 |
| To term | 21 | 20 | 19 | 20 | 23 | 23 | 10 | 12 |
| Live litters | 21 | 20 | 19 | 20 | 23 | 23 | 10 | 8 |
| Implant sites, total | 235 | 232 | 211 | 245 | 282 | 302 | 67 | 58 |
| Average/dam | 11.2 | 11.6 | 11.1 | 12.3 | 12.3 | 13.2 | 6.70 | 4.83 |
| Resorptions | | | | | | | | |
| Total no. | 10 | 8 | 7 | 18 | 10 | 24 | 5 | 9 |
| Dams with 1 or more sites resorbed | 9 | 6 | 3 | 6 | 8 | 5 | 4 | 4 |
| Dams with all sites resorbed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Percent partial resorptions | 42.9 | 30.0 | 15.8 | 30.0 | 34.8 | 21.7 | 40.0 | 33.3 |

TABLE 30. (Continued)

| | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mice | | Rats | | Hamster | | Rabbit | |
| | 400 mg/Kg | Control(0) | 180 mg/Kg | Control(0) | 280 mg/Kg | Control(0) | 206 mg/Kg | Control(0) |
| Percent complete resorptions | - | - | - | - | - | - | - | 33.3 |
| Live fetuses | | | | | | | | |
| Total no. | 223 | 222 | 204 | 227 | 269 | 278 | 61 | 48 |
| Average/dam | 10.6 | 11.1 | 10.7 | 11.4 | 11.7 | 12.1 | 6.10 | 4.00 |
| Dead fetuses | | | | | | | | |
| Total no. | 2 | 2 | 0 | 0 | 3 | 1 | 1 | 1 |
| Dams with 1 or more dead | 2 | 2 | - | - | 3 | 1 | 1 | 1 |
| Dams with all dead | 0 | 0 | - | - | 0 | 0 | - | 0 |
| Percent partial dead | 9.52 | 10.0 | - | - | 13.0 | 4.35 | 10.0 | 8.33 |
| Percent all dead | - | - | - | - | - | - | - | - |
| Average fetus wt., g | 0.93 | 0.91 | 3.91 | 3.73 | 1.87 | 1.83 | 37.4 | 37.0 |

TABLE 31. SUMMARY OF SHORT-TERM STUDY ON THE REPRODUCTIVE EFFECTS OF POTASSIUM NITRITE IN THE GUINEA PIG (2037)

Method:

Species: Guinea pig
Strain: Not given
Sex: Female
Age at Start of Study: Not given
Duration of Study: 100-240 days
Vehicle: Water
Dose Schedule: 0, 300, 1,000, 2,000, 3,000, 4,000, 5,000, 10,000 ppm $KNO_2$ in water (daily intake 18, 45, 154, 182, 192, 244, 577 mg $NO_2$-N per Kg body wt, respectively)
Route of Administration: Oral
Number of Animals per Level: 0,-4; 300 ppm-3; 1,000 ppm, 3; 2,000 ppm, 3; 3,000 ppm, 5; 4,000 ppm, 6; 5,000 ppm; 10,000 ppm, 3

Observations Made:

Sympton Etoloty: No overt symptoms
Weight Gain: Weight gain normal except at 10,000 ppm, $KNO_2$, one seventh of normal. Weight losses in several females after partarition associated with subacute endometritis and cervicitis
Survival: 1 death from apparent toxemia at 5,000 ppm level
Clinical Chemistry: No alterations in serum nitrite, blood urea nitrogen, or serum potassium
Urine Analysis: Not reported
Hematology: Methemoglobin did not exceed 20% of available hemoglobin at 10,000 ppm
Organ Weights: Not reported
Gross Pathology: 3 mummified fetuses, one absorbed at 5,000 ppm
Microscopic Pathology: Placental, uterine and cervical lesions in females in which fetuses died, most extensive at 5,000 ppm
Reproduction: Severe effects at 5,000 and 10,000 ppm levels. No live births. At 5,000 ppm 4 litters with 6 fetuses aborted, 3 mummified and 1 litter produced with 2 fetuses aborted.

Food and Drug Research Laboratories[627, 628] investigated the teratologic effects of sodium and potassium nitrite in mice, rats, hamsters, and rabbits as shown in Tables 32 and 33. The pure compounds were administered by oral intubation at four-dose levels together with negative (water) and positive (aspirin) controls.

Sodium nitrite when administered only to pregnant mice, rats, hamsters, and rabbits at levels up to 23, 3.0, 23, and 23 mg/Kg of body weight for 10, 10, 5, and 13 consecutive days, respectively, did not show any effect on nidation or on maternal or fetal survival. At these levels, the numbers of abnormalities in the soft or skeletal tissues of the sodium nitrite-treated group of mice, rats, and rabbits or in the soft tissues of fetuses of the sodium nitrite-treated hamsters did not differ from the numbers occurring in the controls.

In the rat at 10 mg/Kg body weight, a slight indication of delayed skeletal maturation, especially in the ribs and skull but no increase in terata were observed. It was concluded that sodium nitrite under these test conditions was not a teratogen for the rat.

In the case of hamsters, the administration of sodium nitrite was associated with delayed skeletal maturation which was not dose related. Further studies were recommended to determine whether or not sodium nitrite is teratogenic in the hamster.

When potassium nitrite was administered orally to pregnant mice, rats, hamsters, and rabbits at levels up to 32, 3.0, 32, and 23 mg/Kg of body weight for 10, 10, 5, and 13 consecutive days, respectively, no effects were noted on either nidation or on maternal or fetal survival. At these levels, the numbers of abnormalities observed in either soft or skeletal tissues

TABLE 32. TERATOLOGIC STUDY OF SODIUM NITRITE. (FOOD AND DRUG AND RESEARCH LABORATORIES) [627]

| | Species | | | |
|---|---|---|---|---|
| | Mice | Rats | Hamsters | Rabbits |
| **Method:** | | | | |
| Strain | Albino CD-1 | Albino Wister | Golden | Dutch-belted |
| Sex | Female | Female | - | - |
| Age at Start of Experiment | - (Avg.Wt.29.3 g) | - (Avg.Wt.206 g) | - (Avg.Wt.95.6 g) | - (Avg.Wt.2.73 Kg) |
| Duration | 17 days | 20 days | 14 days | 29 days |
| Vehicle | - | - | - | - |
| Dose Schedule | 0,0.2,1.1,5.0,23.0 mg/Kg - 10 days | 0,0.1,0.5,3.0,10.0 mg/Kg - 10 days | 0,0.2,1.1,5.0,23 mg/Kg - 50 days | 0,0.2,1.1,5.0,23.0 mg/Kg - 13 days |
| Route of Administration | Oral intubation | Oral intubation | Oral intubation | Oral intubation |

**Observations:**

| Dose Level Indicated | 23 mg/Kg | Control(0) | 10.0 mg/Kg | Control(0) | 23 mg/Kg | Control(0) | 23 mg/Kg | Control(0) |
|---|---|---|---|---|---|---|---|---|
| Weight gain (g) | 29.8 | 29.6 | 125 | 127 | 44.5 | 46.2 | 10.0 | -[a] |
| No. mated | 25 | 23 | 23 | 22 | 23 | 24 | 15 | 1.5 |
| No. pregnant | 21 | 22 | 21 | 21 | 23 | 21 | 11 | 10 |
| No. surviving at term | 25 | 23 | 23 | 22 | 23 | 24 | 10 | 6 |
| No. pregnant at term | 21 | 22 | 21 | 21 | 23 | 21 | 6 | 4 |
| Total pregnancies | 21 | 22 | 21 | 21 | 23 | 21 | 11 | 10 |
| Dead or aborted | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 9 |
| To term | 21 | 22 | 21 | 21 | 23 | 21 | 6 | 4 |
| Live litters | 21 | 22 | 21 | 21 | 23 | 21 | 4 | 4 |
| Implant sites, total | 267 | 268 | 238 | 229 | 271 | 255 | 30 | 29 |
| Average/dam | 12.7 | 12.2 | 11.3 | 10.9 | 12.6 | 12.1 | 5.0 | 7.25 |
| Resorptions | | | | | | | | |
| Total no. | 15 | 18 | 4 | 15 | 8 | 11 | 8 | 0 |
| Dams with 1 or more sites resorbed | 11 | 8 | 4 | 6 | 5 | 8 | 4 | - |
| Dams with all sites resorbed | 0 | 0 | 0 | 0 | 0 | 0 | 2 | - |
| Percent partial resorptions | 52.4 | 36.4 | 19.0 | 28.6 | 21.7 | 38.4 | 66.7 | - |

TABLE 32. (Continued)

| | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mice | | Rats | | Hamsters | | Rabbits | |
| | 2.3 mg/Kg | Control(0) | 10.0 mg/Kg | Control(0) | 23 mg/Kg | Control(0) | 23 mg/Kg | Control(0) |
| Percent complete resorptions | - | - | - | - | - | - | 33.3 | - |
| Live fetuses | | | | | | | | |
| Total no. | 250 | 249 | 234 | 214 | 282 | 243 | 22 | 29 |
| Average/dam | 11.9 | 11.3 | 11.1 | 10.2 | 12.2 | 11.6 | 3.67 | 7.25 |
| Dead fetuses | | | | | | | | |
| Total no. | 2 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Dams with 1 or more dead | 2 | 1 | - | - | 1 | 1 | - | - |
| Dams with all dead | 0 | 0 | - | - | 0 | 0 | - | - |
| Percent partial dead | 9.52 | 4.55 | - | - | 4.35 | 4.76 | - | - |
| Percent all dead | - | - | - | - | - | - | - | - |
| Average fetus wt., g | 0.97 | 0.96 | 3.89 | 3.75 | 1.81 | 1.87 | 37.6 | 37.5 |

(a) Not meaningful because of mortalities.

TABLE 33. TERATOLOGIC STUDY OF POTASSIUM NITRITE (FOOD AND DRUG RESEARCH LABORATORIES)[628]

| | Species | | | |
|---|---|---|---|---|
| | Mice | Rats | Hamsters | Rabbits |
| **Method:** | | | | |
| Strain | Albino CD-1 derived | Albino Westar | Golden | Dutch-belted |
| Sex | Female | Female | Female | Female |
| Age at Start of Experiment | - (Avg.Wt.31.5 g) | - (Avg.Wt.206 g) | - (Avg.Wt.99.8 g) | - (Avg.Wt.2.25 Kg) |
| Duration | 17 days | 20 days | 14 days | 29 days |
| Vehicle | - | - | - | - |
| Dose Schedule | 0,0.3,1.5,7.0,32 mg/Kg - 0 days | 0,0.1,0.5,3.0,10.0 mg/Kg - 0 days | 0,0.3,1.5,7.0,32 mg/Kg - 5 days | 0,0.2,1.1,5.0,23.0 mg/Kg - 13 days |
| Route of Administration | Oral intubation | Oral intubation | Oral intubation | Oral intubation |

**Observations:**

| Dose Level Indicated | 32.0 mg/Kg | Control(0) | 10.0 mg/Kg | Control(0) | 32.0 mg/Kg | Control(0) | 23.0 mg/Kg | Control(0) |
|---|---|---|---|---|---|---|---|---|
| Weight gain (g) | 21.0 | 20.4 | 127 | 127 | 38.9 | 41.2 | 240 | -[a] |
| No. mated | 28 | 23 | 23 | 22 | 26 | 24 | 15 | 15 |
| No. pregnant | 21 | 22 | 21 | 21 | 21 | 21 | 11 | 10 |
| No. surviving at term | 28 | 23 | 22 | 22 | 25 | 24 | 11 | 6 |
| No. pregnant at term | 21 | 22 | 21 | 21 | 21 | 21 | 7 | 4 |
| Total pregnancies | 21 | 22 | 21 | 21 | 21 | 21 | 11 | 10 |
| Dead or aborted | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 9 |
| To term | 21 | 22 | 21 | 21 | 21 | 21 | 7 | 4 |
| Live litters | 21 | 22 | 21 | 21 | 21 | 21 | 5 | 4 |
| Implant sites, total | 257 | 268 | 238 | 229 | 250 | 255 | 22 | 29 |
| Average/dam | 12.2 | 12.2 | 11.3 | 10.9 | 11.9 | 12.1 | 3.14 | 7.25 |
| Resorptions | | | | | | | | |
| Total no. | 9 | 18 | 4 | 15 | 18 | 11 | 5 | 0 |
| Dams with 1 or more sites resorbed | 5 | 8 | 3 | 6 | 10 | 8 | 3 | - |
| Dams with all sites resorbed | 0 | 0 | 0 | 0 | 0 | 0 | 2 | - |
| Percent partial resorption | 23.8 | 36.4 | 14.3 | 28.6 | 47.6 | 38.1 | 42.9 | - |

TABLE 33. (Continued)

| | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mice | | Rats | | Hamsters | | Rabbits | |
| | 32.0 mg/Kg | Control(0) | 10.0 mg/Kg | Control(0) | 32.0 mg/Kg | Control(0) | 23.0 mg/Kg | Control(0) |
| Percent complete resorptions | - | - | - | - | - | - | 28.6 | - |
| Live fetuses | | | | | | | | |
| Total no. | 248 | 249 | 234 | 214 | 232 | 243 | 17 | 29 |
| Average/dam | 11.8 | 11.3 | 11.1 | 10.2 | 11.0 | 11.6 | 2.43 | 7.25 |
| Dead fetuses | | | | | | | | |
| Total no. | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Dams with 1 or more dead | - | 1 | - | - | 0 | 1 | - | - |
| Dams with all dead | - | 0 | - | - | 0 | 0 | - | - |
| Percent partial dead | - | 4.55 | - | - | - | 4.76 | - | - |
| Percent all dead | - | - | - | - | - | - | - | - |
| Average fetus wt., g | 0.98 | 0.83 | 3.82 | 3.75 | 1.83 | 1.87 | 34.8 | 37.5 |

(a) Not meaningful because of mortalities.

of potassium nitrite treated mice, rats, and rabbits or in the soft tissues of fetuses of the potassium nitrite-treated group of hamsters did not differ from the numbers occurring in the controls.

At a dose of 10 mg/kg body weight of potassium nitrite in the rat, there was a slight indication of delayed skeletal maturation, especially in the ribs and skull, but no increase in terata was observed. It was concluded that potassium nitrite under these conditions was not a teratogen for the rat.

With hamsters, the administration of potassium nitrite was associated with delayed skeletal maturation which was not dose-related. Further studies of potassium nitrite in the hamster were recommended to determine the possible teratogenicity of this compound in this species.

Nitrosamines. Napalkov and Alexandrov[1523] studied the embryotoxic and teratogenic properties of symmetrical dialkyl nitrosamines, nitrosoanilines, and alkylnitrosoureas in chicken embryos and in pregnant nonimbred rats having a fixed date of pregnancy. Results in rats were recorded on the 17th-21st days of gestation. A total of 50 rats and 3400 fetuses and 400 chicken embryos were examined.

Symmetrical dialkylnitrosamines, nitrosoanilines and alkylnitrosoureas were embryotoxic and increased embryo mortality. However, malformations were observed only with the nitrosoanilines and the alkylnitrosoureas and not with the symmetrical dialkylnitrosamines.

When 0.5 percent ethylurea and 1 percent sodium nitrite were administered to pregnant rats in the diet with a supplemental single dose

of 200 mg/Kg ethylurea by stomach tube, Alexandrov and Janisch[21] observed death of 60 percent of the embryos and malformation of 76 percent of the surviving fetuses. Neither sodium nitrite nor ethylurea alone exerted any embryotoxic or teratogenic effect on the progency of surviving females. The embryotoxic effect disappeared with decreasing concentrations of ethylurea, in the presence of 1 percent nitrite but the teratogenic effect remained.

Mutagenicity

Nitrate. Newell and Maxwell[1540] studied the mutagenic effects of sodium nitrate using host mediated assays, cytogenetic assays and the dominant lethal gene test. Their results (Table 34) indicated that sodium nitrate did not show mutagenic responses in these assays with the exception of human embryonic cells grown in tissue culture where increased numbers of acentric fragments were observed.

Nitrite. In a subsequent study, the mutagenic effects of sodium nitrite were also investigated by Newell and Maxwell[1541] using the same tests as used previously with sodium nitrate.

The results given in Table 35 show that sodium nitrite did not show mutagenic responses in these assays except for the cytogenetic assay using human embryonic lung cells grown in tissue culture. In this latter instance, sharp increases were observed in the number of aberrant cells, and mitotic inhibition was proportional to dose level.

Nitrosamines. Pasternak[1636] investigated the mutagenic effects of nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA), nitrosomethylurea (NMU), nitrosomethylurethane (NMUR),nitrosoethylteriarybutylamine (NETBA),

TABLE 34. SUMMARY OF A STUDY OF MUTAGENIC EFFECTS OF SODIUM NITRATE [1540]

| Test | Test Organism | Concentration o Dose of $NaNO_3$ | Mutagenic Effect |
|---|---|---|---|
| Host-Mediated Assay | Salmonella typhimurium G-46 and TA 1530 | 5% w/v | No mutagenic effect on S. typhimurium |
| | Saccharomyces cerevisiae D-3 | 5% w/v | Mitotic recombination frequency of S. cerevisiae not affected |
| Cytogenetic Assay | Rat (bone marrow chromosomes) | 0,30,800, 1,600 mg/Kg (48 hours) | No adverse effect on metaphase chromosomes |
| | Human embryonic lung cells (WI-38) in tissue culture | 0,10,100,1,000 µg/ml (24 hours) | Definite adverse effect due to increase in numbers of acentric fragments |
| Dominant Lethal Gene Test | Rat (female) | 0,0.2,30,800, 1,600 mg/Kg acute single dose and subacute multiple dose | No evidence of mutagenicity |

TABLE 35. SUMMARY OF A STUDY OF MUTAGENIC EFFECTS OF SODIUM NITRITE[1541]

| Test | Test Organism | Concentration or Dose of $NaNO_3$ | Mutagenic Effect |
|---|---|---|---|
| Host-Mediated Assay | Salmonella typhimurium G-46 and TA 1530 | 5% w/v | No mutagenic effect on S. typhimurium |
| | Saccharomyces cerevisiae D-3 | 5% w/v | Mitotic recombination frequency of S. cerevisiae not affected |
| Cytogenetic Assay | Rat (bone marrow chromosomes) | 0,30,50,70 mg/Kg (48 hours) | No adverse effect on metaphase chromosomes |
| | Human embryonic lung cells (WI-38) in tissue culture | 0,10,100, 1,000 μg/ml (24 hours) | Sharp increase in number of aberrant cells. Mitotic inhibition proportional to dose level |
| Dominant Lethal Gene Test | Rat (female) | 0,0.2,30,50,70 mg/Kg acute single dose and subacute multiple dose | No evidence of mutagenicity |

nitrosomethylbenzylamine (NMBA), nitrosomethylvinylamine (NMVA), and nitrosomethyl piperazine (NMP) using rates of recessively lethal mutations of the X-chromosome of Drosophila as the test method.

NDMA, NDEA, NMU, NMUR, NMBA, NMVA, and NMP all induced increases in the mutation rates in the test system used. NETBA, which is not carcinogenic did not show any effect in the mutation experiments. This investigator pointed out a parallelism between mutagenic and carcinogenic effects of nitrosamines and nitrosamides.

## BIOCHEMICAL ASPECTS

### Breakdown

Nitrates exert their toxic effects after reduction to nitrites by microorganisms[1265, 2181] or by enzymes in animal tissues.[165] Nitrites may undergo a variety of reactions in animal tissues and meat. Nitrite (in the form of nitrous acid) can be converted to nitric oxide which in turn reacts with the myoglobin pigment of muscle to form nitric oxide myoglobin.[633, 1760, 1761, 2418] This latter compound gives the typical pink color of cured meat. This conversion of nitrite occurs in the presence of reductants such as ascorbic acid[2354] cysteine, quinones and nicotinamide adenine dinucleotide (NADH).[633]

The exact steps in the overall production of nitric oxide myoglobin from nitrite are not known. Bunton, et al[268] proposed a reaction sequence between a nitrosating reagent such as nitrous acid with reductones such as ascorbic acid which involves an electrophilic attack of nitrous acid or ascorbic acid to form a nitrite followed by decomposition to yield nitric oxide.

The fate of free nitrate and nitrite in cured meats either during storage or during cooking is not well understood. Rose and Peterson[1797] found that the reducing system of fresh pork destroyed nitrite independently of bacterial contamination. This loss increased as a function of pH decrease and temperature increase up to the protein denaturation temperature range. Factors controlling the oxidation-reduction potential did not control the destruction of nitrite. Furthermore, hemoglobin and 2:6 dichlorobenzenone were able to supplement the electron carriers in meat and increase the loss of nitrite.

According to Eddy, et al[516] substantial quantities of nitrate disappeared from maturing bacon without any accumulation of nitrite. This loss was prevented by reducing bacterial growth on the surface of bacon by swabbing with formaldehyde. They concluded that nitrite may disappear by action of microorganisms and by strictly chemical reactions other than those involving combination with heme and amino compounds.

Nordin[1564] demonstrated that the sodium nitrite content of ham decreases progressively during curing, cooking, and storage. The rate of depletion doubled for every 12 C (22 F) increase in temperature or for every 0.86 unit decrease in pH over the temperature range 4.4 C (40 F) to 104.4 C (220 F). The rate was also proportional to nitrite concentration according to the following equation:

$$\log_{10} (1/2 \text{ life}) = 0.65 - 0.025 (t^{o}C) + 0.35 (pH)$$

In addition, the rate of depletion was not affected by heat denaturation but the above equation did not apply at room temperature unless the product was heat treated.

Nitrite (as nitrous acid) may also react with phenols such as tyrosine. According to Philpot and Small,[1674] nitrous acid reacts with tyrosine to form the corresponding O-nitrosophenol, which in turn undergoes further reaction to form the O-diazophenol.

The reaction of nitrite with amines (chiefly secondary, but also tertiary)[604] to form N-nitrosamines has been discussed previously. N-nitrosamines may also be produced by intestinal bacteria.[846] Mirvish, et al[1424] reported that the reaction between nitrous acid and a variety of amines was blocked by ascorbic acid. They proposed the possibility of blocking the formation of N-nitroso compounds in vivo by the use of ascorbic acid.

Nitrous acid and nitrite also may react with creatine and creatinine.[74, 760] Under acid conditions creatine reacts with nitrite to yield sarcosine and then N-nitrososarcosine, a known weak carcinogen for the rat. Nitrite reacts with creatinine under acid conditions to form either creatinine 5-oxime or 1-methylhydantoin-5-oxime.

The possible formation of N-nitrosamines in vegetables containing nitrite and in cured meats and fish has been the subject of some controversy.[1257, 1671, 2418] Keybets, et al[1096] did not detect N-nitrosamines in spinach containing nitrite. Enders and Ceh[551] state that heating of cured meat or fish leads to a decarboxylation of amino acids to yield the corresponding amines which may in turn react with nitrite to form nitrosamines. However, very high concentrations of nitrite were used in these studies (5 to 50 g. $NaNO_2$ per Kg of meat and 5 to 25 g. $NaNO_2$ per kg of fish) as compared to the 200 ppm level of sodium nitrite permitted in the United States.

Sen, et al[1963, 1964] reported that the cooking of fish rich in amines in the presence of 200 ppm sodium nitrite resulted in the formation of N-nitrosodimethylamine. However, they point out that the analytical methods used, gas-liquid chromatography and thin layer chromatography permit only semiquantitative determination of the N-nitrosamine content.

In the most comprehensive study of N-nitrosamine formation in cured meats reported to date, Fiddler, et al[604] found that frankfurters conforming to U. S. Federal Regulations did not contain sufficient quantities of sodium nitrite to form detectable amounts of N-nitrosodimethylamine under the processing conditions usually employed in the meat industry. In laboratory studies, NDMA was formed in significant quantities when concentrations of sodium nitrite up to 10 times the legal limit were used. They concluded that localized high concentrations of nitrite in emulsions resulting from inadequate homogenization, or extended heating beyond the normal 2-hour processing time may account for the detection of NDMA in some commercial cured meat products cured with permissible levels of sodium nitrite.

## Absorption and Distribution

### Nitrates and Nitrites

Nitrates and nitrites are absorbed in the gastrointestinal tract in animals and man.[269, 1221, 1513, 1992] The reduction of nitrates to nitrites after ingestion by the coliform bacteria and *Clostridium* in non-ruminants and man and by the ruminal flora in cattle and sheep and the resulting induction of methemoglobinemia has been mentioned previously in this report.

In studies with dogs, maximum absorption of sodium nitrate after a single dose of 10.3 mEq/kg as indicated by plasma concentrations did not occur until approximately 4 hours after administration. Oral administration of sodium nitrite (138-148 mg/kg) to dogs resulted in respiratory difficulty and paralytic symptoms within 3 to 4 minutes.[1513] Postmortem information after 25 minutes showed only small amounts of nitrite in the stomach and traces in the liver, kidneys and intestines indicating rapid absorption. Although dosage of sodium nitrite in the latter study was greater than in the former, it appears that nitrite is more rapidly absorbed than nitrate (see also 269, 1221, 1992).

Nitrites depress the activity of the gastrointestinal tract. According to Sleeth and VanLiere,[2036] a 0.065 g. oral dose of sodium nitrite delayed gastric emptying time by 23.6 percent in adult human subjects.

Popov[1697] investigated the effect of potassium nitrate and nitrite assimilation of dietary components by rats. Doses of 4.4 mg potassium nitrate (20 mg/kg) and 0.33 mg of potassium nitrite (1.5 mg/kg) were fed in the diet in 24-hour periods with total doses of 30.8 mg $KNO_3$ and 2.31 mg $KNO_2$ over the test period. Analyses of the feces showed a statistically significant decrease in the assimilation of fats in rats fed diets containing either potassium nitrate or nitrite, and a lesser decrease in assimilation of albumen and ash elements. However, carbohydrate assimilation was not affected.

Conversion of Nitrates and Nitrites to Nitrosamines

The question of the possible conversion of nitrites to nitrosamines in the human gastrointestinal tract has been raised by several investigators.[1256, 1258, 1867, 1869] Sander[1867] demonstrated the production of nitrosamines in human gastric juice *in vitro* from the reaction of sodium nitrite (50 μg/ml) with L-proline, diethylamine, dimethylamine, and ephedrine.

In a subsequent study with 31 human volunteers, Sander and Seif[1874] demonstrated the reduction of ingested nitrate to nitrite *in vivo* in the human stomach. The extent of this reduction depended on the pH of the gastric juice; the greatest conversions were obtained at pH 5.0, the least at pH 1.5. When sodium nitrite was administered to human subjects simultaneously with diphenylamine, nitrosamines were detected in specimens of the gastric contents in 16 of the subjects. Nitrosamine formation was enhanced by a gradual decrease in the pH of the gastric contents. The results of this study are summarized in Table 36.

Alam, et al[19, 20] presented evidence for the synthesis of N-nitrosopiperidine from piperidine and sodium nitrate and nitrite in the gastrointestinal tract of the rat both *in vitro*, in small intestine washings and in the gastric juice, and *in vivo* in the stomach and in the small intestine. Yields of N-nitrosopiperidine from nitrite by *in vivo* synthesis were 5 to 159 μg in the small intestine, and 11 to 40 μg in the stomach and were slightly higher than from nitrate (11-34 μg in the small intestine and 8-44 μg in the stomach). These authors point out that recoveries were

TABLE 36. FATE OF NITRATE IN THE HUMAN STOMACH AFTER INGESTION WITH DIPHENYLAMINE [1874]

| Time After Consumption (minutes) | Gastric Juice Content[a] | |
|---|---|---|
| | Nitrite (μg/ml) at pH 5.0 | Diphenylnitrosamine (μg) at pH 3.0 |
| Empty stomach | 0-1 | -- |
| 10 | 0.1-2.2 | -- |
| 20 | 0.5-3.5 | 2.0-8.5 |
| 30 | 2.0-5.6 | 0.5-8.5 |
| 40 | 1.7-7.0 | 7.5-8.5 |
| 50 | 0.1-3.0 | 8.5-10.5 |

(a) Values are ranges in 31 human volunteers.

low and that no correction was made for in vivo absorption of N-nitrosopiperidine during the 40-minute incubation period used.

## Metabolism and Excretion

Bernheim and Dixon[165] demonstrated the reduction of nitrate to nitrite by rat and guinea pig muscle and by the livers of the ox, sheep, pig, rabbit, dog, and chicken. Rat liver reduced 5 to 10 times as much nitrate per unit of weight per unit of time as did rat muscle.

The administration of 20 mEq/kg of sodium nitrate orally to dogs did not result in any marked changes in blood pressure or renal hemodynamics nor was any evidence of toxicity observed according to Greene and Hiatt.[757] The glomerular filtration rate and the renal plasma flow increased slightly. At doses of 7.5 mEq/kg or more, 80-90 percent of the nitrate administered was recovered in the urine (Table 37). The rate of sodium excretion was markedly greater after administration of sodium nitrate as compared to that observed after administration of equivalent doses of sodium as sodium chloride.

Hiatt[896] studied the diuretic effects of oral doses of sodium and potassium nitrates as compared to other salts and diuretic agents in three female dogs. Net sodium and potassium excretion, net volume excretion, and net osmolar excretion were determined. Table 38 presents a summary of the results. Net sodium excretion was used as the diuretic index. Potassium nitrate was more effective than sodium nitrate as a diuretic agent as measured by net sodium excretion; the difference in net potassium excretion was not significant. Both sodium and potassium nitrate had a greater diuretic effect than sodium chloride but less than Mercuhydrin, Diamox and ammonium nitrate.

TABLE 37. EXCRETION OF ORALLY ADMINISTERED SODIUM NITRATE IN THE URINE OF DOGS[757]

| Total Dose (meq/kg) | Time After Dose (hrs) | Recovery (percent) |
|---|---|---|
| 4.8 (in food) | 24 | 67 |
| 7.4 (stomach tube) | 24 | 66 |
| 7.5 (in food) | 20 | 90 |
| 10.3 (in food) | 23 | 81 |

TABLE 38. SUMMARY OF DIURETIC EFFECTS OF SODIUM AND POTASSIUM NITRATES IN DOGS[896]

| Compound | Dose and Route | Mean Net Value per 24 Hours | | | |
|---|---|---|---|---|---|
| | | Sodium[a] Excretion meq/Kg | Potassium[a] Excretion meq/Kg | Volume[b] Excretion ml/Kg | Osmolar[b] Excretion milliosmols/Kg |
| $NaNO_3$ | 7.5 mM/Kg oral | 0.52 | 0.71 | 11.03 | 11.39 |
| $KNO_3$ | 7.5 mM/Kg oral | 1.99 | 0.07 | 15.70 | 12.44 |
| $NH_4NO_3$ | 7.5 mM/Kg oral | 3.13 | 2.30 | 19.63 | 10.50 |
| NaCl | 7.5 mM/Kg oral | -0.59 | 0.02 | 3.46 | 7.75 |
| Diamox | 500 mg oral | 2.85 | 2.35 | 17.07 | 3.50 |
| Mercuhydrin | 2 ml intravenous injection | 3.50 | 0.03 | 19.34 | 1.13 |
| Vehicle alone[c] | -- | 1.08 | 1.34 | 23.45 | 20.65 |

(a) Na or K excreted - (control Na or K excreted + Na or K administered)

(b) Total excreted - mean control value excreted

(c) Vehicle - 50 g Lonolac in 300 ml of water.

Kilgore, et al[1106] reported the results of nitrate balance studies in rabbits. Their urinary nitrate recovery values agreed with those obtained in dogs by Greene and Hiatt[757] cited previously. Recoveries in 24 hours varied from 35.3 percent at an oral dose of 22 mg/kg $NaNO_3$ to 56.1 percent at 198 mg/kg $NaNO_3$. Only small amounts of nitrate were detected in the feces. In one experiment in which 5 rabbits were fed sodium nitrate at the 200 mg/kg level using a diet of Purina rabbit chow (total nitrate fed, 2023 mg), 774 mg of nitrite and 7.61 mg nitrite were recovered in the urine. They concluded that nitrate was primarily reduced to nitrite in the body, and that the nitrite is absorbed and reacts with hemoglobin in the blood. However, some of the nitrate could not be accounted for and no conclusions could be given on its fate.

## Effects on Enzymes and Other Biochemical Parameters

### Effects on Enzymes

According to Philpot and Small,[1675] sodium nitrite in acid solution (pH 3.7-4.5) reacts with pepsin to give a photosensitive diazo compound having 50 percent of the original activity. The reaction rate is proportional to the concentrations of undissociated nitrous acid and unchanged pepsin but depends only slightly on the nitrite concentration. The decrease in activity was attributed to the reaction of nitrous acid with the tyrosine groups of pepsin.

The oral administration of sodium nitrite to guinea pigs at the 0.5 and 1.0 percent levels resulted in decreased lactic (LDH) and succinic

(SDH) dehydrogenese activity in the myocardium and kidneys.[2269] LDH activity was considered to be a better index of nitrite toxicosis than SDH. The depletion of these enzymes was considered to be evidence that hypoxia is the mode of action of nitrite on tissues. The loss of body weight or poor weight gain observed in guinea pigs drinking the sodium nitrite solutions was attributed to the possible depression of enterokinase and pancreatic lipase.

It can be concluded that the information available on the effects of nitrate and nitrite on enzymes is limited and further research, particularly on the effects of these compounds on respiratory enzymes and key enzymes in intermediary metabolism is warranted.

Effects on Vitamin A

The effects of nitrate and nitrite on Vitamin A nutrition in cattle, sheep, and poultry have been studied extensively. According to Pugh and Garner,[1721] carotene and vitaman A alcohol are destroyed readily by nitrite solutions at pH values below 5.0. However, β-apo-8' and β-apo-12' carotenal were more resistant than β-carotene and vitamin A alcohol to attack by nitrite.

Emerick and Olson[539] reported that the liver storage in rats of orally administered doses of vitamin A decreased significantly in rats fed 0.5 percent sodium nitrite but not with 3 percent sodium nitrate. However, the liver storage of vitamin A in rats administered β-carotene decreased significantly when either nitrate or nitrite was fed. The effect of nitrate in the presence of β-carotene was attributed to a lowered absorption of carotene.

Subsequently, Phillips[1669] found reduced quantities of vitamin A in the livers of rats fed 1 percent potassium nitrite in diets containing either vitamin A or β-carotene. After prolonged feeding of nitrite, vitamin A storage in the liver was reduced only when vitamin A alcohol or β-carotene were administered, but little effect was observed with vitamin A aldelyde. Nitrite ingestion did not affect the ability of rats to store vitamin A in the liver or affect the rate of disappearance of vitamin A from the liver. These results indicated that β-carotene and vitamin A alcohol were degraded by nitrite in the digestive tract prior to absorption and that nitrite did not affect the absorption of carotene or its conversion to vitamin A, or the function of the liver.

## Effects on Endocrine Function

As early as 1927, Barlow[113] reported that vasodilating substances such as sodium nitrite (15 mg dose) increased the external pancreatic secretory rate in dogs. This effect was attributed to improvement in the blood supply to the splanchnic organs.

Dietary potassium nitrate fed at the 0.5 percent level to rats, and 15 percent level to sheep for 6 days affected the normal functioning of the thyroid gland as measured by decreased uptake of $I^{131}$.[194] Further studies that the upstake of $I^{131}$ by rats decreased after 7 hours on a diet containing 2.5 percent potassium nitrate. However, the rats were able to overcome this effect after two weeks.[195]

Effects on Muscle

The contraction of frog sartorius muscle was potentiated by nitrate ion when all of the chloride in Ringer's solution was replaced with nitrate.[1042] Furthermore, nitrate ion in Ringer's solution prolonged the active state and the plateau of full activity of frog sartorius muscle. Nitrate also reduced the tetanic tension of the muscle.[1781] There is a lack of information in the literature on any effects of nitrite on muscle.

Effects on Blood Pressure and Erythrocytes

Fregly[143] studied the effects of administering potassium nitrate over a 16-week period in the drinking water on the blood pressure of rats. Ingestion of a 0.3M $KNO_3$ solution gave maximal elevation of systolic blood pressure. A gradual loss of body weight took place during the test period. Ratios of organ weight to body weight of the kidneys, testis and adrenals increased; organ weight ratios of the heart, seminal vesicles, prostate, thyroid and thymus were not affected by the administration of potassium nitrate. No conclusion could be drawn on the mechanisms involved in blood pressure elevation by potassium nitrate although time of administration, renal concentrating ability and changes in acid-base balance may have been contributing factors.

Nitrite in molar ratios to heme over the range 1:4 to 64:1, caused increased rates of oxidation of hemoglobin with Heinz body formation and loss of osmotic integrity in human erythrocytes.[825] The mode of action of nitrite was considered to be that of oxidation of -SH groups of globin and other erythrocyte components and possible deamination of cellular proteins.

Effects on Animal Cell Tissue Cultures

Pace, et al[1617] found that the proliferation of mouse liver cells in tissue cultrue was not affected adversely by sodium nitrate at concentrations of 200 mg-percent or lower. However, some retardation was observed in L and HeLa cell cultures at sodium nitrate concentrations as low as 10 mg-percent. All three cell lines were inhibited at all concentrations of sodium nitrite tested (10, 50, 100, and 200 mg-percent); the extent of inhibition increased with increasing concentrations of sodium nitrite.

Effects of Microorganisms

The effects of curing salts on food spoilage and food poisoning microorganisms have been the subject of numerous investigations. Nitrite exerts primarily a bacteriostatic effect and bacteria vary widely in their susceptibility to nitrite. Tarr[2180] found that the growth of various species of Achromobacter, Flavobacterium, Pseudomonas, Micrococcus, Escherichia and Aerobacter were inhibited by 0.02 percent sodium nitrite. Furthermore, pH of the medium markedly affects the bacteriostatic effect of nitrite. The extent of inhibition increases as pH decreases to about 4.5 which parallels the increase in the fraction of undissociated nitrous acid.[2183] Below pH 4.5, nitric oxide (NO) and nitrate are formed. Under anaerobic conditions no further reaction takes place, but under aerobic conditions, the nitric oxide can be oxidized to nitrogen dioxide ($NO_2$) and this in turn is converted to nitric and nitrous acids in an aqueous system.[1972]

The bacteriostatic effect of sodium nitrite on Staphylococcus aureus, Streptococcus salivarious and Streptoccus mitis was much greater when glucose was autoclaved in the test medium than when glucose was added after autoclaving. The nitrate tolerance of these organisms was restored to the level observed when glucose was autoclaved separately by the addition of sulfhydryl compounds such as cysteine or glutathione to the medium autoclaved with glucose.(320)

Nitric oxide (NO) had no appreciable effect under anerobic conditions (vacuum) on Clostridium sporogenes, Staphylococcus aureus, and Streptococcus durans according to Shank, et al.(1972) However, nitrite addition (and nitrous acid formation) in the presence of nitric oxide resulted in substantial reductions in the numbers of these organisms. They concluded that nitrous acid was responsible for the observed changes in the bacterial flora in cured meats.

At this point it is appropriate to analyze the information available in the literature on the effects of nitrates and nitrites on food poisoning and food spoilage microorganisms, particularly the anaerobes such as Clostridium botulinum and C.perfringens, and facultative organisms such as Staphylococcus aureus. Since nitrite but not nitrate shows antimicrobial activity this discussion will emphasize nitrite.(267, 753, 2000, 2183)

Table 39 presents a summary of studies on the effects of nitrite on Clostridium botulinum both in laboratory culture media and in meat. In general, nitrite inhibits C.botulinum types A, B, E, and F, the inhibitory concentration depends upon the type and strain, the number of

TABLE 39. SUMMARY OF SELECTED STUDIES ON THE EFFECTS OF NITRITE ON CLOSTRIDIUM BOTULINUM

| Organism | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| Clostridium botulinum types A & B | Glucose broth and plain broth | 0.149-0.588% (1490-5880 ppm) | Growth inhibited at 0.588%, toxin formation inhibited by 0.490 and 0.588% $NaNO_2$ | (2172) |
| | Pork infusion and egg meat | 0.0588-0.392% (588-3920 ppm) | No inhibition of growth or toxin formation | (2172) |
| C.botulinum | Fish digest broth | 0.02% (200 ppm) | Growth inhibited at pH5.6 or lower at 37 C over 14 days with initial inoculum of 17.7 x $10^6$ vegetative cells/ml or below pH4.6 with 2.5 x $10^6$ spores/ml | (2182) |
| C.botulinum types A & B | Cooked, sliced ham (vacuum packaged), jellied pork tongue | 0-450 ppm | Toxicity observed with 300 ppm $NaNO_2$ in 25 days or less at 25 C with ham; or with 450 ppm $NaNO_2$ in 45 days at 20 C with jellied pork tongue | (1680) |
| C.botulinum types A,B,E, & F | PT medium (1654) | 0-2560 ppm (unheated) 0-320 ppm (heated) 0-60 ppm (unheated) | Growth inhibited at 5-10 ppm except 40 ppm for one Type B strain | (1653) |
| C.botulinum type E | Trypticase - peptone glucose & trypticase peptone sucrose yeast media | 100, 200 ppm | No growth with 100 & 200 ppm $NaNO_2$ in 4 strains and of 6 at pH6.6, 10 C $10^6$ spores/ml | (547) |
| C.botulinum types B | PT medium | 0,20,50,150 ppm | PT medium + 20 ppm $NaNO_2$ heated to 110 C for 20 min. was inhibitory (inhibitor nondialyzable) | |

TABLE 39. (Continued)

| Organism | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| C.botulinum type B | Meat suspension | 0,75,150,200 ppm | Meat suspension heated with 150 ppm $NaNO_2$, inhibitory (inhibitor dialyzable) | (1024) |
| C.botulinum types A & B | Phosphate buffer pH6.2, meat | 200-2000 ppm | 200 ppm nitrite had no effect on destruction of spores during heating or on germination of spores after heating<br><br>400-600 ppm showed greater descruction of spores by heat and greater germination of spores after heating | (1679) |
| C.botulinum types A & B | Ground pork | 0,75,150,300 ppm | No toxin produced at brine concentration of 4.6% and 300 ppm $NaNO_2$ at thermal process of $F_o$=0.6 or at 146 ppm $NaNO_2$, 5.5% brine $10^6$ spores/g at $F_o$=0.6 | (1678) |
| C.botulinum type B | PT medium, reinforced clostridial medium, liver veal medium, Wynne fluid medium meat (50% water) | 0,20,50,100,150, 250,500 ppm | Inhibitory concentration varied with medium, inhibition in all media at 250 ppm $NaNO_2$ underprocesses, normal process, overprocessed meat required 500, 150, and 50 ppm $NaNO_2$ for inhibition of outgrowth, respectively | (1023) |

TABLE 39. (Continued)

| Organism | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| C.botulinum types A & B | Pork hams (boneless) | 0,50,100,200,300, 400,500 ppm | With inoculum of 100 spores/g, no toxin with 200 ppm $NaNO_2$ or more during 6 mos. storage at 80 F. With inoculum of 10,000 spores/g, no toxin with 500 ppm, $NaNO_2$ during 6 mos. storage at 80 F | (753) |
| C.botulinum types A,B, & E | Minced meat | 64-182 ppm | For vegetative cells, $NaNO_2$ added before heating, $ED_{50}$ = 83, 71, and 78 ppm for types A, B, & E, respectively.<br><br>For spores, $NaNO_2$ added before heating, $ED_{50}$ = 64, 67, 99 ppm for types A, B, & E, respectively; $NaNO_2$ added after heating, $ED_{50}$ = 114, 156, 182 ppm for types A, B, & E, respectively. | (82) |

spores present, pH, temperature, prior heating of nitrite, and sodium chloride concentration.[82, 547, 753, 1023, 1024, 1653, 1678]

Of particular interest in this connection are the observations of Perigo, et al[1654] and Perigo and Roberts[1653] that sodium nitrite heated in a laboratory medium is highly inhibitory to the Clostridia, including C.botulinum and gives a greater inhibition than would be attributed to nitrite alone. The exact nature of this inhibiting effect is unknown.

Wasserman and Huhtamen[2349] demonstrated that this inhibitory effect called the "Perigo" effect is not a nitrosamine, and that several nitrosamines tested did not inhibit clostridia. The composition of the medium, however, does have an effect since the Perigo effect against C.botulinum was produced in Perigo type medium (PT),[1654] and Reinforced Clostridial Medium (RCM) when they were heated with 20 ppm of sodium nitrite but not in Liver Veal Medium and Wynne Fluid Medium when these media were heated with 100 ppm of sodium nitrite.[1023]

Ashworth and Spencer[82] also observed a Perigo-type effect against C.botulinum in minced pork containing nitrite. $ED_{50}$ values (level of residual nitrite to give inhibition in 50 percent of the samples) obtained with spores and vegetative cells of C.botulinum types A, B, and E were higher after heating than before heating. They concluded that the Perigo effect may play some role in the safety and stability of canned cured meats.

It can be concluded that nitrites exert a significant inhibitory effect on C.botulinum in heated cured meat products. The effect of heating on the enhancement of the inhibitory of nitrite against C.botulinum is of considerable significance and further research is needed to determine the exact nature of this inhibitory effect in cured meat.

It is also of interest to consider the effects of nitrite on other clostridia, particularly the food poisoning organism Clostridium perfringens and the anaerobic spoilage organism Clostridium sporogenes PA3679. Tables 40 and 41 present the results of selected studies reported in the literature on the effects of nitrite on these organisms.

Nitrite inhibited the outgrowth of C.perfringens spores in some studies. However, in hams inoculated with C.perfringens, the organism could be isolated after curing and smoking.[737] Again, a Perigo effect of nitrite as a result of heating has been observed with C.perfringens.[1653] However, the relation of this inhibition observed under laboratory conditions to the effects of nitrite in cured meats contaminated with C.perfringens has not been established.

Clostridium sporogenes PA3679 is also inhibited by nitrite (Table 41). Again, the inhibitory concentration depends upon pH, temperature, and composition of the medium and a Perigo effect is observed with nitrite.[82, 267, 1654, 2172, 2182]

Duncan and Foster[500-502] reported that sodium nitrate and salt protected the spores from heat injury but sodium nitrite did not. Nitrite was strongly inhibitory at concentrations of 0.01 - 0.09 percent at pH 6.0 and blocked cell division at 0.06 percent and 0.08 - 1 percent concentrations at pH 6.0 and 7.0, respectively. They, as have others, observed a pronounced stimulatory effect of nitrite on PA3679 spore germination at high concentrations (3.45 percent), elevated temperature (45 C) and at acid pH levels (6.0).

Ashworth and Spencer[82] confirmed a Perigo effect in minced pork containing nitrite. They pointed out that this effect has only been demonstrated in a model meat system and that attempts to magnify this effect by altering the conditions that lead to this inhibition failed.

TABLE 40. SUMMARY OF SELECTED STUDIES ON THE EFFECTS OF NITRITE ON CLOSTRIDIUM PERFRINGENS

| Strain | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| 18 strains | Fluid thioglycolate | 300,400,500 ppm (+800, 10,000,12,000 ppm ($NaNO_3$)) | Inhibition observed at 400 ppm $NaNO_2$, 3700 ppm $NaNO_3$ and 6% (w/v) NaCl | (737) |
| | Fresh ham | 200 ppm (+500 ppm $NaNO_3$) | Spore survivals: ranged from 0.1 to 39% with 3% NaCl depending on treatment of inoculum | (737) |
| | Cured ham | 980 ppm (+1500 ppm $NaNO_3$) in pumping brine, 1300 ppm $NaNO_2$ (+1300 ppm $NaNO_3$ in cover pickle) | Spores survived curing and smoking | (737) |
| 5 classical strains, 3 food poisoning strains | PT medium | pH7.0 0-2560 ppm (unheated)<br>0-320 ppm (heated)<br>pH6.0 0-240 ppm (unheated)<br>0-60 ppm (heated) | Inhibition at 640-2560 ppm<br>Inhibition at 5-20 ppm<br>Inhibition at 60-240 ppm<br>Inhibition at 5-15 ppm | (1653) |
| FD-1 NCTC 8798 | Brain heart infusion and yeast extract | 0.01 & 0.02 percent | 0.02 and 0.01 percent prevented out growth of heat sensitive and heat resistance spores, respectively. Nitrite induced germination at higher concentrations | (1199) |

TABLE 41. SUMMARY OF SELECTED STUDIES ON [illegible] EFFECTS OF NITRITE ON CLOSTRIDIUM SPOROGENES

| Strain | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| Nos.10,11,14 | Glucose broth and plain broth | 0.149-0.588% (1490-5880 ppm) | Growth inhibited at 0.588% | (2172) |
| Ditto | Egg meat medium and pork intusion medium | 0.0588-0.392% (588-3920 ppm) | No inhibition observed at any concentrations of $NaNO_2$ in this range | (2172) |
| -- | Pasteurized meat | 0.63% (300 ppm) | Growth observed in the presence of 0.03% $NaNO_2$ | (1015) |
| No. 459 | Fish digest broth | 0.02% (200 ppm) | Growth inhibited at pH5.6 or lower at 37 C over 14 days with initial inoculum of $19.6 \times 10^6$ vegetative cells/ml over 14 days or at pH6.1 with $5.2 \times 10^6$ spores/ml | (2182) |
| PA3679 | Comminuted pork trimmings | 0.04-0.08% (400-800 ppm) | No spoilage noted at 0.08% $NaNO_2$ up to 4 weeks at 37 C with initial inoculum of 50 spores/g. Spoilage delayed at 0.037% $NaNO_2$ or more | (267) |
| PA3679 | Beef-pork emulsions | 78 ppm | No spoilage noted in the presence of 3.5% brine at $F_o$ = 0.10, with initial inoculum of spores/g after 90 days at 100 F | (2101) |
| PA3679 | Reinforced clostridial agar | 0,100,200,400 800,1200,1600 ppm | Increased inhibition with decreasing pH when spores were heated at $F_o$ = 0.0015 to 1.5. Tenfold increase in inhibitory effect of $NaNO_2$ when pH decreased from 7.0 to 6.0 | (1787 |

TABLE 41. (Continued)

| Strain | Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|---|
| PA3679 | PT (1654) | 0-1500 ppm | With inoculum of 8 x $10^6$ spores/ml, inhibitory effect of $NaNO_2$ greater when autoclaved then unautoclaved at pH6.0 and above | (1654) |
| PA3679 | PT (1654) | pH7.0 0-2560 ppm (unheated)<br>0-320 ppm (heated)<br>pH6.0 0-240 ppm (unheated)<br>0-60 ppm (heated) | Inhibition at 640-1280 ppm<br>Inhibition at 5-10 ppm<br>Inhibition at 60-80 ppm<br>Inhibition at 5-7.5 ppm | (1653) |
| PA3679 | Liver veal agar | 0-0.2% (0-2000 ppm) | 100 ppm nitrite was inhibitory at pH60 and did not protect against heat injury; 3.45% $NaNO_2$ at 45 C and pH6.0 stimulated spore germination | (500,501,502) |
| NCTC 8594 | Minced pork | 0,150,200,300,400 ppm | Inhibition of vegetative cells by nitrite enhanced by heating $ED_{50}$ vegetative = 71 ppm (heated); 114 ppm (unheated) | (82) |

Table 42 presents a summary of studies on the effects of nitrite on Staphylococcus aureus. Again, the degree of inhibition observed depends upon the pH of the system. Tarr[2182] found that 0.02 percent $NaNO_2$ inhibited S.aureus at an initial pH of 5.6 or lower, and that 1.0 percent $NaNO_2$ was bactericidal to S.aureus at pH 5.6 but not at 7.2 This increased inhibition of S. aureus by nitrite with decreasing pH was confirmed by Castellani and Niven, [320] Shank, et al,[1972] and Buchanan and Solberg,[259] and can be attributed to the increase in the concentration of undissociated nitrous acid accompanying the decrease in pH.

Lechowich, et al[1219] reported that food poisoning staphylococci grew well in ground pork muscle that contained any permissible and palatable concentration of curing salts. Anaerobic growth of staphylococci did not occur when the pH of the meat was lowered to 9.8 to 5.0, even when nitrite was absent. When staphylococci were injected into hams with the pump pickle, they survived curing, but did not survive heating in a smokehouse to an internal temperature of 137 F.

The inhibition of S.aureus by nitrite is a function of the partial pressure of oxygen ($pO_2$) as well as the nitrite concentration and initial pH according to Buchanan and Solberg.[259] Under aerobic conditions, S.aureus metabolizes nitrite, but not under anaerobic conditions. They concluded that vacuum packaging of meats cured with nitrite provides protection against S.aureus outgrowth.

In any discussion of food poisoning by Staphylococcus aureus, consideration must be given to the enterotoxins secreted by the organism. Markus and Silverman[1334] did not observe any effect of sodium chloride,

TABLE 42. SUMMARY OF STUDIES ON THE EFFECTS OF NITRITE ON STAPHYLOCCUS AUREUS

| Medium | Concentration of Nitrite ($NaNO_2$) | Observations | References |
|---|---|---|---|
| Nutrient broth | 10% | Initial count of $1.99 \times 10^8$ per ml reduced 28,700 and 0 after 30 min. and 6 hrs. exposure at pH5.40 | (2182) |
| Glucose-tryptone-yeast extract-phosphate | 40-4000 ppm | Inhibition increased with decreasing pH, under aerobic conditions. Anaerobic growth inhibited by 40 ppm $NaNO_2$ when glucose was introduced with the medium, 1000 ppm when glucose was added aseptically. Nitrite tolerance restored by cysteine or glutathione | (320) |
| Ground pork muscle | 0,100,200,500 ppm | In presence of 5.0% NaCl, pH5-6, 30 C, only partial inhibition of growth at 500 ppm $NaNO_2$ | (1219) |
| Curing pickle | 1.8 g/l | With inoculum of 20,000 ml in presence of 60 salometer brine and 1.2 g $NaNO_3$ per l at 5 C over 50% reduction in numbers after 48 hrs. (Meat juices had a protective effect) | (1219) |
| Ham | 8 g/l in curing pickle | Staphylococci ($5 \times 10^4$ per g). Injected into ham with curing pickle ($60^o$ brine 1,2 g $NaNO_3$/liter) survived curing but were destroyed during heating in a smokehouse to internal temperature of 137 F | (1219) |
| Brain heart infusion broth | 0,200,500,1000 ppm | At 200 ppm $NaNO_2$, greatest inhibition at pH6.3 anaerobically, bactericidal effect at 500 ppm $NaNO_2$ at pH6.3 anaerobically | (259) |

sodium nitrate or sodium nitrite on the production of staphylococcal enterotoxin A per unit of growth. For staphylococcal enterotoxin B, McLean, et al[1369] found that sodium nitrite in the range 50 to 200 ppm did not affect production of this toxin. However, a curing salts mixture of 2 percent sodium chloride, 120 ppm sodium nitrite and 200 ppm sodium nitrate reduced enterotoxin B production significantly.

## Drug Interaction

It has been pointed out previously in this report that the vasodilating effects of nitrites have been known for many years. However, only recently has the possibility of adverse drug-nitrite interactions in the body been given any consideration.

Sander[1867] observed the reaction of sodium nitrite with ephedrine to form N-nitrosamines in human gastric juice in vitro. More recently, Greenblatt, et al[754] studied the nitrosation of phenmetrazine (3-methyl-2-phenylmorpholine), a drug used for appetite control, by nitrite in rabbits and rats in vivo. Nitrite and phenmetrazine were administered to two rabbits in a 4:1 molar ration (4mM $NaNO_2$ to 1mM phenmetrazine) by injection into the stomach, followed by termination after 5 hours and removal of the stomach contents. In a second experiment stomach contents were removed after 1, 2, 3, 4, and 5 hours through an indwelling catheter. In the rats 0.8mM sodium and 0.2mM phenmetrazine were administered by injection. The analyses of the stomach contents revealed the formation of the nitrosamine, N-nitroso phenmetrazine (3-methyl-2 phenyl-N-nitrosomorpholine) in rabbits to the extent of 13 to 18 percent of the theoretical amount and in rats, 23 to 48

percent of the theoretical amount. In rabbits, the yield increased with increasing nitrite concentration. No N-nitrosophenmetrazine was detected when nitrate was substituted for nitrite.

Subsequently, Lijinsky, et al[1256] studied the reactions of sodium nitrite with representative drugs having a tertiary nitrogen structure including the following: an antibiotic, oxytetracycline; an analgesic, aminopyrine, an antialcoholic, disulfuram; a respiratory stimulant, nikethamide; an oral hypoglycemic, tolazamide; and in addition, piperine, found in pepper. The reactions were performed at 37 C in the pH range 3.5 to 8.5 for 4 to 20 hours in the presence of excess nitrite. In all cases, measurable amounts of nitrosamines were formed but the highest yields were obtained with aminopyrine and oxytetracycline. In a previous study, Lijinsky and Greenblatt[1258] reported that rats given small doses of aminopyrine and nitrite by stomach tube died from acute N-nitrosodimethylamine hepatotoxicity.

It should be pointed out that the concentrations used by Lijinsky, et al[1256], are much higher than would be found in the human stomach. Also in the studies by Greenblatt, et al[754] both the drug and nitrite were injected into the stomach. In vivo studies in selected laboratory animals using the oral route of administration are needed to ascertain the extent of formation of nitrosamines from nitrite and various amine-type drugs in the gastrointestinal tract and the nature of any carcinogenic action resulting from such nitrosamine formation.

## CONSUMER EXPOSURE INFORMATION

At the time of preparation of this report, data from the National Academy of Sciences - National Research Council Questionnaire on nitrates and nitrites were not available.

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