FOREWORD INTRODUCTION

SODIUM NITRITE

CAS N°: 7632-00-0

SIDS Initial Assessment Report

For

SIAM 20

Paris, France, 19-22 April 2005

1. Chemical Name: Sodium Nitrite

2. CAS Number: 7632-00-0

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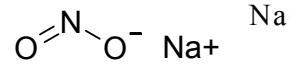
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	Sodium Nitrite Consortium
• Process used	Industry collected data, prepared the updated the IUCLID dossier, and drafted versions of the SIAR and SIAP.
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme?	This substance is sponsored by Japan under the ICCA Initiative and is submitted for first discussion at SIAM 20.
7. Review Process Prior to the SIAM:	The Japanese government peer-reviewed the documents and audited selected studies.
8. Quality check process:	The Japanese government peer-review committee performed spot checks on randomly selected endpoints and compared original studies with data in the SIDS Dossier.
9. Date of Submission:	25 July, 2005
10. Date of last Update:	22 July, 2005
11. Comments:	No

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	7632-00-0
Chemical Name	Sodium nitrite
Structural Formula	 $Na(NO_2)^-$

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Sodium nitrite has been reviewed by a number of international organizations: JECFA (Joint FAO/WHO Expert Committee on Food Additives); National Academy of Sciences (NAS); US National Institute of Environmental Health Sciences (NIEHS); National Institute of Public Health and the Environmental Hygiene, Netherlands; US National Toxicology Program (NTP); and California EPA (CAL/EPA).

Nitrite in blood is highly reactive with haemoglobin and causes methaemoglobinaemia. Ferrous iron associated with haemoglobin is oxidized by nitrite to ferric iron, leading to the formation of methaemoglobin. Humans are considered to be more sensitive than rats in this respect.

The primary acute effect of sodium nitrite in rats and mice is methaemoglobinaemia. Methaemoglobin concentrations in SD rats increased from 45% to 80% over 1 hour after an oral dose of sodium nitrite at 150 mg/kg bw and they returned to normal levels within 24 hours in surviving rats.

LD_{50} values by gavage are 214 mg/kg bw (males) and 216 mg/kg bw (females) in mice. In an acute inhalation study (which could not be validated) methaemoglobin levels in female rats were significantly increased after 4 hours exposure to 10 mg/m^3 sodium nitrite. The increase was judged not to be haematologically significant. No significant increase was observed in exposed males. There were no toxicologically significant effects on animals maintained for 14 days post exposure. No information on acute dermal toxicity is available.

Based on the available information, sodium nitrite is a moderate eye irritant, but is non-irritant to skin in rabbits. No studies are available investigating the sensitising potential of sodium nitrite in animals. No cases of sensitisation have been reported in humans.

In a repeated dose toxicity study [NTP] male and female F344/N rats were exposed to 0, 375, 750, 1500, 3000 or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 30, 55, 115, 200, or 310 mg/kg bw/day in males and 0, 40, 80, 130, 225, or 345 mg/kg bw/day in females) in drinking water for 14 weeks. Methaemoglobin levels were significantly elevated in all treated groups compared to the controls by the end of the treatment period. For males, mean methaemoglobin levels after 14 weeks were 0.03±0.01, 0.08±0.01, 0.12±0.02, 0.25±0.07, 0.71±0.20 and 3.38±0.80 g/dL at doses of 0, 30, 55, 115, 200, and 310 mg/kg bw/day. For females, mean methaemoglobin levels after 14 weeks were 0.06±0.02, 0.14±0.02, 0.16±0.02, 0.48±0.05, 0.99±0.20 and 2.27±0.54 g/dL at doses of 0, 40, 80, 130, 225 and 345 mg/kg bw/day. The NOAELs were not determined (increased methaemoglobinaemia). The LOAELs for other endpoints were 115 mg/kg bw/day (decreased sperm motility) in males and 225 mg/kg bw/day (increased relative weight of the kidney and spleen) in females .

In a second 14-week repeated dose toxicity study [NTP] male and female B6C3F1 mice were exposed to 0, 375, 750, 1500, 3000 or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1230 mg/kg bw/day in females) in drinking water. Methaemoglobin levels were not reported however there were no clinical signs of toxicity. The LOAELs were 750 mg/kg bw/day (extramedullary haematopoiesis in the spleen, degeneration of the testis) in males and 445 mg/kg bw/day (extramedullary haematopoiesis in the spleen) in females.

In a two-year chronic toxicity/carcinogenicity study [NTP] male and female F344/N rats were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day in males and 0, 40, 80 or 150 mg/kg bw/day in females) in drinking water. There were no clinical findings related to exposure. Methaemoglobin levels were measured at two weeks and three months. At both 2 weeks and three

months, methaemoglobin levels were high at night when the rats were actively feeding and drinking and low during the day when the rats were less active. Methaemoglobin levels tended to increase with increasing dosage.

In a second two-year study [NTP] male and female B6C3F1 mice were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water. There were no clinical findings related to exposure. At 12 months, no significant increase in methaemoglobin level was observed in either sex at any dose.

Based on the two-year studies, the NOAELs for rats were 130 mg/kg bw/day in males and 150 mg/kg bw/day in females. For mice the NOAELs were 220 mg/kg bw/day in males and 165 mg/kg bw/day in females

Sodium nitrite is a direct-acting, base-pair substitution mutagen in organisms ranging from bacteria to mammalian cells *in vitro*. This substance induced chromosomal aberrations in mammalian cells *in vitro*. There is evidence of potential *in vivo* genotoxicity. The substance tested positive in a micronucleus test (peripheral blood) when mice were dosed by gavage at 10 – 20 mg/kg bw (4 times at 24 hrs intervals) but was negative in a second study where mice were dosed via drinking water at dosed up to 900 mg/kg bw/day (females) for 14 weeks. In a chromosomal aberration test, pregnant rats were dosed with 210 mg/kg bw/day for 13 days. Positive results for the induction of chromosomal aberrations in bone marrow of the parents and liver cells of embryos were reported.

In a two-year chronic toxicity/carcinogenicity study [NTP] male and female F344/N rats were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day for males and 0, 40, 80 or 150 mg/kg bw/day for females) in drinking water. The incidences of hyperplasia of the forestomach epithelium in high dose males (44/50) and females (40/50) were significantly greater than those in the control groups (12/50 males, 8/50 females). The incidence of fibroadenoma of the mammary gland was significantly increased in 80 mg/kg bw/day females, and the incidences of multiple fibroadenoma were increased in 40 and 80 mg/kg bw/day females; however these neoplasms occur with a high background incidence and no increase was seen in the high dose group. The incidences of mononuclear cell leukemia were significantly decreased in 70 and 130 mg/kg bw/day males (7/50 and 3/50, respectively) and 80 and 150 mg/kg bw/day females (1/50 and 1/50, respectively) compared with controls (17/50 males, 15/50 females). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in F344/N rats at approximate doses of up to 130 mg/kg bw/day in males and 150 mg/kg bw/day in females over a two year period.

In another NTP study male and female B6C3F1 mice were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water for two years. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a positive trend (1/50, 0/50, 1/50 and 5/50 at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively). The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in 220 mg/kg bw/day males (10/50) than in the controls (0/50). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in male B6C3F1 mice at doses up to approximately 220 mg/kg bw/day over a two year period. There is equivocal evidence of carcinogenic activity in female mice, based on the positive trend of squamous cell papilloma or carcinoma (combined) in the forestomach.

Various other carcinogenicity studies in rats were negative. Moreover, some even showed a reduction in tumor risk (e.g. lymphoma or leukemia). WHO concluded that there was no evidence of carcinogenic activity of sodium nitrite in rats and mice based on the findings of NTP carcinogenicity studies.

There is evidence for transfer of sodium nitrite to fetuses in rats and mice. Reproductive success in the F1 generation was not affected. Increase in mortality of pre- and postnatal offspring and decrease in body weight of preweaning pups were observed in rat dams given a diet containing sodium nitrite at 0.0125% (10.75 mg/kg bw/day), 0.025% (21.5 mg/kg bw/day) and 0.05% (43 mg/kg bw/day), and the NOAEL is considered to be 10.75 mg/kg bw/day. Reproductive toxicity by continuous breeding in the mice was conducted with drinking water at doses of 125, 260 and 425 mg/kg bw/day, and no adverse effect on reproductive performance or necropsy endpoint were observed. The NOAEL is estimated to be 425 mg/kg bw/day. Sodium nitrite caused maternal anemia and the incidence of abortion and fetal mortality increased when administered to pregnant guinea pigs in drinking water and LOAEL is considered to be at 60 mg/kg bw/day.

From the weight of evidence, sodium nitrite appears to affect erythropoiesis, haematological parameters and brain development resulting in mortality and poor growth of offspring.

In humans, sodium nitrite causes smooth muscle relaxation, methaemoglobinaemia, and cyanosis. Infants are particularly sensitive. A large proportion of haemoglobin in infants is in the foetal haemoglobin form, which is more

readily oxidised to methaemoglobin than adult haemoglobin. Further, reduced nicotinamide-adenine dinucleotide (NADH)-dependent methaemoglobin reductase, the enzyme responsible for reduction of methaemoglobin back to normal haemoglobin, has only about half the activity present in adults.

Environment

Sodium nitrite is white or slightly yellow hygroscopic granules, rod or powder, which is very soluble in water (820 g/L at 20 °C). Melting point, boiling point, vapour pressure and partition coefficient are 271 °C, >320 °C (decomposes), 9.9E-17 hPa (25°C) and log Kow = -3.7, respectively. Fugacity model Mackay level III calculations suggest that the substance will distribute mainly to soil if released to the air or soil compartments separately or to all three compartments simultaneously and almost exclusively to water if released to the water compartment. Estimated value of Henry's constant is 2.06E-07 atm-m^3/mole. This substance dissociates immediately into sodium and nitrite ions in water. The nitrite ion is a component of the nitrogen cycle. In the environment, bacteria of the genus Nitrobacter oxidise nitrites to nitrates. Nitrates are reduced to nitrogen by anaerobic bacteria present in soil and sediment. The estimated BCF is 3.162 and hence bioaccumulation is not significant. Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 82.3 days.

The LC_{50} values for the acute toxicity of sodium nitrite to fish reported in the literature vary widely between the species tested; LC_{50} (96h) = 0.54 mg $NaNO_2$/L for *Oncorhynchus mykiss*; LC_{50} (96h) = 35 mg $NaNO_2$/L for *Ictalurus punctatus*; LC_{50} (96h) = 691.0 mg $NaNO_2$/L for *Micropterus salmoides*; and LC_{50} (96h) = 1010.4 mg $NaNO_2$/L for *Anguilla japonica*, for example. This difference has been attributed to the ability of certain species, such as eels, bass and sunfish to prevent nitrite from crossing the gill membrane and entering the blood, whilst other species such as rainbow trout concentrate nitrite in their blood. The range of toxicity values reported for some species of fish varies widely and is believed to be dependant on the quality of the water used in the test with pH, chloride and calcium ion concentration all having an influence. In particular, chloride ion concentration has been shown to be important, with increasing concentrations leading to a decrease in the toxicity of nitrite. As with fish, there is variation in toxicity between invertebrate species. Sodium nitrite is toxic to invertebrates such as *Cherax quadricarinatus* (LC_{50} (96h) = 4.93 mg $NaNO_2$/L and *Thamnocephalus platyurus* (LC_{50} (24h) = 3.9 mg $NaNO_2$/L), whereas other species, such as *Procambarus clarkii* (LC_{50} (96h) = 18.7 mg $NaNO_2$/L) and *Penaeus paulensis* are much less sensitive (LC_{50} (96h) = 539.2 mg $NaNO_2$/L). The presence of chloride ions has been found to mitigate nitrite toxicity in some species. Acute toxicity to green alga (*Desmodesmus subspicatus*) is > 100 mg/L (72-h E_rC_{50} and E_bC_{50}) [OECD TG 201].

No data is available for chronic toxicity of sodium nitrite in fish. In invertebrates, an 80-day NOEC of 9.86 mg $NaNO_2$/L for *Penaeus monodon* has been reported. The NOEC value in green alga (*Desmodesmus subspicatus*) is 100 mg/L (72-h for growth rate and biomass) [OECD TG 201].

For other aquatic organisms, the EC_{50} (48h, deformation) and LC_{50} (48h) for the protozoa *Spirostomum ambiguum* were 421 and 533 mg $NaNO_2$/L, respectively; for the microalgae *Tetraselmis chuii* the EC_{50} (96h, mobility) and NOEC (96h, mobility) were 7886 and 3740 mg $NaNO_2$/L, respectively.

Exposure

Total production of sodium nitrite in Japan was 10,000 - 50,000 t/year in 2001. Worldwide production of sodium nitrite is not available.

This substance is used in closed system, for non dispersive use, and also for wide dispersive use. Workers are recommended to wear protective gear such as a mask, rubber gloves and goggles to prevent exposure. There are no available official recommendations or regulations for occupational exposure limits to this substance. This substance is widely used in various industries in the category including agricultural, basic chemicals, chemical industry, and others. The use in synthesis includes as raw material for caprolactam and others. This substance is used widely as food/foodstuff additives, corrosion inhibitor, and so forth.

The nitrite ion is ubiquitous in the environment, where it forms part of the nitrogen cycle. The source of nitrogen is natural or anthropogenic. Fertilizers are considered to be the main anthropogenic source of nitrogen, although anthropogenic nitrogen oxide and dioxide present in the atmosphere from combustion processes are also sources of nitrite and nitrate in soils and surface waters, delivered via acid rain. Naturally occurring nitrogen oxide and dioxide in the atmosphere are also possible sources of nitrite. It should be noted that although the nitrite ion (NO_2^-) may cause a concern when assessing the potential eutrophication hazard including drinking water quality in certain regions, the use of this substance ($NaNO_2$) as a fertilizer has not been reported. Therefore this substance has a potential of eutrophication, but its influence is lower than that of the fertilizers.

The most common source of exposure of anthropogenic sodium nitrite to consumers is from its use in cured meat products. Exposure to nitrite also occurs from vegetables and drinking water. Nitrite can be formed in the body through reduction of nitrate by enteric bacteria and mammalian nitrate reductase. The Joint FAO/WHO Expert Committee on Food Additives established an acceptable daily nitrite intake of 0 to 0.07 mg/kg bw/day. Various countries have set limits for nitrite through water quality regulations.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical possesses properties indicating a hazard to human health (acute toxicity, irritation, repeated toxicity, mutagenicity, and reproductive toxicity) and the environment (acute toxicity). Given the wide dispersive use of this substance, member countries are invited to perform an exposure assessment, and if necessary a risk assessment for these uses. It is acknowledged that some uses (e.g. as a food additive) as well as the presence in drinking water are already regulated in many member countries. It is recommended that the information on possible total exposure from regulated and non-regulated use be shared between regulatory agencies.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 7632-00-0
IUPAC Name: Sodium nitrite
Molecular Formula: NO_2Na
Structural Formula: $Na^+(NO_2)^-$
Molecular Weight: 69.00
Synonyms: Nitrous Acid, Sodium Salt.
Nitrous acid sodium salt (1:1)

1.2 Purity/Impurities/Additives

Moisture: < 0.3 % w/w, water insoluble matter: <0.05 % w/w

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	White or slightly yellow hygroscopic granules, rods or powder	Merck Index, 2001
Melting point	271 °C	Merck Index, 2001
Boiling point	>320 °C (decomposes)	Merck Index, 2001
Density	2.1 (20 degree)	MERCK MSDS 2004
Vapour pressure	9.9E-17 hPa @ 25°C	UBE Industries, 2005
Water solubility	820 g/L (20 °C)	ICSC, 2000
Partition coefficient n-octanol/water (log value)	-3.7	ICSC, 2000; MERCK MSDS 2004
Dissociation constant, pKa	3.27	Takahama *et al*, 2002
Henry's law constant	2.06E-07 atm-m^3/mole	UBE Industries, 2005

2 GENERAL INFORMATION ON EXPOSURE

Environmental and/or exposure to sodium nitrite or the nitrite ion from industrial sources may occur during production or from use. However, there is also exposure from the nitrite ion that is distributed ubiquitously in natural water or soils, present in food or generated endogenously.

2.1 Production Volumes and Use Pattern

Total production of sodium nitrite in Japan was 10,000 - 50,000 t/year in 2001. Worldwide production of sodium nitrite is not available.

Sodium nitrite is widely used in various industries in categories including agricultural, basic chemicals, chemical industry, electrical/electronic engineering industry, fuel industry, metal

extraction, refining and processing of metals, paints/lacquers and varnishes industry, polymers industry, public domain, textile processing industry and others.

Sodium nitrite is used as a raw material for the production of caprolactam polymers and antioxidants for synthetic polymers. It is used as a colour fixative and preservative in meats and fish. It is also used in: adhesives, binding agents, anti-freezing agents, cleaning/washing agents, disinfectants, colouring agents, construction materials additives, corrosive inhibitors, cutting fluids, fillers, food/foodstuff additives, heat transferring agents, intermediates, laboratory chemicals, lubricants and additives, non agricultural pesticides, oxidizing agents, pesticides, pharmaceuticals, process regulators, reducing agents, stabilizers, surface-active agents.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

The nitrite ion is ubiquitous in the environment, where it forms part of the nitrogen cycle. The source of nitrogen is natural or anthropogenic. Fertilizers are considered to be the main anthropogenic source of nitrogen, although anthropogenic nitrogen oxide and dioxide present in the atmosphere from combustion processes are also sources of nitrite and nitrate in soils and surface waters, delivered via acid rain. Naturally occurring nitrogen oxide and dioxide in the atmosphere are also possible sources of nitrite. It should be noted that although the nitrite ion (NO_2^-) may cause a concern when assessing the potential eutrophication hazard including drinking water quality in certain regions, the use of this substance ($NaNO_2$) as a fertilizer has not been reported. Therefore this substance has a potential of eutrophication, but its influence is lower than that of the fertilizers. Various countries have set limits for nitrite through water quality regulations. Limit values permissible in drinking water are 0.05 mg/L (as nitrite-N, Japan), 0.5 mg/L (as nitrite, EU), 1mg/L (as nitrite-N, US EPA) and 3 mg/L (acute) and 0.2 mg/L (chronic) (as nitrite, WHO).

2.2.2 Photodegradation

Indirect photo-oxidation by hydroxy radicals (1500000 molecule/cm^3) is predicted to occur with a half-life estimated at 82.3 days (calculated using AOPWIN v1.91 at 25 °C, rate constant, 1.30 x 10^{-13} cm^3/molecule/sec, 12-hour day) [UBE Industries, 2005].

2.2.3 Stability in Water

This substance dissociates immediately into sodium and nitrite ions in water.

2.2.4 Transport between Environmental Compartments

Fugacity Model Mackay level III calculations [UBE Industries, 2005] using EPI Suite v3.12 indicate that the substance will distribute mainly to soil if released to the air or soil compartments separately or to all three compartments simultaneously and almost exclusively to water if released to the water compartment.

Table 2 Environmental Distribution of Sodium Nitrite Using Fugacity Model Mackay Level III

	1000 kg/h emission to these compartments separately			Simultaneous 1000 kg/h emission to air, water and soil compartments
	Air	Water	Soil	
In air	5.0	0	0.3	1.78
In water	25.0	99.7	22.3	40.2
In soil	69.9	0.1	77.4	58.0
In sediment	0.1	0.2	0.0	0.07

2.2.5 Biodegradation

The nitrite ion is a component of the nitrogen cycle. In the environment, bacteria of the genus Nitrobacter oxidise nitrites to nitrates. Nitrates are reduced to nitrogen by anaerobic bacteria present in soil and sediment.

2.2.6 Bioaccumulation

An estimated BCF of 3.162 was calculated by EPI Suite v3.12 using the default Log Pow value of 0.06 [UBE Industries, 2005]. Sodium nitrite is known to be metabolised in fish, hence there is low potential for bioaccumulation.

2.2.7 Other Information on Environmental Fate

No information available.

2.3 Human Exposure

2.3.1 Occupational Exposure

Occupational exposures at production sites may occur by the inhalation or dermal route.

The atmospheric concentration was measured at two production sites [JISHA, 2004]. Air samples were collected at a rate of 2.0 L/min through a suction tube placed at the breathing zone of the worker, trapped in a filter in a collection tube and analysed by LC. The monitoring data are shown in Table 3. The concentrations in the operations at site 1 (powder production) were higher than those in the operations at site 2 (liquid production).

Workers are recommended to wear protective gear such as a mask, rubber gloves and goggles to prevent exposure. There are no available official recommendations or regulations for occupational exposure limits to this substance.

Table 3 Work Place Monitoring Data For Sodium Nitrite

	Monitoring Data (Maximum Concentration) mg/m^3	Frequency times/day	Working time hrs/day	Maximum EHEinh mg/kg/day
Operation in site 1 (powder production)				
Paper bag filling	0.110	1	6.00	1.18×10^{-2}
Sampling (process)	0.308	2	0.06	3.30×10^{-4}
Sampling (product)	0.803	1	0.03	4.30×10^{-4}
Analysis work	<0.022	2	0.34	1.34×10^{-4}
Operation in site 2 (liquid production)				
Tank car operation	<0.008	2	0.60	8.57×10^{-5}
Sampling (process)	0.046	2	0.34	2.79×10^{-4}
Sampling (product)	<0.014	1	0.08	2.00×10^{-5}

EHEinh: Estimated Human Exposure via inhalation (calculated by using the default value of 1.25 m^3/h and 70 kg)

2.3.2 Consumer Exposure

Diet constitutes an important source of exposure to both nitrite and nitrate. The major dietary source of nitrate is vegetables. Lettuce, spinach, celery and beetroot commonly contain more than 1g nitrate/kg fresh weight and may reach 3-4 g/kg [Walker, 1990]. Nitrite occurs in plants at low concentrations, normally between 1-2 mg/kg fresh weight and rarely over 10 mg/kg, although potatoes have been reported to contain 2-60 mg/kg, with a mean concentration of 19 mg/kg [MAFF, 1992]. Mean estimates of nitrate intake range from 31 – 185 mg/day in various European countries, with vegetables supplying 80-85% [Gangolli, et al, 1994]. The intake of nitrite is much lower in various European countries and averages 0.7 – 8.7 mg/day, with both vegetables and cured meats being the major sources.

As discussed in Section 3.1.1, nitrate can be reduced in the body to nitrite by both enteric bacteria and mammalian nitrate reductase activity.

The Joint FAO/WHO Expert Committee on Food Additives established an acceptable daily nitrite intake of 0 to 0.07 mg/kg bw/day. [JECFA, 2003]. This regulation is enforced in OECD countries. Various countries have set limits for nitrite through water quality regulations (see Section 2.2.1).

3 HUMAN HEALTH HAZARDS

There are several review/evaluation documents on sodium nitrite issued by pertinent international or national organizations. JECFA (Joint FAO/WHO Expert Committee on Food Additives) has issued a series of updated evaluation documents [e.g. WHO, 2004]. National Academy of Sciences (NAS, 1981), National Institute of Environmental Health Sciences of U.S.A. (NIEHS, 1970), or National Institute of Public Health and the Environmental Hygiene, Netherlands, (1986) issued a document regarding drinking water, which included the review/evaluation on human health hazard. National Toxicology Program document (NTP, 1990, 2001) includes a review on the toxicology publications in their reports. With regard to reproductive toxicity California EPA (CAL/EPA, 2000) is a noteworthy review.

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Nitrite in blood is highly reactive with haemoglobin and causes methaemoglobinaemia. Ferrous iron associated with haemoglobin is oxidized by nitrite to ferric iron, leading to the formation of methaemoglobin. The oxygen-carrying capacity of methaemoglobin is much less than that of haemoglobin. Humans are considered to be more sensitive than rat in this respect. The primary acute toxic effects of sodium nitrite in rats and mice are resulted from methaemoglobinaemia.

Reduction of nitrate (NO_3^-) to nitrite (NO_2^-) occurs by mammalian nitrate reductase and nitrate reductase activity of microorganisms in the oral cavity and upper gastrointestinal tract [NAS, 1981; Walker, 1996; WHO, 1996b]. In particular, oral microorganisms are responsible for significant levels of nitrate reduction. Salivary nitrate concentrations are considered directly related to an orally ingested amount of nitrate. Allowing for considerable inter-individual variations it has been estimated that 25% of nitrate ingested by humans are secreted in the saliva. Of this 25% approximately 20 % (i.e., about 5 % of the ingested dose) is reduced to nitrite in humans.

The enzyme methaemoglobin reductase catalyzes the reduction of methaemoglobin to haemoglobin and protects red blood cells against oxidative damage.

The secondary toxic effects of acute sodium nitrite in animals result in vasodilation, relaxation of smooth muscle, and lowering of blood pressure.'

Studies in Animals

In vitro Studies

An *in vitro* study was conducted using erythrocytes of different species [Klimmek *et al*., 1988]. After incubation of erythrocytes with sodium nitrite at 2.5 mmol/L, the highest ferrihaemoglobin formation was observed in dogs followed by cattle and then cats. The degree of ferrihaemoglobin formation in human erythrocytes was similar to that in cats. The degree of ferrihaemoglobin formation was lowest in rabbit erythrocytes.

In another *in vitro* study, Calabrese *et al.* (1983) showed that 50% methaemoglobin was formed when 1 mL of human whole blood was mixed with 10 µL of a 3 mmol/L solution of sodium nitrite, whereas the same concentration of sodium nitrite induced only 14% methaemoglobin formation with rat blood. The difference in sensitivity is probably due to the fivefold difference in erythrocyte methaemoglobin reductase activity between humans and rats [Smith and Beutler, 1966].

In vivo Studies

Sodium nitrite at 0.3 mmol/kg bw was given intravenously to rats, rabbits and cats [Klimmek *et al.*, 1988]. Sodium nitrite induced ferrihaemoglobin formation with maxima of 47.7+/-1.3% at 90 min in cats, 7.5+/-1.0% at 10 min in rabbits and 18.4+/-0.0% at 30 min in rats. Despite the five times greater ferrihaemoglobin maxima due to treatment with sodium nitrite in cats compared to rabbits, respiratory rate increased three times less. Total haemoglobin was not influenced by nitrite.

Imaizumi et al (1980) reported that maximum levels of methaemoglobin (45 – 80%) were reached one hour after dosing Sprague-Dawley rats with 150 mg/kg bw sodium nitrite. The concentration returned to normal after 24 hours if the animal survived.

In Sprague-Dawley rats receiving a single dose of 30 mg/kg bw of sodium nitrite in aqueous solution by gavage (10 - 15% of LD_{50}), plasma nitrite and methaemoglobin levels were increased after 2.8 minutes and maximum effects (plasma nitrite = 15%, methaemoglobin = 12%) were observed after 22.5 minutes. After three hours both parameters had returned to normal levels [Hirneth & Classen, 1984].

Friedman et al (1972)investigated the gastric adsorption of sodium nitrite in male Swiss ICR/Ha mice. The mice were dosed by gavage with 150 µg sodium nitrite in 0.1 mL aqueous solution. Animals, in groups of 13 – 18, were killed at one, 10, 20 and 30 minutes after dosing. Stomachs, together with attached 5 mm segments of the esophagus and duodenum, were removed and analysed separately for sodium nitrite. Sodium nitrite was found to disappear rapidly from the mouse stomach, with 85 and 95% losses seen at 10 and 30 minutes, respectively.

In rats, infusion of sodium nitrite at a range of doses up to 1000 µmol/kg bw over 5 minutes, resulted in a dose-dependent increase in plasma levels of nitrite and a rapid conversion of nitrite into nitrate. Sodium nitrite decreased mean arterial pressure dose-dependently but no marked effects on heart rate were observed [Vleeming et al, 1997].

Conclusion

Nitrite in blood is highly reactive with haemoglobin and causes methaemoglobinemia. Ferrous iron associated with haemoglobin is oxidized by nitrite to ferric iron, leading to the formation of methaemoglobin. Humans are considered to be more sensitive than rats in this respect.

The primary acute effect of sodium nitrite in rats and mice is methaemoglobinemia. Methaemoglobin concentrations in SD rats increased from 45% to 80% over 1 hour after an oral

dose of sodium nitrite at 150 mg/kg bw and they returned to normal levels within 24 hours in surviving rats.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

One study for acute inhalation toxicity in rats (which could not be validated) is reported in the literature [ECB,2000]. Rats were exposed to sodium nitrite aerosols generated from the aqueous solution for four hours. The target exposure concentration was 10 or 100 mg/m^3 (achieved; 95.1 mg/m^3). Methaemoglobin levels were measured after exposure and the remaining animals maintained for 14 days. Methaemoglobin was significantly increased above concurrent control values only in females exposed to 10 mg/m3 only, however this increase was judged not to be haematologically significant as the value was within the range seen for controls animals. There was no significant methaemoglobin increase in exposed males. There were no toxicologically significant effects on animals maintained for 14 days post exposure.

Dermal

No information is available on acute dermal toxicity.

Oral

Four studies are available for acute oral toxicity, one of which is considered reliable. In this study [Riemann, 1950] male and female mice (10 animals/sex/dose) were dosed by gavage with 100, 150, 200, 250 and 300 mg/kg of a 0.5 – 2% aqueous solution of sodium nitrite. All animals that died were found to have methaemoglobin in their blood, although the levels are not reported. Mice receiving the larger doses died within a few minutes and all other mice (except one) that died did so within 24 hours. The LD_{50} value was 214 mg/kg for males and 216 mg/kg for females.

Similar values were obtained from the other available studies; LD_{50} values of 77 and 130 mg/kg were reported for fasted and fed BD rats, respectively [Druckery et al, 1963]; 150 mg/kg for fasted Sprague-Dawley rats [Imaizumi et al, 1980]; and 124 mg/kg for New Zealand White rabbits [Dollahite and Rowe, 1974].

Other Routes of Exposure

No reliable studies are available.

Studies in Humans

Dermal

Saito *et al* (1996) report a case where a four year old boy was treated with two liniment solutions containing sodium nitrite at 30 g/L (Liniment A) and 140 g/L (Liniment B). Liniment A was applied all over the boy's body, causing listlessness and vomiting. Liniment B was applied all over the boy's body a few days later. The boy went into shock and suffered severe cyanosis. He was hospitalised immediately, but died after two hours in intensive care. The boy's blood methaemoglobin level was found to be 76%. In a study using rats, the authors confirmed percutaneous absorption of nitrite from both of the liniment formulations [Saito *et al*, 1997].

Oral

There are numerous case reports concerning the acute toxicity of sodium nitrite in humans available in the literature, as illustrated by the following examples: Gowans (1990) reported the fatal case of a nurse who probably ingested a 1g tablet of sodium nitrite (670 mg NO_2^-). Death occurred two hours after admission to hospital. Post mortem methaemoglobin level was 35%, implying a much higher level on admission. Serum nitrite level was 13 mg/L. Finan et al (1998) reported a case of methaemoglobinaemia associated with three previously healthy children (two four year old boys and a two year old girl). One of the children had mistaken a bag of sodium nitrite crystals for sugar and added it to cups of tea at concentrations of 5100, 5000 and 4900 mg/L. Methaemoglobin levels of 77% and 38% were measured for two of the children. Centers for Disease Control (1997) report two cases of methemoglobinaemia attributable to nitrite contamination of potable water through boiler fluid additives. In the first of these, 49 schoolchildren were affected after eating soup which had been diluted with hot water from the tap. The soup was found to contain 459 ppm nitrite. Methaemoglobinaemia was diagnosed in 59% of the children, with levels between 3 – 47%. In the second case, six workers were found to have methaemoglobin levels of between 6 – 16% after drinking coffee contaminated with 300 ppm nitrite.

Infants under 3 months old are particularly sensitive to nitrite. A large proportion of haemoglobin in these infants is in the foetal haemoglobin form, which is more readily oxidised to methaemoglobin than adult haemoglobin. Further, reduced nicotinamide-adenine dinucleotide (NADH)-dependent methaemoglobin reductase, the enzyme responsible for reduction of methaemoglobin back to normal haemoglobin, has only about half the activity present in adults [ATSDR, 2001].

Most clinical case data refer to neonates developing methaemoglobinaemia after drinking water or water-based formulations with high nitrate or nitrite content. The great majority of cases (well-water methaemoglobinaemia) occurred when nitrate levels in drinking water exceeded 100 mg NO_3^- /L. It is generally accepted that water nitrate content of 50 mg/L is safe even for neonates. Assuming normal liquid intake of 150 mL/kg bw/day by neonates, nitrate intake of 7.5 mg NO_3^-/kg bw/day is considered safe.

Other review reports are as follows. The lowest acute oral lethal dose of nitrite reported for humans varied from 27-255 mg/kg bw, in which the lowest figures applied for children and elderly people. Nitrite is also more toxic to young infants (3 months) than adults giving rise to relatively higher methaemoglobin levels in the blood. The lowest toxic dose reported was 1 mg NO_2/kg bw, whereas in another study 0.5-5 mg NO_2/kg bw did not cause any toxic effect [National Institute of Public Health and Environmental Hygiene, Netherlands, 1986].

Conclusion

LD_{50} values by gavage are 214 mg/kg (males) and 216 mg/kg (females) in mice. In an acute inhalation study (which could not be validated) methaemoglobin levels in female rats were significantly increased after 4 hours exposure to 10 mg/m^3 sodium nitrite. The increase was judged not to be haematologically significant. No significant increase was observed in exposed males. There were no toxicologically significant effects on animals maintained for 14 days post exposure. No information on acute dermal toxicity is available.

In humans, nitrite causes methaemoglobinaemia and cyanosis. Fatal poisoning cases of infants resulting from ingestion of nitrates in water or spinach are recorded. Lethal poisonings at doses of 27–255 mg/kg bw from anthropogenic sodium nitrite are also reported.

3.1.3 Irritation

Skin Irritation

Studies in Animals

In a reliable study for skin irritation in rabbits, performed using a method similar to OECD TG 404 but not under GLP, approximately 500 mg of the substance was applied to the shaved backs of 6 male New Zealand White rabbits and covered with a semi-occlusive dressing for four hours. The animals were examined one hour, one, two and three days after removal of the chemical. Some slight irritation was observed one hour after removal of the substance, but all signs had disappeared by the one day observation and the substance is not considered to be a skin irritant. [Southwood, 1985].

Eye Irritation

Studies in Animals

In a reliable study for eye irritation in rabbits, performed using a method similar to OECD TG 405 but not under GLP, 100 mg of substance was applied into the conjunctival sac of the left eye of six female New Zealand White Rabbits. The eyes of three of the rabbits were irrigated with water for two minutes 30 – 60 seconds after application of the substance. Conjunctival effects were seen in all animals and consisted of moderate redness, mild chemosis and severe discharge. All signs of irritation had disappeared by twelve days. No corneal effects were observed [Southwood, 1985].

Conclusion

This substance is a moderate eye irritant, but is non-irritant to skin in rabbits.

3.1.4 Sensitisation

No studies are available in animals investigating the sensitising potential of sodium nitrite. As this substance is endogenously generated, sensitisation potential is not expected. No evidence of sensitisation in humans has been reported.

Conclusion

No studies are available in animals investigating the sensitising potential of sodium nitrite. No cases of sensitisation have been reported in humans.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Oral

Reliable studies are listed in Table 4.

In a NTP study (2001) groups of male and female F344/N rats (10 animals/sex/group) were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 30, 55, 115, 200, or 310 mg sodium nitrite/kg bw/day in males and 0, 40, 80, 130, 225, or 345 mg/kg bw/day in females) in drinking water for 14 weeks. Clinical pathology study groups of 15 male and 15 female rats were exposed to the same concentrations for 70 or 71 days. One 225 mg/kg bw/day female died before the end of the study. Body weights of 200 and 310 mg/kg bw/day males and 345 mg/kg bw/day females were significantly less than those of the controls. Water consumption by 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females was less than that by the controls at weeks 2 and 14. Clinical findings related to sodium nitrite

exposure included brown discoloration in the eyes and cyanosis of the mouth, tongue, ears, and feet of 200 and 310 mg/kg bw/day males and of 130 mg/kg bw/day and higher females. Reticulocyte counts were increased in 200 and 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females. The erythron was decreased on day 19 but increased by week 14 in 310 mg/gk bw/day males and 345 mg/kg bw/day females. Methaemoglobin levels were significantly elevated in all treated groups compared to the controls by the end of the treatment period. For males, mean methaemoglobin levels after 14 weeks were 0.03±0.01, 0.08±0.01, 0.12±0.02, 0.25±0.07, 0.71±0.20 and 3.38±0.80 g/dL at doses of 0, 30, 55, 115, 200, and 310 mg/kg bw/day. For females, mean methaemoglobin levels after 14 weeks were 0.06±0.02, 0.14±0.02, 0.16±0.02, 0.48±0.05, 0.99±0.20 and 2.27±0.54 g/dL at doses of 0, 40, 80, 130, 225 and 345 mg/kg bw/day. The NOAELs were not determined. The relative kidney and spleen weights of 200 and 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females were significantly greater than those of the controls. Sperm motility in 115 and 310 mg/kg bw/day males was significantly decreased. Increased erythropoietic activity in the bone marrow of exposed males and females was observed. The incidences of squamous cell hyperplasia of the forestomach in 310 mg/kg bw/day males and 345 mg/kg bw/day females were significantly increased.

In a second 14-week study [NTP, 2001] groups of male and female $B6C3F_1$ mice (10 animals/sex/group) were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1,230 mg/kg bw/day in females) in drinking water for 14 weeks. Body weights of 990 mg/kg bw/day males were significantly less than those of the controls. Water consumption by males exposed to 1,500 ppm or greater was slightly less than that by the controls at week 13. Methaemoglobin concentrations were not reported however there were no clinical signs of toxicity. Relative spleen weights of 750 and 990 mg/kg bw/day males and absolute and relative heart, kidney, liver, and spleen weights of 840 and 1230 mg/kg bw/day females females were greater than those of the control groups. Sperm motility was decreased in 990 mg/kg bw/day males, and the estrous cycles of 445 and 1230 mg/kg bw/day females were significantly longer than in the controls. There were increased incidences of squamous cell hyperplasia of the forestomach in 990 mg/kg bw/day males and 1230 mg/kg bw/day females, extramedullary hematopoiesis of the spleen in 750 and 990 mg/kg bw/day males and 445 mg/kg bw/day or greater females, and degeneration of the testis in 750 and 990 mg/kg bw/day males.

In a NTP study (2001) groups of male and female F344/N rats (50 animals/sex/group) were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day for males and 0, 40, 80 or 150 mg/kg bw/day for females) in drinking water for two years. Survival of exposed groups was similar to that of the controls (29/50, 38/50, 36/50 and 36/50 for males at doses of 0, 35, 70 and 130 mg/kg bw/day, respectively and 33/50, 31/50, 36/50 and 33/50 for females at 0, 40, 80 or 150 mg/kg bw/day, respectively). Mean body weights of 130 mg/kg bw/day males and 150 mg/kg bw/day females were less than those of the controls throughout the study. Water consumption by high dose males and females was less than that by the controls throughout the study and that by the other exposed groups was generally less after week 14. Methaemoglobin levels were measured at two weeks and three months. At both 2 weeks and three months, methaemoglobin levels were high at night when the rats were actively feeding and drinking and low during the day when the rats were less active. Methaemoglobin levels tended to increase with increasing dosage.

In another NTP study (2001) groups of male and female $B6C3F_1$ mice (50 animals/sex/group) were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water for two years. Survival of exposed groups was similar to that of the controls (39/50, 45/50, 42/50 and 39/50 for males at doses of 0, 60, 120 or 220 mg/kg bw/day,

respectively and 40/50, 34/50, 37/50 and 41/50 for females at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively). Mean body weights of 165 mg/kg bw/day females were less than those of the controls throughout the study. Exposed groups generally consumed less water than the control groups. At 12 months, no significant increase in methaemoglobin level was observed in either sex at any dose.

In a two year study, groups of male rats (eight animals/group) received drinking water containing 0, 100, 1000, 2000 or 3000 mg sodium nitrite/L (equivalent to approximately 0, 10, 100, 250 or 350 mg/kg bw/day, respectively) [Shuval and Gruener, 1972]. There were no significant differences in growth, development, mortality or total haemoglobin levels between the control and treated groups. However, the methaemoglobin levels in the groups receiving 100, 250 and 350 mg/kg bw/day sodium nitrite were raised significantly throughout the study and averaged 5, 12 and 22% of total haemoglobin, respectively. The main histopathological changes occurred in the lungs and heart. Focal degeneration and fibrosis of the heart muscle were observed in animals receiving the highest dose of nitrite. The coronary arteries were thin and dilated in these aged animals, instead of thickened and narrow as is usually seen at that age. Changes in the lungs consisted of dilatation of the bronchi with infiltration of lymphocytes and alveolar hyperinflation. Such changes were observed in rats receiving 100, 250 and 350 mg/kg bw/day sodium nitrite.

Table 4 Oral repeated dose toxicity

Species /strain	Dose level	Exposure Time	Effects observed	LO(A)EL/NO(A)EL/MTD	Reference
Rat (male, and female), F344	0, 375, 750, 1,500, 3,000, 5,000 ppm in drinking water (0, 30, 55, 115, 200 or 300 mg/kg bw/day in males; 0, 40, 80, 130, 225 or 345 mg/kg bw/day in females)	14 weeks	Methaemoglobin formation Males: decreased sperm motility Females: increased weight kidney & spleen	NOAEL : not obtained (all showed methaemoglobin formation) LOAEL: Males = 115 mg/kg bw/day Females = 225 mg/kg bw/day	NTP, 2001
Mouse (male and female), $B6C3F_1$	0, 375, 750, 1,500, 3,000, 5,000 ppm in drinking water (0, 90, 190, 345, 750 or 990 mg/kg bw/day in males; 0, 120, 240, 445, 840 or 1230 mg/kg bw/day in females)	14 weeks	Males: extramedullary haematopoiesis in spleen, degeneration of testis Females: extramedullary haematopoiesis in spleen	LOAEL: Males = 750 mg/kg bw/day Females = 445 mg/kg bw/day	NTP, 2001
Rat (male and female), F344/N	0, 750, 1,500, 3,000 ppm in drinking water (0, 35, 70, 130 mg/kg bw/day in males; 0, 40, 80, 150 mg/kg bw/day in females)	2 years	None	NOAEL Males = 130 mg/kg bw/day Females = 150 mg/kg bw/day	NTP, 2001

Species /strain	Dose level	Exposure Time	Effects observed	LO(A)EL/NO(A)EL/MTD	Reference
Mouse(male and female), $B6C3F_1$	0, 750, 1,500, 3,000 ppm in drinking water (0, 60, 120, 220 mg/kg bw/day in males; 0, 45, 90, 165 mg/kg bw/day in females)	2 years	None	NOAEL Males = 220 mg/kg bw/day Females = 165 mg/kg bw/day	NTP, 2001
Rat (male)	0, 100, 1,000, 2,000, 3,000 mg/L in drinking water (0, 10, 100, 250, or 350 mg/kg bw/day	24 months	Histological changes in the lung and heart	NOEL : 10 mg/kg bw/day (equivalent to 6.7 mg NO_2/kg bw/day)	Shuval and Gruener, 1972
Rat (male and female) F344	0, 500, 1,250, 2,500, 5,000, 10,000 ppm in drinking water	42 days	Mortality/methae moglobin formation	MTD: 2,500 ppm	Maekawa *et al.*, 1982
Rat (male), Sprague Dawley	0, 2,000 ppm in drinking water	14 months	Lower body and liver weights and plasma vitamin E levels, higher GSH levels and higher incidence of pulmonary lesions. Methaemoglobin formation	LOEL: 2000 ppm	Chow *et al*., 1980
Rat (male), Sprague Dawley	0, 200 ppm in drinking water	16 weeks	Methaemoglobin formation (minimal)	LOEL : 200 ppm	Chow *et al*., 1980
Rat (male), Wistar (Riv:TOX)	Control (36 mmol/L KCl), 3.6, 12, 36 mmol/L KNO_2 in drinking water	90 days	Hypertrophy of adrenal zona glommerulosa	LOEL: 12 mmol/L KNO_2 (equivalent to 54 mg NO_2/kg bw/day)	Boink *et al*., 1996
Rat (male and female), Wistar (Bor; WISW)	0, 100, 300, 1,000, 3,000 mg/L KNO_2 in drinking water	90 days	Hypertrophy of adrenal zona glommerulosa	LOEL: 100 mg KNO_2/L (equivalent to 5.4 mg NO_2/kg bw/day)	Til *et al*., 1988

Studies in Humans

Oral

In U.S.A, 320 cases of infant methaemoglobinaemia have been reported to be associated with the use of nitrate-containing (converted to nitrite de novo) well water [NIEHS, 1970]. Other cases, particular in Europe, have been associated with the consumption of high nitrite containing vegetables, particularly spinach [Sander and Jacobi, 1967; NIEHS, 1970; Hack and Dowes, 1983].

Conclusion

The NOAELs were not determined in the rat 14-week repeated dose toxicity study as all treated groups showed elevated methaemoglobin concentrations. The LOAELs for other endpoints were 225 mg/kg bw/day in females (increased relative weight of kidney and spleen) and 115 mg/kg bw/day in males (decreased sperm motility). The LOAELs in the mouse 14-week repeated dose

toxicity study were 445 mg/kg bw/day in females (extramedullary haematopoiesis in the spleen) and 750 mg/kg bw/day in males (extramedullary haematopoiesis in the spleen and degeneration of the testis). Based on the two-year NTP studies, the NOAELs for rats were 130 mg/kg bw/day in males and 150 mg/kg bw/day in females. For mice the NOAELs were 220 mg/kg bw/day in males and 165 mg/kg bw/day in females.

In a two-year study in male rats the NOEL was 10 mg/kg bw/day (Focal degeneration and fibrosis of the heart, dilatation of the bronchi with infiltration of lymphocytes and alveolar hyperinflation in lungs) equivalent to 6.7 mg NO_2/kg bw/day. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily nitrite intake of 0 to 0.07 mg NO_2/kg bw/day by applying a safety factor of 100 to this NOEL.

3.1.6 Mutagenicity

A variety of reliable mutagenicity tests on sodium nitrite are available (Table 5, 6). Sodium nitrite is mutagenic and clastogenic *in vitro*. There are, however, conflicting reports regarding genotoxicity *in vivo*. Although the important feature of this substance for genotoxicity is the formation of nitrosamines or nitrosamides by reaction with secondary amines or amides, repsectively, such issues are beyond the scope of this document and will be reported elsewhere. Therefore, the results on mutagenicity of this substance alone *in vitro* and *in vivo* are summarized below.

Studies in Animals

In vitro Studies

Positive results have been reported for sodium nitrite, with and without metabolic activation (S9 mix), in Salmonella gene mutation studies with strains that revert by base-pair substitution [Ehrenberg *et al*., 1980; Katz *et al*., 1980; Ishidate *et al*., 1984; Brams *et al*., 1987; Zeiger *et al*., 1992; Balimandawa *et al*., 1994]. Typically, Salmonella Mutagenicity Tests conducted by NTP showed that there was positive response in TA100 at doses of 6,666, 10,000 ug/plate without metabolic activation, while negative response in TA98 [Zeiger *et al*., 1992]. Positive responses were obtained by using TA100, TA1530 and TA1535 (base-pair substitution) at doses of 1,000, 2,500 and 5,000 mg/plate, whereas TA102 (base-pair substitution), YG1024, DG400 and DJ460 (frameshift mutation) were inactive with and without metabolic activation [Balimandawa *et al.,* 1994]. Forward mutation [Kosako and Nishioka, 1982] and DNA damage [De Flora *et al*., 1984] were also observed in *Escherichia coli* tester strains after exposure to sodium nitrite in the presence of S9 mix. Furthermore, sodium nitrite-induced gene mutations were reported in *Saccharomyces cerevisiae* [Fahrig, 1979].

Sodium nitrite was reported to induce gene mutations, chromosomal aberrations and sister chromatid exchanges in cultured mammalian cells.

Tsuda *et al* (1973) reported that addition of sodium nitrite (0.05 or 0.1 mol/L) to cell cultures obtained from newborn Syrian hamsters resulted in transformation after 21 days. Ishidate & Odashima (1977) reported that sodium nitrite induced chromosomal aberrations in Chinese hamster lung cells (CHL) when tested at doses up to 0.53 mg/mL (the 50% growth inhibition dose) without exogenous metabolic activation. Tsuda *et al* (1981) also reported a significant increase in the incidence of chromosomal aberrations in Chinese hamster V79-H3 cells treated with 50 or 100 mmol/L of sodium nitrite without exogenous metabolic activation. Tsuda & Kato (1977) exposed Syrian hamster embryo cells to sodium nitrite (0, 5, 10, 20, 30, 50 mmol/L) without exogenous metabolic activation. Significant, dose-dependant increases in chromosomal aberrations were observed. Abe & Sasaki (1977) treated Chinese hamster D-6 cells with sodium nitrite (1 or 3 mmol/L) without exogenous metabolic activation. Dose-dependant increases in the number of chromosomal aberrations and sister chromatid exchanges were observed. Tsuda *et al* (1981) also

reported a significant increase in sister chromatid exchanges in Chinese hamster V79-H3 cells treated with 50 or 100 mmol/L of sodium nitrite without exogenous metabolic activation. HeLa S3 cells incubated for 1 to 36 hours had increased levels of unscheduled DNA synthesis (DNA repair) at concentrations above 1 mmol/L sodium nitrite [Lynch *et al*., 1983].

In vivo Studies

Inui *et al* (1979b) treated Syrian golden hamster embryos *in utero* by dosing the pregnant females by gavage with 0, 125, 250 or 500 mg/kg aqueous sodium nitrite solution on the 11^{th} or 12^{th} day of pregnancy. The fetuses were excised 24 hours after dosing and cells cultured. Marked dose-dependant increases in micronucleus formation, induction of 8-azaguanine- and ouabain-resistant mutations and morphological or neoplastic transformations in the embryo cells were observed. However, there was no marked increase in the frequency of chromosomal aberrations.

El Nahas *et al* (1984) reported positive results for the induction of chromosomal aberrations in bone marrow cells of pregnant female albino rats exposed to 210 mg/kg bw/day in drinking water for 13 days. In this same study, the liver cells of embryos exposed trans- placentally for the first 13 days of gestation also showed increased numbers of chromosomal aberrations. In another study, SCE induction increased with increasing dose in bone marrow cells of Swiss albino mice treated with 2.5 to 200 mg/kg bw sodium nitrite by intraperitoneal injection [Giri *et al*., 1986].

NTP (2001) have performed several *in vivo* studies. In the first of these, sodium nitrite was administered by intraperitoneal injection at 0, 6.25, 12.5, 25, 50, 100 or 200 mg/kg bw to male F344/N rats three times at 24-hour intervals. 200 mg/kg was found to be the lethal dose. No significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in any of the dose groups. The initial trial was judged to be positive, based on the trend test (P=0.001); however, results of a repeat trial, in which doses of 0, 25 or 50 mg/kg bw were tested, were negative, and the rat bone marrow micronucleus test with sodium nitrite was judged to be negative overall. A similar study in which male $B6C3F_1$ mice were administered 0, 7.81, 15.63, 31.25, 65.5, 125 or 250 mg/kg bw also gave negative results. In the third *in vivo* study, a peripheral blood micronucleus test, male and female mice were administered 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite in drinking water for 14 weeks. The equivalent average daily doses were approximately 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1,230 mg/kg bw/day in females. There was no significant increase in the frequency of micronucleated normochromatic erythrocytes in either males or females.

Diaz-Barriga Arceo *et al* (2002) administered 0, 10, 15 or 20 mg/kg of sodium nitrite orally four times at 24-hour intervals and examined peripheral blood samples after 96 hours. The treated animals showed a statistically significant increase (p=0.05) in micronucleated polychromatic erythrocytes at all doses compared with the 0h values (MNPCE/1000 PCE = 1.2+/-0.58 (0h), 4.8+/-0.37 (96h) at 10 mg/kg; 0.4+/-0.24 (0h), 5.0+/-0.31 (96h) at 15 mg/kg; and 1.0+/-0.31 (0h), 5.8+/-0.85 (96h) at 20 mg/kg). No statistical differences were observed in the PE/NE ratio when their results were compared before and after treatment, suggesting that sodium nitrite produced no significant influence on normal bone marrow activity in this study.

Conclusion

Sodium nitrite is a direct-acting, base-pair substitution mutagen in organisms ranging from bacteria to mammalian cells *in vitro*. This substance induced chromosomal aberrations in mammalian cells *in vitro*. There is evidence of potential *in vivo* genotoxicity.

Table 5 Genotoxicity studies in vitro

Type of test	Test system	Dose	Result	Reference
Bacterial test (reverse mutation)	*S.typhymurium* TA1535, TA1537, TA92, TA94, TA98, TA100	1,000-10,000ug/plate (TA1535, TA100), 10-5,000ug/plate (TA1537, TA92, TA94, TA98)	Positive with and without metabolic activation (TA1535, TA100)	Ishidate *et al.,* 1984
Bacterial test (reverse mutation)	*S.typhymurium* TA1535, TA1537, TA98, TA100	25-2500ug/plate	Positive with and without metabolic activation (TA1535, TA100)	Katz *et al.,* 1980
Bacterial test (reverse mutation)	*S.typhymurium* TA1530,TA1535, TA1538, TA100, TA102, YG1024, DG400, DJ460	1-5mg/plate	Positive with and without metabolic activation (TA1530, TA1535, TA100)	Balimandawa *et al.,* 1994
Bacterial test (reverse mutation)	*S.typhymurium* TA97, TA98, TA100	100-1000ug/plate	Positive with metabolic activation (TA100)	Brams *et al.,* 1987
Bacterial test (forward mutation)	*Escherichia coli* WPuvrA/pKM101	1,000-10,000ug/plate	Positive with and without metabolic activation	Kosako & Nishioka, 1982
Bacterial test (reverse mutation)	*S.typhymurium*, TA98	23.4-375ug/plate	Positive with metabolic activation	Ehrenberg *et al.,* 1980
Bacterial test (reverse mutation)	*S.typhymurium*, TA98, TA100	100-10,000ug/plate	Positive with and without metabolic activation	Zeiger *et al.*, 1992
Bacterial test (DNA repair test)	*Escherichia coli* WP2, WP67, CM871	MIC without S9 mix (ug): WP2; 2500, WP67; 2500, CM871; 625 MIC with S9 mix (ug): WP2; 3000, WP67; 3000, CM871; 1250	Positive with and without metabolic activation	De Flora, *et al.,* 1984
Bacterial test (UV induced forward mutation)	*Saccharomyces cerevisiae* (diploid strain MPI)	0.058-0.43mM	Positive without metabolic activation	Fahrig 1979
Chromosomal aberration	C3H Mouse mammary carcinoma cell line EM3A	0.001-0.1mol/L	Positive without metabolic activation	Kodama *et al.,* 1976
Chromosomal aberration	Chinese hamster lung cell line	0.25-1.0mg/mL	Positive without metabolic activation	Ishidate *et al.,* 1977
Chromosomal aberration	Syrian hamster embryo cell	5-50mmol/L	Positive without metabolic activation	Tsuda & Kato, 1977
Chromosomal aberration & sister chromatid exchange	Chinese hamster cell line V79-H3	10-100mmol/L	Positive without metabolic activation	Tsuda *et al.,* 1981
Chromosomal aberration & sister chromatid exchange	Chinese hamster cell line D-6	0.001-0.003M	Positive without metabolic activation	Abe & Sasaki, 1977

Type of test	Test system	Dose	Result	Reference
Sister chromatid exchange	Human peripheral blood lymphocyte	0.003-0.03mol/L	Positive without metabolic activation	Inoue *et al.,* 1985
DNA repair assay	HelaS3 Carcinoma cell	0.0000001-0.006M	Positive without metabolic activation	Lynch *et al.,* 1983

Table 6 Genotoxicity studies in vivo

Type of test	Test system	Dose	Result	Reference
Micronucleus & chromosomal aberration test	Syrian golden hamster (embryonic cells)	125, 250, 500mg/kg bw (single gavage)	Positive: micronucleus test Negative: chromosomal aberration	Inui *et al.,* 1979b
Chromosomal aberration test	Albino pregnant rat (bone marrow & liver)	Mean 210mg/kg bw/day for 0-18 days (drinking water)	Positive: pregnant adult bone marrow & embryonic liver	El Nahas *et al.,* 1984
Sister chromatid exchange test (bone marrow)	Swiss mouse	2.5-200mg/kg bw (single *ip* injection)	Positive	Giri *et al.,* 1986
Heritable translocation assay	C3H Mouse (male)	60, 120mg/kg bw/ day for 14 days	Negative: no heritable defects in F1 germ cells	Alavantic *et al.,* 1988
MN Test (bone marrow)	F344/N Rat (male)	6.25-200mg/kg bw (three times at 24-hour intervals, *ip*)	Negative	NTP, 2001
MN Test (bone marrow)	$B6C3F_1$ Mouse(male)	7.81-250mg/kg bw (three times at 24-hour intervals, *ip*)	Negative	NTP, 2001
MN Test (peripheral blood)	$B6C3F_1$ Mouse(male & female)	90-900mg/kg bw (male) 120-1,230mg/kg bw (female) (drinking water, 14-week)	Negative	NTP, 2001
MN Test (peripheral blood)	Mouse (NIH, male)	10-20mg/kg bw (four times at 24-hour intervals, *po*)	Positive	Diaz-Barriga Arcero *et al.,* 2002
8-Azaguanine-resistant mutation & neoplastic transformation assay	Syrian golden hamster (embryonic cells)	25, 50, 100mg/kg bw (single gavage)	Positive	Inui *et al.,* 1979a

3.1.7 Carcinogenicity

The possibility of carcinogenicity of nitrite and/or nitrate associated with endogenous formation of N-nitroso compounds has been investigated. It has been shown in several controlled laboratory studies that, when both nitrite and N-nitrosable compounds are present together at high level, N-nitroso compounds are formed endogenously. The committee of JECFA's 44th meeting, however,

noted that quantitative data were available only on N-nitrosocompounds which are readily formed endogenously, such as N-nitrosoproline, which is not carcinogenic. One long term study of toxicity and carcinogenicity was recently conducted, where rats were fed with fish meal concomitantly with sodium nitrite [Furukawa *et al*., 2000]. Dose (fish meal)-related increase in incidences and multiplicity of atypical renal tubules, adenomas and renal cell carcinomas were found. However the diets used in this study were nutritionally inappropriate and the study does not provide additional insight for the safety evaluation of sodium nitrite [WHO, 2004]. Therefore, the safety evaluation should be based on the toxicity study on nitrite.

In vivo Studies in Animals

Reliable studies on carcinogenic potential of sodium nitrite are summarized in Table 7.

In a NTP study (2001) groups of male and female F344/N rats (50 animals/sex/group) were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day for males and 0, 40, 80 or 150 mg/kg bw/day for females) in drinking water for two years. Survival of exposed groups was similar to that of the controls (29/50, 38/50, 36/50 and 36/50 for males at doses of 0, 35, 70 and 130 mg/kg bw/day, respectively and 33/50, 31/50, 36/50 and 33/50 for females at 0, 40, 80 or 150 mg/kg bw/day, respectively). Mean body weights of 130 mg/kg bw/day males and 150 mg/kg bw/day females were less than those of the controls throughout the study. Water consumption by high dose males and females was less than that by the controls throughout the study and that by the other exposed groups was generally less after week 14 The incidences of hyperplasia of the forestomach epithelium in 130 mg/kg bw/day males (44/50) and 150 mg/kg bw/day females (40/50) were significantly greater than those in the control groups (12/50 males, 8/50 females). The incidence of fibroadenoma of the mammary gland was significantly increased in 80 mg/kg bw/day females, and the incidences of multiple fibroadenoma were increased in 40 and 80 mg/kg bw/day females; however these neoplasms occur with a high background incidence and no increase was seen in the 150 mg/kg bw/day group. The incidences of mononuclear cell leukemia were significantly decreased in 70 or 130 mg/kg bw/day males (7/50 and 3/50, respectively) and 80 or 150 mg/kg bw/day females (1/50 and 1/50, respectively) compared with controls (17/50 males, 15/50 females). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in F344/N rats exposed at up to 3000 ppm (approximately 130 mg/kg bw/day in males and 150 mg/kg bw/day in females) in drinking water over a two year period.

In another NTP study (2001) groups of male and female $B6C3F_1$ mice (50 animals/sex/group) were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water for two years. Survival of exposed groups was similar to that of the controls (39/50, 45/50, 42/50 and 39/50 for males at doses of 0, 60, 120 or 220 mg/kg bw/day, respectively and 40/50, 34/50, 37/50 and 41/50 for females at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively). Mean body weights of 165 mg/kg bw/day females were less than those of the controls throughout the study. Exposed groups generally consumed less water than the control groups. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a positive trend (1/50, 0/50, 1/50 and 5/50 at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively). The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in 220 mg/kg bw/day males (10/50) than in the controls (0/50). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in male $B6C3F_1$ mice exposed at up to 3000 ppm (approximately 220 mg/kg bw/day) in drinking water over a two year period. There is equivocal evidence of carcinogenic activity in female mice, based on the positive trend of squamous cell papilloma or carcinoma (combined) in the forestomach.

In a third study, groups of male and female F-344 rats (50 animals/sex/group) were given 20 mL of

0, 0.125 or 0.250% sodium nitrite/rat/day in their drinking water for two years. There were no significant differences in the incidence of tumors between control and test groups, apart from a lower incidence of mononuclear cell leukaemia amongst the test groups compared with controls. This was attributed to slight atrophy of the haematopoietic organs [Maekawa et al, 1982].

Although some findings regarded as equivocal, result is conclusively understood as lack of carcinogenicity of this substance (the view is consistent with that of WHO, 2004]).

There were a further two studies that employed multiple doses with control groups of both sexes, equal to or more than 104 week of test period and more than 50 animals per group. These studies were performed using methods regarded as similar to OECD guidelines. Despite lack of GLP information, they are subjected to evaluation [Gruener and Shuval. *et al*., 1973; Taylor and Lijinsky, 1975]. Neither of these studies showed increased tumor incidences in treated animals compared to the controls.

Due to the tremendous amount of related information, it would be useful to refer the conclusion of authoritative review for the studies, except for NTP studies. An expert US Committee [NAS, 1981] reviewed about 20 studies on nitrite that could be used to evaluate carcinogenic potential. Several of the studies reviewed, although limited, did involve chronic administration of high levels of nitrite to large groups of animals. The committee concluded that these studies gave no indication that nitrite was carcinogenic to rats, mice or guinea pigs.

Table 7 Carcinogenicity Studies

Species /strain	Dose level	Exposure Time	Results	Reference
Rat (male and female), F344/N	0, 750, 1,500, 3,000 ppm in drinking water	2 years	Non-neoplastic effects : epithelial hyperplasia of forestomach (male and female) Neoplastic effects : none	NTP, 2001
Mouse(male and female), $B6C3F_1$	0, 750, 1,500, 3,000 ppm in drinking water	2 years	Non-neoplastic effects : epithelial hyperplasia of glandular stomach (males) Neoplastic effects : Positive trend in squamous cell papilloma or carcinoma (combined) in forestomach (females)	NTP, 2001
Rat (male and female), F 344/N	0, 0.125, 0.25% in drinking water	104 weeks + 16 weeks	Lower incidence of mononuclear cell leukaemia amongst the test groups compared with controls	Maekawa *et al.*, 1982

Studies in Humans

No adequate epidemiology studies of sodium nitrite and human cancer were found in literature.

Conclusion

There is no evidence of carcinogenic activity of sodium nitrite in F344/N rats exposed at up to 3000 ppm (approximately 130 mg/kg bw/day in males and 150 mg/kg bw/day in females) in drinking water over a two year period. There is no evidence of carcinogenic activity of sodium nitrite in male $B6C3F_1$ mice exposed at up to 3000 ppm (approximately 220 mg/kg bw/day) in drinking water over a two year period. There is equivocal evidence of carcinogenic activity in female mice, based on the positive trend of squamous cell papilloma or carcinoma (combined) in the forestomach.

NTP carcinogenicity studies concluded that there was no evidence of carcinogenic activity of sodium nitrite in rats or mice except equivocal findings of epitherial hyperplasia, papiloma or carcinoma of forestomach or glandular stomach. Overall evaluation leads to the conclusion that nitrite ingestion via drinking water did not show evident carcinogenicity. This NTP view is consistent with that of the recent view of WHO [WHO, 2004].

3.1.8 Toxicity for Reproduction

There is evidence for transfer of sodium nitrite to foetuses in rats and mice. There were no studies available for reproductive and developmental toxicity study of sodium nitrite under standard protocols in rats. There are four studies in mice under the protocol inferable similarity to standard method. Pre- and postnatal exposure to sodium nitrite had adverse effects on haematological parameters, including dose-dependent decreases in Hb content, RBC counts, and MVC value in mice offspring. Guinea pig was used to investigate the maternal effect on offspring, because those parameters of the mother guinea pig are susceptible to nitrite administration and eased by co-administration of ascorbic acid.

Studies in Animals

Effects on Reproduction

At a dose of 0.31 g/kg bw in drinking water for the whole gestation period, sodium nitrite did not affect reproductive parameters of female C57BL/6 mice [Anderson et al., 1985]. Pregnant Swiss CD-1 mice given sodium nitrite up to 0.24% in drinking water caused reduced maternal water consumption, but not body weight gain [Chapin and Sloane, 1997]. In the comprehensive continuous breeding study of sodium nitrite in accordance with NTP protocol, reproductive success in the F1 generation was not affected (0.24 %, approximately 425 mg/kg bw/day). Oral intubation of sodium nitrite on day 13 of gestation up to 120 mg/kg bw/day had no effect on dams [Khera, 1982]. Reproductive performance, such as parental weight gain, food consumption, mortality, fertility, pregnancy maintenance or gestation length was not affected in male or female rats given sodium nitrite at 0.0125, 0.025 and 0.05% (10.75, 21.5 and 43 mg/kg bw/day) in feed before and during breeding [Vorhees et al., 1984]. The NOAEL of reproduction was considered to be 43 mg/kg bw/day. In general, available studies range over a wide diversity of protocols and endpoints and not employing standard study designs. This diversity limits the extent to which general comparison can be made among studies.

Sodium nitrite (60 mg/kg bw/day), in drinking water, administered to pregnant guinea pigs produced maternal anemia and increase in the incidences of abortion and fetal mortality [Sinha and Sleight, 1971]. In guinea pigs, administration of 45 mg sodium nitrite/kg bw/day by s.c. injection during the last week of gestation resulted in abortion in ascorbic acid-deficient females. Neither ascorbic acid deficiency alone, nor sodium nitrate in the presence of sufficient ascorbic acid, was associated with excess abortions. Authors concluded that maternal anemia was the causative agent for reproductive toxicity. The LOAEL for reproductive toxicity was considered to be 60 mg/kg bw/day.

Developmental Toxicity

Sodium nitrite administered by oral intubation to pregnant mice from 0 to 14, 16, or 18 days of gestation (0.5 mg/mouse per day) did not cause embryonic/fetal mortality, changes in fetal weight or increased incidence of skeletal malformations. Sodium nitrite caused fetal hepatic erythropoiesis, probably related to fetal methaemoglobinaemia [Globus and Samuel, 1978]. The comprehensive and continuous breeding study of sodium nitrite was conducted in accordance with NTP protocol [Chapin and Sloane, 1997]. The sodium nitrite (0.06, 0.12, 0.24% in drinking water) did not affect the number of litters per pair, the number of pups per litter, or the viability or weight

of pups (125, 260, 425 mg/kg bw/day) and no adverse effect on reproductive performance or necropsy endpoint were observed. The NOAEL is estimated to be 425 mg/kg bw/day.

Increases in fetal and pup mortality and decreases in body weight of preweaning pups were observed in rat given, for whole gestation period and 90 days post natal, diet containing sodium nitrite at 0.025% and 0.05% (21.5, 43 mg/kg bw/day), but not at 0.0125% (10.75 mg/kg bw/day) [Vorhees *et al.*, 1984]. Thus, the NOAEL is considered to be 10.75 mg/kg bw/day.

In addition, oral intubation of sodium nitrite on day 13 of gestation up to 120 mg/kg bw/day had no effect on dams or pups [Khera, 1982].

No gross abnormalities were noted in any live or aborted fetuses [Kociba and Sleight, 1970]. Co-administration of methylene blue, a MetHb antagonist, exerted a protective effect on maternal anemia and fetal development.

The following studies are systematic and therefore noteworthy.

Pregnant Wistar rats were given drinking water containing sodium nitrite at 2 g/L from gestation day 13 until parturition [Nyakas *et al.*, 1990, 1994a, 1994b]. Simple learning in response to either reward or aversive stimulus was not affected in two-month old male offspring. Discriminatory learning of both visual and auditory cues, however, was impaired in the treated animals, as of long term retention of a conditioned passive avoidance response up to 24 month old. The authors related the observation to higher organ weight of adrenal and prenatal hypoxia, leading to retarded development of certain neurotransmitter pathways [Nyakas *et al.*, 1994a, 1994b]. These studies were performed in rats of single dose group and did not show the number of dams or dam assignment of tested pups. No confirmative or supporting study has appeared thereafter.

Studies in Humans

Effects on Fertility

No studies in humans that evaluated directly the potential for prenatal exposure to sodium nitrite to cause adverse effects on fetal viability, growth, morphology or functional parameters were available.

Conclusion

The NOAEL of reproduction in mice was 425 mg/kg bw/day in drinking water and the NOAEL in rats was 43 mg/kg bw/day in diet. The LOAEL in guinea pigs was 60 mg/kg bw/day in drinking water. Increase in mortality of pre- and postnatal offspring and decrease in body weight of preweaning pups were observed in rat dams given a diet containing sodium nitrite at 0.025% to 0.5%. Sodium nitrite caused maternal anaemia and increase in the incidence of abortion and fetal mortality when administered to pregnant guinea pigs in drinking water at 60 mg/kg bw/day.

3.2 Initial Assessment for Human Health

Sodium nitrite has been reviewed by a number of international organizations: JECFA (Joint FAO/WHO Expert Committee on Food Additives); National Academy of Sciences (NAS); US National Institute of Environmental Health Sciences (NIEHS); National Institute of Public Health and the Environmental Hygiene, Netherlands; US National Toxicology Program (NTP); and California EPA (CAL/EPA).

Nitrite in blood is highly reactive with haemoglobin and causes methaemoglobinaemia. Ferrous iron associated with haemoglobin is oxidized by nitrite to ferric iron, leading to the formation of methaemoglobin. Humans are considered to be more sensitive than rats in this respect.

The primary acute effect of sodium nitrite in rats and mice is methaemoglobinaemia. Methaemoglobin concentrations in SD rats increased to 45% to 80% over 1 hour after an oral dose of sodium nitrite at 150 mg/kg bw, and they returned to normal levels within 24 hours in surviving rats.

LD50 values by gavage are 214 mg/kg bw (males) and 216 mg/kg bw (females) in mice. In an acute inhalation study (which could not be validated) methaemoglobin levels in female rats were significantly increased after 4 hours exposure to 10 mg/m3 sodium nitrite. The increase was judged not to be haematologically significant. No significant increase was observed in exposed males. There were no toxicologically significant effects on animals maintained for 14 days post exposure. No information on acute dermal toxicity is available.

Sodium nitrite is a moderate eye irritant, but is non-irritant to skin in rabbits. No studies are available investigating the sensitising potential of sodium nitrite in animals. No cases of sensitisation have been reported in humans.

In a repeated dose toxicity study [NTP] male and female F344/N rats were exposed to 0, 375, 750, 1500, 3000 or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 30, 55, 115, 200, or 310 mg/kg bw/day in males and 0, 40, 80, 130, 225, or 345 mg/kg bw/day in females) in drinking water for 14 weeks. Methaemoglobin levels were significantly elevated in all treated groups compared to the controls by the end of the treatment period. For males, mean methaemoglobin levels after 14 weeks were 0.03±0.01, 0.08±0.01, 0.12±0.02, 0.25±0.07, 0.71±0.20 and 3.38±0.80 g/dL at doses of 0, 30, 55, 115, 200, and 310 mg/kg bw/day. For females, mean methaemoglobin levels after 14 weeks were 0.06±0.02, 0.14±0.02, 0.16±0.02, 0.48±0.05, 0.99±0.20 and 2.27±0.54 g/dL at doses of 0, 40, 80, 130, 225 and 345 mg/kg bw/day. The NOAELs were not determined (increased methaemoglobinaemia). The LOAELs for other endpoints were 225 mg/kg bw/day (increased relative weight of the kidney and spleen) in females and 115 mg/kg bw/day (decreased sperm motility) in males.

In a second 14-week repeated dose toxicity study [NTP] male and female B6C3F1 mice were exposed to 0, 375, 750, 1500, 3000 or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1230 mg/kg bw/day in females) in drinking water. Methaemoglobin levels were not reported however there were no clinical signs of toxicity. The LOAELs were 445 mg/kg bw/day (extramedullary hematopoiesis in the spleen) in females and 750 mg/kg bw/day (extramedullary haematopoiesis in the spleen, degeneration of the testis) in males.

In a two-year chronic toxicity/carcinogenicity study [NTP] male and female F344/N rats were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day in males and 0, 40, 80 or 150 mg/kg bw/day in females) in drinking water. There were no clinical findings related to exposure. Methaemoglobin levels were measured at two weeks and three months. At both 2 weeks and three months, methaemoglobin levels were high at night when the rats were actively feeding and drinking and low during the day when the rats were less active. Methaemoglobin levels tended to increase with increasing dosage.

In a second two-year study [NTP] male and female B6C3F1 mice were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water. There were no clinical findings related to exposure. At 12 months, no significant increase in methaemoglobin level was observed in either sex at any dose.

Based on the NTP two-year studies, the NOAELs for rats were 130 mg/kg bw/day in males and 150 mg/kg bw/day in females. For mice the NOAELs were 220 mg/kg bw/day in males and 165 mg/kg bw/day in females.

In a two-year study in male rats the NOEL was 10 mg/kg bw/day, equivalent to 6.7 mg NO_2/kg bw/day. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily nitrite intake of 0 to 0.07 mg NO_2/kg bw/day by applying a safety factor of 100 to this NOEL.

Sodium nitrite is a direct-acting, base-pair substitution mutagen in organisms ranging from bacteria to mammalian cells in vitro. This substance induced chromosomal aberrations in mammalian cells in vitro. There is evidence of potential in vivo genotoxicity. The substance tested positive in a micronucleus test (peripheral blood) when mice were dosed by gavage at 10 – 20 mg/kg bw (4 times at 24 hrs intervals) but was negative in a second study where mice were dosed via drinking water at dosed up to 900 mg/kg bw/day (females) for 14 weeks. In a chromosomal aberration test, pregnant rats were dosed with 210 mg/kg bw/day for 13 days. Positive results for the induction of chromosomal aberrations in bone marrow of the parents and liver cells of embryos were reported.

In a two-year chronic toxicity/carcinogenicity study [NTP] male and female F344/N rats were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day for males and 0, 40, 80 or 150 mg/kg bw/day for females) in drinking water. The incidences of hyperplasia of the forestomach epithelium in high dose males (44/50) and females (40/50) were significantly greater than those in the control groups (12/50 males, 8/50 females). The incidence of fibroadenoma of the mammary gland was significantly increased in 80 mg/kg bw/day females, and the incidences of multiple fibroadenoma were increased in 40 and 80 mg/kg bw/day females; however these neoplasms occur with a high background incidence and no increase was seen in the high dose group. The incidences of mononuclear cell leukemia were significantly decreased in 70 and 130 mg/kg bw/day males (7/50 and 3/50, respectively) and 80 and 150 mg/kg bw/day females (1/50 and 1/50, respectively) compared with controls (17/50 males, 15/50 females). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in F344/N rats at approximate doses of up to 130 mg/kg bw/day in males and 150 mg/kg bw/day in females over a two year period.

In another NTP study male and female B6C3F1 mice were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females) in drinking water for two years. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a positive trend (1/50, 0/50, 1/50 and 5/50 at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively). The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in 220 mg/kg bw/day males (10/50) than in the controls (0/50). Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in male B6C3F1 mice at doses up to approximately 220 mg/kg bw/day over a two year period. There is equivocal evidence of carcinogenic activity in female mice, based on the positive trend of squamous cell papilloma or carcinoma (combined) in the forestomach.

Various other carcinogenicity studies in rats were negative. Moreover, some even showed a reduction in tumor risk (e.g. lymphoma or leukemia). WHO concluded that there was no evidence of carcinogenic activity of sodium nitrite in rats and mice based on the findings of NTP carcinogenicity studies.

There is evidence for transfer of sodium nitrite to fetuses in rats and mice. Reproductive success in the F1 generation was not affected. Increase in mortality of pre- and postnatal offspring and decrease in body weight of preweaning pups were observed in rat dams given a diet containing

sodium nitrite at 0.0125% (10.75 mg/kg bw/day), 0.025% (21.5 mg/kg bw/day) and 0.05% (43 mg/kg bw/day), and the NOAEL is considered to be 10.75 mg/kg bw/day. Reproductive toxicity by continuous breeding in the mice was conducted with drinking water at doses of 125, 260 and 425 mg/kg bw/day, and no adverse effect on reproductive performance or necropsy endpoint were observed. The NOAEL is estimated to be 425 mg/kg bw/day. Sodium nitrite caused maternal anemia and the incidence of abortion and fetal mortality increased when administered to pregnant guinea pigs in drinking water and LOAEL is considered to be at 60 mg/kg bw/day.

From the weight of evidence, sodium nitrite appears to affect erythropoiesis, hematological parameters and brain development resulting in mortality and poor growth of offspring.

In humans, sodium nitrite causes smooth muscle relaxation, methaemoglobinemia, and cyanosis. Infants are particularly sensitive. A large proportion of haemoglobin in infants is in the foetal haemoglobin form, which is more readily oxidised to methaemoglobin than adult haemoglobin. Further, reduced nicotinamide-adenine dinucleotide (NADH)-dependent methaemoglobin reductase, the enzyme responsible for reduction of methaemoglobin back to normal haemoglobin, has only about half the activity present in adults.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The reliable toxicity data of aquatic organisms are summarized in Tables 8 to 11. (The values were converted from NO_2-N into $NaNO_2$ in those cases where the concentrations were represented as NO_2-N in the original reports).

Acute Toxicity Test Results

Fish

A large number of reliable acute toxicity tests for fish are reported in the literature (Table 8). The LC_{50} values obtained vary widely between the species tested; LC_{50} (96h) = 0.54 mg $NaNO_2$/L for *Oncorhynchus mykiss* [Russo et al, 1981]; LC_{50} (96h) = 35 mg $NaNO_2$/L for *Ictalurus punctatus*, LC_{50} (96h) = 691.0 mg $NaNO_2$/L for *Micropterus salmoides* [Palachek & Tomasso, 1984]; and LC_{50} (96h) = 1010.4 mg $NaNO_2$/L for *Anguilla japonica* [Yamagata & Niwa, 1979]. The reason for this difference has been attributed to the ability of certain species, such as eels, bass and sunfish to prevent nitrite from crossing the gill membrane and entering the blood, whilst other species such as rainbow trout concentrate nitrite in their blood [Palachek & Tomasso, 1984]. The range of toxicity values reported for some species of fish varies widely and is believed to be dependant on the quality of the water used in the test with pH, chloride and calcium ion concentration all having an influence. In particular, chloride ion concentration has been shown to be important, with increasing concentrations leading to a decrease in the toxicity of nitrite [for example, Russo et al, 1981].

Invertebrates

The reliable data available for the acute toxicity of sodium nitrite to invertebrates are summarised in Table 9. As with fish, there is variation in toxicity between species. Sodium nitrite is toxic to invertebrates such as *Cherax quadricarinatus* (LC_{50} (96h) = 4.93 mg $NaNO_2$/L)[Rouse *et al*, 1995], whereas other species, such as *Procambarus clarkii* (LC_{50} (96h) = 42 mg $NaNO_2$/L) [Gutzmer & Tomasso, 1985] and *Penaeus paulensis* are much less sensitive (LC_{50} (96h) = 539.2 mg $NaNO_2$/L) [Cavalli *et al*, 1996]. Gutzmer & Tomasso (1985) reported that increased levels of chloride ions in the test water decreased the sensitivity of *Procambarus clarkii* to nitrite.

Algae

One reliable study is available. In an algal growth inhibition study [OECD TG 201], green alga (*Desmodesmus subspicatus,* formerly known as *Scenedesmus subspicatus*) was exposed under static conditions to sodium nitrite at nominal concentrations of 0 and 100 mg/L for 72 hours. The 72-h E_rC_{50} and the 72-h E_bC_{50} were > 100 mg/L [JAFA, 2005].

Chronic Toxicity Test Results

Fish

No studies available for this endpoint.

Invertebrates

Chen & Chen (1992) reported a NOEC (80 day, growth) of 9.86 mg $NaNO_2$/L for *Penaeus monodon* (jumbo tiger prawns).

Algae

One reliable study is available. In an algal growth inhibition study [OECD TG 201], green alga (*Desmodesmus subspicatus*) was exposed under static conditions to sodium nitrite at nominal concentrations of 0 and 100 mg/L for 72 hours. The NOEC was 100 mg/L for growth rate and biomass.

Toxicity to Microorganisms

Two reliable studies are available. Nalecz-Jawecki and Sawicki (1998) studied the effect of sodium nitrite on deformation (morphological changes such as shortening, bending of the cell, etc.) and mortality of Spirostomun ambiguum (protozoa). The 48-hour EC_{50} and LC_{50} were 421 and 533 mg/L (expressed as $NaNO_2$), respectively. Ostrensky and Lemos (1993) reported a 96h EC_{50} (mobility) of 7886 mg $NaNO_2$/L and 96h NOEC (mobility) of 3740 $NaNO_2$/L for *Tetraselmis chuii*.

Table 8 Summary of acute/prolonged toxicity of sodium nitrite on fish

Organism	Test duration	Result	Reference
Fish			
Rainbow trout, Donaldson trout (*Oncorhynchus mykiss*)	8 d (flow through)	LC_{50} = 0.69 - 1.92 mg/L*	Russo *et al*., 1974
	8 d (flow through)	LC_0 > 15.2 mg/L*	Rodriguez-Moreno & Tarazona, 1994
	6 d (flow through)	LC_0 > 69.7 mg/L	Stormer *et al.,* 1996
	96 h (static)	LC_{50} = 3.89 mg/L* LC_0 = 2.66 mg/L*	Buhl & Hamilton, 2000
	96 h (flow through)	LC_{50} = 27.7 mg/L*	Bortz, 1977
	96 h (flow through)	LC_{50} = 0.94 - 1.92 mg/L*	Russo *et al*., 1974
	96 h (flow through)	LC_{50} = 0.94 - 1.38 mg/L*	Russo and Thurston, 1977
	96 h (flow through)	LC_{50} = 0.54 - 26.3 mg/L*	Russo *et al*., 1981
	96 h (semistatic)	LC_{50} = 1.49 - 153.8 mg/L*	Wedemeyer & Yasutake, 1978
Cutthroat trout (*Oncorhynchus clarki*)	96 h (flow through) 11 d (flow through) 36 d (flow through)	LC_{50} = 2.73 mg/L* LC_{50} = 1.92 mg/L* LC_{50} = 1.82 mg/L*	Thurston *et al*., 1978
Fathead minnow (*Pimephales promelas*)	9 6 d (flow through)	LC_{50} =11.3 mg/L*	Russo & Thurston, 1977
Grass carp, white amur (*Ctenopharyngodon idella*)	120 h (static)	120-h LC_{50} = 7.39 mg/L*	Alcaraz & Espina, 1994
Channel catfish (*Ictalurus punctatus*)	96 h (static)	LC_{50} = 35.0 mg/L*	Palachek & Tomasso, 1984
	96 h (static)	LC_{50} = 7.55 mg/L	Konikoff, 1975
Tilapia (*Tilapia aurea*)	96 h (static)	LC_{50} = 79.8 mg/L*	Palachek & Tomasso, 1984
Flatfish (*Paralichtys orbignyanus*)	96 h (semistatic)	LC_{50} = 118.3 - 150.7 mg/L*	Bianchini *et al.,* 1996
Japanese eel (*Anguilla japonica*)	96 h (static)	LC_{50} = 1,010.4 mg/L*	Yamagata & Niwa, 1979
Largemouth bass (*Micropterus salmoides*)	96 h (static)	LC_{50} = 691.0 mg/L*	Palachek & Tomasso, 1984
Common eel (*Anguilla anguilla*)	96 h (semistatic)	LC_{50} = 708.2 mg/L*	Kamstra, 1996
	96 h (static)	LC_{50} = 739.3 mg/L*	Yamagata and Niwa, 1979
	50d (flow through)	LC_0 = 98.6 mg/L*	Kamstra, 1996

*: The values were converted from NO_2-N into $NaNO_2$.

Table 9 Summary of acute toxicity of sodium nitrite on invertebrates

Organism	Test duration	Result	Reference
Invertebrates			
Australian redclaw crayfish (*Cherax quadricarinatus*)	96 h (static)	LC_{50} = 4.93 mg/L*	Rouse *et al*., 1995
	96 h (semistatic)	LC_{50} = 126.7 mg/L*	Meade & Watts, 1995
Blue crab (*Callinectes sapidus*)	96 h (semistatic)	LC_{50} = 351.4 - 460.3 mg/L*	Ary & Poirrier, 1989
Fleshy prawn (*Penaeus chinensis*)	96 h (semistatic) 192 h (semistatic)	LC_{50} = 182.9 mg/L* LC_{50} = 113.1 mg/L*	Chen *et al*., 1990a
Giant Malaysian prawn (*Macrobrachium rosenbergi*)	96 h (semistatic) 192 h (semistatic)	LC_{50} = 42.4 mg/L* LC_{50} = 22.2 mg/L*	Armstrong *et al*., 1976
Greasyback shrimp (*Metapeaeus ensis*)	120 h (semistatic)	LC_{50} = 34.8 mg/L* NOEC = 3.5 mg/L*	Chen, & Nan, 1991
Hard clam (*Mercenaria mercenia*)	96 h (static)	EC_{50} = 1100 - 1200 mg/L	Epifanio & Srna, 1975
Jumbo tiger prawn (*Penaeus monodon*)	240 h (semistatic)	EC_{50} = 522.4 mg/L*	Chen *et al*., 1990b
Northern white shrimp (*Penaeus setiferus*)	72 h (static)	EC_{50} = 851.7 mg/L*	Alcaraz *et al*, 1997
Oyster *(Crassostrea virginica*)	96 h (static)	EC_{50} = 660 -800 mg/L	Epifanio & Srna, 1975
Red swamp crayfish (*Procambarus clarkii*)	96 h (static)	LC_{50} = 42-112 mg/L	Gutzmer & Tomasso, 1985
San paulo shrimp (*Penaeus paulensis*)	96 h (semistatic)	LC_{50} = 539.2 mg/L*	Cavalli *et al*., 1996

* : The values were converted from NO_2-N into $NaNO_2$.

Table 10 Summary of acute toxicity of sodium nitrite on algae

Organism	Test duration	Result	Reference
Algae			
Green Alga (*Desmodesmus subspicatus*)	72 h (static)	E_rC_{50} > 100 mg/L E_bC_{50} > 100 mg/L	JAFA, 2005

Table 11 Summary of chronic toxicity of sodium nitrite on invertebrates

Organism	Test duration	Result	Reference
Invertebrates			
Jumbo tiger prawn (*Penaeus monodon*)	80 d (semistatic)	-Mortality LC_{50} > 95.6 mg/L* -EC_{50} for weight gain EC_{50} = 114.9 mg/L* NOEC = 9.86 mg/L*	Chen & Chen, 1992

* : The values were converted from NO_2-N into $NaNO_2$.

Table 12 Summary of chronic toxicity of sodium nitrite on algae

Organism	Test duration	Result	Reference
Algae			
Green Alga (*Desmodesmus subspicatus*)	72 h (static)	NOEC = 100 mg/L (growth rate and biomass)	JAFA, 2005

Table 13 Summary of toxicity of sodium nitrite on other organisms

Organism	Test duration	Result	Reference
Prasinophyte (*Tetraselmis chuii*)	96 h	(Mobility) EC_{50} = 7886 mg/L* NOEC = 3,740 mg/L*	Ostrensky & Lemos, 1993
Protozoa *(Spirostomum ambiguum)*	48 h (Static)	EC_{50} (deformation) = 421 mg/L* LC_{50} = 533 mg/L*	Nalecz-Jawecki & Sawicki, 1998

- * : The values were converted from NO_2-N into $NaNO_2$.

4.2 Terrestrial Effects

No reliable data are available.

4.3 Other Environmental Effects

No reliable data are available

4.4 Initial Assessment for the Environment

Sodium nitrite is white or slightly yellow hygroscopic granules, rod or powder, which is very soluble in water (820 g/L at 20 °C). Melting point, boiling point, vapour pressure and partition coefficient are 271°C, >320°C (decomposes), 9.9E-17 hPa (25°C) and log Kow = -3.7, respectively. Fugacity model Mackay level III calculations suggest that the substance will distribute mainly to soil if released to the air or soil compartments separately or to all three compartments simultaneously and almost exclusively to water if released to the water compartment. Estimated value of Henry's constant is 2.06E-07 atm-m^3/mole. This substance dissociates immediately into sodium and nitrite ions in water. The nitrite ion is a component of the nitrogen cycle. In the environment, bacteria of the genus Nitrobacter oxidise nitrites to nitrates. Nitrates are reduced to nitrogen by anaerobic bacteria present in soil and sediment. The estimated BCF is 3.162 and hence bioaccumulation is not significant. Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 82.3 days.

The LC_{50} values for the acute toxicity of sodium nitrite to fish reported in the literature vary widely between the species tested; LC_{50} (96h) = 0.54 mg $NaNO_2$/L for *Oncorhynchus mykiss*; LC_{50} (96h) = 35 mg $NaNO_2$/L for *Ictalurus punctatus*; LC_{50} (96h) = 691.0 mg $NaNO_2$/L for *Micropterus salmoides*; and LC_{50} (96h) = 1010.4 mg $NaNO_2$/L for *Anguilla japonica*, for example. This difference has been attributed to the ability of certain species, such as eels, bass and sunfish to prevent nitrite from crossing the gill membrane and entering the blood, whilst other species such as rainbow trout concentrate nitrite in their blood. The range of toxicity values reported for some species of fish varies widely and is believed to be dependant on the quality of the water used in the test with pH, chloride and calcium ion concentration all having an influence. In particular, chloride ion concentration has been shown to be important, with increasing concentrations leading to a decrease in the toxicity of nitrite. As with fish, there is variation in toxicity between invertebrate

species. Sodium nitrite is toxic to invertebrates such as *Cherax quadricarinatus* (LC_{50} (96h) = 4.93 mg $NaNO_2$/L, whereas other species, such as *Procambarus clarkii* (LC_{50} (96h) = 18.7 mg $NaNO_2$/L) and *Penaeus paulensis* are much less sensitive (LC_{50} (96h) = 539.2 mg $NaNO_2$/L). The presence of chloride ions has been found to mitigate nitrite toxicity in some species. Acute toxicity to green alga (*Desmodesmus subspicatus*) is > 100 mg/L (72-h E_rC_{50} and E_bC_{50}) [OECD TG 201].

No data is available for chronic toxicity of sodium nitrite in fish. In invertebrates, an 80-day NOEC of 9.86 mg $NaNO_2$/L for *Penaeus monodon* has been reported. The NOEC value in green alga (*Desmodesmus subspicatus*) is 100 mg/L (72-h for growth rate and biomass) [OECD TG 201].

For other aquatic organisms, the EC_{50} (48h, deformation) and LC_{50} (48h) for the protozoa *Spirostomum ambiguum* were 421 and 533 mg $NaNO_2$/L, respectively; for the microalgae *Tetraselmis chuii* the EC_{50} (96h, mobility) and NOEC (96h, mobility) were 7886 and 3740 mg $NaNO_2$/L, respectively.

5 RECOMMENDATIONS

Human Health: The chemical is a candidate for further work.

Environment: The chemical is a candidate for further work.

The chemical possesses properties indicating a hazard to human health (acute toxicity, irritation, repeated toxicity, mutagenicity, and reproductive toxicity) and the environment (acute toxicity). Given the wide dispersive use of this substance, member countries are invited to perform an exposure assessment, and if necessary a risk assessment for these uses. It is acknowledged that some uses (e.g. as a food additive) as well as the presence in drinking water are already regulated in many member countries. It is recommended that the information on possible total exposure from regulated and non-regulated use be shared between regulatory agencies.

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S I D S

D o s s i e r

Existing Chemical ID: 7632-00-0
CAS No. 7632-00-0
EINECS Name sodium nitrite
EC No. 231-555-9
TSCA Name Nitrous acid, sodium salt
Molecular Formula HNO2.Na

Producer Related Part
Company: Safepharm Laboratories
Creation date: 28-FEB-2005

Substance Related Part
Company: Safepharm Laboratories
Creation date: 28-FEB-2005

Memo: Sodium Nitrite ICCA HPV SIAM 20

Printing date: 04-JAN-2006
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Date of last Update: 04-JAN-2006

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Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: Ube Industries Ltd.
Contact Person: Mr. Etsuro Ito Date:
Street: Seavans North Bldg., 1-2-1, Shibaura, Minato-ku
Town: 105-8449 Tokyo
Country: Japan
Phone: +81-3-5419-66242
Telefax: +81-3-5419-6242
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25-APR-2005

Type: cooperating company
Name: Nissan Chemical Industries Ltd.
Contact : Kazuo Nagashima Date:
Street: 7-1, Kanda-Nishiki-cho 3-chome, Chiyoda-ku
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Email: nagashimak@nissanchem.co.jp

25-APR-2005

Type: cooperating company
Name: Mitsubishi Chemical Corporation
Contact Person: Yasukazu Uchida Date:
Street: Dai-ichi Tamachi Building, 33-8, Shiba 5-chome, Minato-ku
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\@mc.m-kagaku.co.jp

25-APR-2005

Type: cooperating company
Name: Sumitomo Chemical Co. Ltd.
Contact Person: Tsuneo Nara Date:
Street: 27-1, Shinkawa 2-chome, Chuo-ku
Town: 104-8260 Tokyo
Country: Japan
Phone: +81-3-5543-5196
Telefax: +81-3-5543-5909
Email: narat@sc.sumitomo-chem.co.jp

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

Name of recip.: Mr. Motohiko Kato, Ministry of Foreign Affairs, Economic Affairs Bureau, Second International Organizations Div.
Street: 2-2-1 kasumigaseki, Chiyoda-ku
Town: 100-8919

Country: Japan
Phone: +81-3-3581-0018
Telefax: +81-3-3581-9470

29-APR-2005

1.0.4 Details on Category/Template

1.1.0 Substance Identification

IUPAC Name: sodium nitrite
Smiles Code: [Na]ON=O
Mol. Formula: NaNO2
Mol. Weight: 69.00

29-APR-2005

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: inorganic
Physical status: solid
Purity: >= 99 - % w/w
Colour: white or slightly yellow

Remark: Appearance: powdery crystal
Impurity: Moisture: < 0.3 % w/w, insolubility for water <0.05 % w/w
29-APR-2005 (186)

Purity type: other
Substance type: inorganic
Physical status: solid
Purity: 96 - 98 % w/w
Colour: white or slightly yellow, hygroscopic granules, rods, or powder.
Very slowly oxidixes to nitrate in air.

Purity type: typical for marketed substance
Substance type: inorganic
Physical status: solid
Purity: >= 97 - % w/w
Colour: white or slightly yellow
Odour: no

29-APR-2005 (162)

1.1.2 Spectra

1.2 Synonyms and Tradenames

Anti-Rust

29-JUN-1995

E 250

29-JUN-1995

Erinitrit

29-JUN-1995

Filmerine

29-JUN-1995

Konservierungsstoff E 250

29-JUN-1995

Na-Nitrit

06-MAY-1997

NaNO2

07-OCT-1994

Natriumnitrit

02-DEC-1992

NCI-C02084

29-JUN-1995

Nitrito sodico

20-MAY-1994

Nitrous acid sodium salt (1:1)

02-DEC-1992

Nitrous acid, sodium salt

29-APR-2005

Nitrous acid, sodium salt (8CI, 9CI)

02-DEC-1992

Salpetrige Säure, Na-Salz

07-OCT-1994

Salpetrigsaures Natrium

02-JUN-1998

Sodio nitrito

20-MAY-1994

Sodium nitrite

02-DEC-1992

Sodium nitrite 30%

13-APR-1994

Synfat 1004

29-JUN-1995

1.3 Impurities

Purity type: typical for marketed substance
CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2O
Contents: <= .3 - % w/w

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
29-APR-2005 (186)

Purity type: typical for marketed substance
EINECS-Name: water insoluble matter
Contents: <= .05 - % w/w

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
29-APR-2005 (186)

1.4 Additives

1.5 Total Quantity

Quantity: 10000 - 50000 tonnes produced in 2001

Remark: Amount produced in Japan. Worldwide production of sodium nitrite is not available
27-MAY-2005 (122) (176)

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: (O) oxidizing
(T) toxic
(N) dangerous for the environment
(E) For substances ascribed Nota E the risk phrases R20, R22 to R28 and all combinations of these risk phrases shall be preceded by the word 'also'. E.g. R23 'also' toxic by inhalation
Specific limits: yes
R-Phrases: (8) Contact with combustible material may cause fire

(25) Toxic if swallowed
(50) Very toxic to aquatic organisms
S-Phrases: (1/2) Keep locked up and out of reach of children
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
(61) Avoid release to the environment. Refer to special instructions/Safety data sets

29-APR-2005

1.6.2 Classification

Classified: as in Directive 67/548/EEC
Class of danger: dangerous for the environment
R-Phrases: (50) Very toxic to aquatic organisms

29-APR-2005

Classified: as in Directive 67/548/EEC
Class of danger: oxidizing
R-Phrases: (8) Contact with combustible material may cause fire

03-MAY-2005

Classified: as in Directive 67/548/EEC
Class of danger: toxic
R-Phrases: (25) Toxic if swallowed

03-MAY-2005

1.6.3 Packaging

Memo: 20 kg, 30 kg
Packaging: paper bag (polyethylene; inner packaging), flexible container

29-APR-2005 (186)

1.7 Use Pattern

Type: type
Category: Non dispersive use

29-APR-2005

Type: type
Category: Use in closed system

29-APR-2005

Type: type
Category: Wide dispersive use

29-APR-2005

Type: industrial
Category: Agricultural industry

29-APR-2005 (169)

Type: industrial
Category: Basic industry: basic chemicals

29-APR-2005

Type: industrial
Category: Chemical industry: used in synthesis

29-APR-2005

Type: industrial
Category: Electrical/electronic engineering industry

29-APR-2005 (169)

Type: industrial
Category: Fuel industry

29-APR-2005 (169)

Type: industrial
Category: Metal extraction, refining and processing of metals

29-APR-2005

Type: industrial
Category: Paints, lacquers and varnishes industry

29-APR-2005

Type: industrial
Category: Personal and domestic use

29-APR-2005 (169)

Type: industrial
Category: Polymers industry

29-APR-2005

Type: industrial
Category: Public domain

29-APR-2005

Type: industrial
Category: Textile processing industry

29-APR-2005

Type: industrial
Category: other: raw material for caprolactam

29-APR-2005

Type: industrial

Category: other

29-APR-2005

Type: use
Category: Adhesive, binding agents

29-APR-2005 (169)

Type: use
Category: Anti-freezing agents

29-APR-2005 (169)

Type: use
Category: Cleaning/washing agents and disinfectants

29-APR-2005 (169)

Type: use
Category: Colouring agents

29-APR-2005

Type: use
Category: Construction materials additives

29-APR-2005 (169)

Type: use
Category: Corrosive inhibitors

29-APR-2005

Type: use
Category: Fillers

29-APR-2005

Type: use
Category: Food/foodstuff additives

29-APR-2005

Type: use
Category: Heat transferring agents

29-APR-2005

Type: use
Category: Intermediates

29-APR-2005

Type: use
Category: Laboratory chemicals

29-APR-2005

Type: use

Category: Lubricants and additives

29-APR-2005 (169)

Type: use
Category: Non agricultural pesticides

29-APR-2005 (169)

Type: use
Category: Oxidizing agents

29-APR-2005

Type: use
Category: Pesticides

29-APR-2005

Type: use
Category: Pharmaceuticals

29-APR-2005

Type: use
Category: Process regulators

29-APR-2005 (169)

Type: use
Category: Reducing agents

29-APR-2005

Type: use
Category: Stabilizers

29-APR-2005

Type: use
Category: Surface-active agents

29-APR-2005 (169)

Type: use
Category: other: cutting fluids

29-APR-2005 (169)

Type: use
Category: other: paint, lacquers and varnishes

29-APR-2005 (169)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)

Remark: kein MAK-Wert festgelegt
29-APR-2005 (182)

Type of limit: TLV (US)
Limit value: 5.6 mg/m3
Short term exposure
Limit value: 9.4 mg/m3
Schedule: 15 minute(s)
Frequency: 4 times

Remark: Exposure limit values not assigned for sodium nitrite in solution 30%
Exposure limit values referred to nitrogen dioxide
27-MAY-2005 (2)

Type of limit: TLV (US)
Limit value: 31 mg/m3

Remark: Exposure limit values no assigned for sodium nitrite in solution 30%
Exposure limit values referred nitric oxide
27-MAY-2005 (2)

Type of limit: other: skin, (HUNGARY)
Short term exposure
Limit value: 1 mg/m3

Remark: Jan, 1993
29-APR-2005

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: KBwS (DE)
Labelled by: KBwS (DE)
Class of danger: 2 (water polluting)

29-APR-2005

1.8.4 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: yes

Remark: group of materials concerns 4c: poisonous materials
29-APR-2005 (170)

1.8.5 Air Pollution

Classified by: TA-Luft (DE)
Labelled by: TA-Luft (DE)
Number: 3.1.3 (total dust)

Remark: No classes for inorganic materials except the final list in 3.1.6 TA air. Dust missions, limit after 3.1.3.
29-APR-2005

1.8.6 Listings e.g. Chemical Inventories

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

Remark: Reaction between ammonia and caustic soda
29-APR-2005 (54)

Remark: Toxic substance used in closed systems. Therefore exposure only occurs as a result of loss of containment.
(ICI Chemicals & Polymers Limited Runcorn, Cheshire)
29-APR-2005

1.11 Additional Remarks

Memo: Work place monitoring data

Method: Air sample was suctioned at the breathing zone of the worker at the suction rate of 2.0 L/min. and trapped in a filter through a collection tube and analyzed by LC. Workers are recommended to wear protective gear such as a mask, rubber gloves and goggles to prevent exposure.

Result: -Monitoring Data (Maximum Concentration) (mg/m3)
--Operation in site 1 (powder production)
Paper bag filling: 0.110
Sampling from process: 0.308
Sampling from product: 0.803
Analysis work: <0.022
--Operation in site 2 (liquid production)
Tank car operation: <0.08
Analysis work (process): 0.046
Analysis work (product): <0.014

-Frequency (Time/day)
Paper bag filling: 20 days/month
Sampling from process: 2
Sampling from product: 1
Analysis work: 2

--Operation in site 2 (liquid production)
Tank car operation: 2
Analysis work (process): 2
Analysis work (product): 4 times/month

-Working time (hrs/day)
--Operation in site 1 (powder production)
Paper bag filling: 6
Sampling from process: 0.03
Sampling from product: 0.030
Analysis work: 0.17
--Operation in site 2 (liquid production)
Tank car operation: 0.3
Analysis work (process): 0.17
Analysis work (product): 0.08

-Maximum EHEinh(mg/kg/day)
--Operation in site 1 (powder production)
Paper bag filling: 1.30 x 10-2
Sampling from process: 3.61 x 10-4
Sampling from product: 4.71 x 10-4
Analysis work: 1.33 x 10-4
--Operation in site 2 (liquid production)
Tank car operation: 8.56 x 10-4
Analysis work (process): 2.79 x 10-4
Analysis work (product): 4.00 x 10-8

The concentrations at operation in site 1 (powder production) were higher than those at operation in site 2 (liquid production).

Test condition: Analysis column: Geilack GL-IC-A25
Separate liquid : 4 mmol/L Na2CO3
Feed rate : 1.0 mL/L
Column temp. : 40 degree C
Detector system : conduct metric detection
Injection volume : 25 micro-L

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
29-APR-2005 (95)

Memo: The environmental limitation

Remark: The environmental limitations; 10 mg/L (the sum of nitrite-nitrogen plus nitrate-nitrogen) in JAPAN
06-JAN-2004

Memo: Water quality-based limitations

Remark: JAPAN:
Guideline value; 0.05 mg/L (nitrite-nitrogen) for a tap water quality.

Water quality-based limitations; 10 mg/L (the sum of nitrite-nitrogen plus nitrate-nitrogen)

WHO:
acute; 3 mg/L (as nitrite)
chronic; 0.2 mg/L (as nitrite)

EU:

0.5 mg/L (as nitrite)

USEPA:
1 mg/L
19-JAN-2005 (123) (196)

Remark: This product has a 5.1, 23c RID/ADR/ADNR classification and UN number is 1500.
13-JAN-2005

Remark: - Code of the public health. Art. 5149 A 5211. Table C.

- Directive 64/54/The EEC of the 15.11.1963 (preservatives).
Arrete of 20.7.1979 (metal nitrites, salts niters).
Concern: E 250.

- water pollution - KBwS classification (OF)
- labelling KBwS (OF)
- category of danger (wGK): 2

- major risks of accident:
- Directive 82/501/The EEC (Seveso)
- Substance not listed.
21-JAN-2005

1.12 Last Literature Search

Type of Search: Internal and External

Remark: ACGIH
AQUIRE (CIS, STN)
BEILSTEIN (STN)
BIOSIS (STN, Dialog)
CHEMCATS (STN)
CHRIS (CIS, CHEM-BANK)
CSCHEM (STN)
ChemFinder
ECDIN
GMELIN (STN)
HODOC(STN)
HSDB (CIS, STN, DataStar, CHEM-BANK)
IARC
IRIS (CIS, CHEM-BANK)
IUCLIDMSDS-CCOHS (STN, Dialog)
MEDLINE (STN, Dialog, Datastar)
MSDS-OHS (STN)
NCI
NIOSHOHMTADS (CIS, CHEM-BANK)
NIOSHTIC(STN, Dialog)
PROMT(STN, Dialog)
REGISTRY (STN, Dialog)
RTECS(STN, CIS, Dialog, CHEM-BANK)
SPECINFO (STN)
SRC PhysPro Database(SRC: Syracuse Research Corporation)
TOXCENTER (STN)
TOXFILE (Dialog, Datastar)
TSCATS (CIS)

Date of the literature search: 6 Jan, 2004

29-APR-2005

1.13 Reviews

Memo: NTP (2001). Toxicology and Carcinogenesis Studies of Sodium Nitrite (CAS No. 7632-00-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program Report No. NTP TR 495. NIH Publication No. 01-3954.

03-JUN-2005

Memo: JECFA (FAO/WHO Joint Expert Committee on Food Additives) (2003). Food Additives Series: 50

15-JUL-2005

Memo: National Academy of Sciences (NAS) (1981). The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds. National Academy Press, Washington.

15-JUL-2005

Memo: National Institute of Environmental Health Sciences (NIEHS) (1970). Nitrates, Nitrites, and Methemoglobinemia. Research Triangle Park, N.C.: NTIS;

15-JUL-2005

Memo: National Institute of Public Health and Environmental Hygiene, Netherlands (1986). Nitrate: Basis Document Effects. Project Number 840820.

15-JUL-2005

Memo: CAL/EPA (California Environmental Protection Agency) (2000). Evidence on Developmental and Reproductive Toxicity of Sodium Nitrite, Reproductive and Cancer Hazard Assessment Section (RCHAS), Office of Environmental Health Assessment (OEHHA)-

2.1 Melting Point

Value: = 271 degree C
Sublimation: no
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
27-MAY-2005 (120)

Value: = 280 degree C
Decomposition: yes at degree C
Sublimation: no

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (83) (119)

Value: = 280 degree C
Decomposition: yes at degree C
Sublimation: no

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (14)

Value: 281 degree C
GLP: no data

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (61)

2.2 Boiling Point

Value: > 320 degree C
Decomposition: yes
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
27-MAY-2005 (78) (82) (83) (120)

2.3 Density

Type: density
Value: = 2.1 g/cm³ at 20 degree C

Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (14) (119)

Type: density
Value: = 2.17 g/cm³

Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions
27-MAY-2005 (120)

Type: density
Value: = 2.2 g/cm³

Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (83) (102)

Type: density
Value: 2.135 g/cm³ at 26 degree C

Method: other
GLP: no data

Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (173)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: at 25 degree C

Method: other (calculated): MPBWIN v1.41
Year: 2005

Result: Vapour Pressure = 9.9E-17 hPa (7.44E-17 mm Hg) [Modified Grain Method]
Test condition: Inputs:

Boiling Point: 706.27°C (estimated)
Melting Point: 308.95°C (estimated)

Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
31-MAY-2005 (185)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: -3.7

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
15-JAN-2004 (14) (83) (119)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 820 g/l at 20 degree C

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
16-JAN-2004 (83)

Solubility in: Water
Value: = 816 g/l at 15 degree C

Remark: Solubility at 0°C = 720 g/L
Solubility at 100°C = 1630 g/L
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (176)

Solubility in: Water
Value: = 818 g/l at 20 degree C
pH value: 8 - 9
Conc.: 100 g/l at 20 degree C

Remark: Solubility at 80°C = 1355 g/L
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (14)

Remark: Freely soluble in water. Solutions of 40% may be achieved at 20 °C.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (82)

Remark: very soluble in water 80 % at 20 degree C,
slightly soluble in Ethnol 0.3 % and Methanol 0.45%
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
27-MAY-2005 (15)

2.6.2 Surface Tension

2.7 Flash Point

Remark: Not combustible but enhances combustion of other substances. Many reactions may cause fire or explosion. Gives off irritating or toxic fumes (or gases) in a fire.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (83)

2.8 Auto Flammability

Value:

Remark: See section 2.7
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
27-MAY-2005

2.9 Flammability

Remark: See section 2.7
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

2.10 Explosive Properties

Remark: May explode on heating above 530 degree C.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (83)

Result: not explosive

Method: other: Stahlhuelsentest (BAM)

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
14-MAY-2004 (14)

Result: other

Method: other
Year: 1985
GLP: no data

Result: Explodes at 537 C.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
14-MAY-2004 (75)

2.11 Oxidizing Properties

Remark: See section 2.10
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
14-MAY-2004 (83)

Result: other

Method: other
Year: 1985
GLP: no data

Result: Strong oxidizing agent.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
27-MAY-2005 (75)

Result: other

Method: other
GLP: no data

Remark: Sodium nitrite is a strong oxidising agent at high temperature and also is a strong supporter of combustion.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
27-MAY-2005 (82)

2.12 Dissociation Constant

12-JAN-2005 (174)

Acid-base Const.: pKa = 3.27

Year: 2002

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (174)

2.13 Viscosity

2.14 Additional Remarks

Memo: The substance decomposes on contact with acids producing toxic fumes (nitrogen oxides). The substance is a strong oxidant and reacts with combustible and reducing materials causing fire and explosion hazard. The solution in water is a weak

27-MAY-2005 (83)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1500000 molecule/cm³
Rate constant: .00000000000013 cm³/(molecule * sec)
Degradation: 50 % after 82.3 day(s)

Method: other (calculated): AOPWIN v1.91
Year: 2005

Test condition: Inputs:

12-hour day
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (185)

3.1.2 Stability in Water

Remark: This substance dissociates immediately into sodium and nitrite ions in water
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
27-MAY-2005

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Method: other: Calculated using EPIWIN V 3.12 Level III Fugacity Model
Year: 2005

Remark: The substance will distribute mainly to soil if released to the air or soil compartments separately or to all three compartments simultaneously and almost exclusively to water if released to the water compartment.

Result:

	1000 kg/h emission to these compartments separately			Simultaneous 1000 kg/h emission to air, water and soil compartments
	Air	Water	Soil	
In air	5.0	0.0	0.3	1.78
In water	25.0	99.7	22.3	40.20
In soil	69.9	0.1	77.4	58.00
In sediment	0.1	0.2	0.0	0.07

Test condition: Inputs:

Molecular weight: 69
Henry's Law Constant 2.06E-07 atm-m3/mole (Henrywin program)

Vapour pressure: 7.44E-17 mm Hg (Mpbpwin program)
Liquid VP: 4.79E-14 mm Hg (super cooled)
Melting point: 309°C (mpbpwin program)
Log Kow: -2.37 (Kowwin program)
Soil Koc: 0.00175 (calc by model)

Half-lives (hr) (based on Biowin (Ultimate) and Aopwin):
Air: 1975
Water: 360
Soil: 720
Sediment: 3240
Biowin estimate: 3.047 weeks

Reliability: (2) valid with restrictions
27-MAY-2005 (185)

3.3.2 Distribution

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Remark: The nitrite ion is a component of the nitrogen cycle. In the environment, bacteria of the genus Notribacter oxidise nitrites to nitrates. Nitrates are reduced to nitrogen by anaerobic bacteria present in soil and sediment.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
27-MAY-2005

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

BCF: 3.16

Method: other: calculation using BCF Program v2.15
Year: 2005

Result: BCF = 3.16, Log BCF = 0.5
Test condition: Input:

Log Kow = 0.06 (BCF estimate)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (185)

3.8 Additional Remarks

Memo: Henry's Law Constant

Result: Henry's Law Constant at 25°C = 2.06E-07 atm-m3/mole (bond estimate)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
27-MAY-2005 (185)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 : = .11 - 5.34

Method: other: Russo et al
Year: 1981

Method: METHOD FOLLOWED: Russo et al method.

Four series of 96-h bioassays were conducted using rainbow trout. Series 1 (12 test) and Series II (22 tests) were conducted over the pH range 6.4 - 9.0. The two series were conducted on two different size ranges of fish and in two different years. Series III (six tests) was conducted at pH 7 using three different acids (H2SO4, H3PO4 and HNO3) for pH reduction. Series IV (four tests) was conducted over the pH range 7.5 - 8.6 at chloride concentrations above background.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
LC50 values and their 95% confidence interval endpoints were calculated using a computer program developed for the Trimmed Spearman-Karber Method; the calculations were performed using an XDS Sgma 7 computer.

METHOD OF CALCULATION:
No data

ANALYTICAL METHODS: Nitrite concentration determined using the method described by EPA (1974).
References:
US EPA (1974) Methods for Chemical Analysis of Water and Wastes. EPA-625-/6-74-003. Methods Development and Quality Assurance Research Laboratory, National Environmental research Center, Cincinnati, OH. 215-216

Remark: Effect of pH:
As pH increased, the toxicity in terms of NO2-N decreased.

Effect of sulfate, phosphate and nitrate ions:
To determine what might be the effect of using an acid other than sulfuric acid to reduce pH in the test water, thus adding different anions. The LC50 values obtained were lowest in the presence of sulfate and highest in the presence of nitrate.

Effect of chloride ion:
The 96-h LC50 values were an order of magnitude higher than, and followed the same trend as, those obtained for the fish tested over the same pH range without chloride addition (series I and II). Fish varied in size from 28 - 244 g in the chloride added experiments, but this was not considered to be a factor in results.

Result: The lowest 96-h LC50 was 0.11 mg/L NO2-N (equivalent to 0.54 mg/L NaNO2).

The highest 96-h LC50 was 5.35 mg/L NO2-N (equivalent to 26.3 mg/L NaNO2), in the presence of 10 mg/L chloride.

Table 1: Acute toxicity of nitrite at different pH values to rainbow trout (Series I)

Avg (mean) Fish size Wt (g)	Length (cm)	Fish per tank	Acid or base	Alkalinity mg/L CaCO3 (range)	pH (range)	96h-LC50 mg/L NO2-N (95% CI)
285	28.1	5	H3PO4	109 (108-113)	6.44 (6.28-6.56)	0.21 (0.13-0.34)
171	23.2	10	H3PO4	171 (170-171)	7.50 (7.47-7.53)	0.20 (0.17-0.24)
46.7	15.3	20	H3PO4	174 (172-175)	7.52 (7.44-7.63)	0.32 (0.26-0.39)
53.1	15.7	20	NONE	176 (175-177)	7.68 (7.58-7.79)	0.27 (0.22-0.32)
387	29.7	5	NONE	159 (158-160)	7.74 (7.69-7.80)	0.24 (0.17-0.33)
60.5	16.6	20	NONE	177 (175-180)	7.76 (7.71-7.83)	0.27 (0.23-0.32)
188	23.6	10	NONE	172 (171-172)	7.81 (7.76-7.86)	0.19 (0.15-0.24)
24.3	12.3	20	NONE	179 (178-180)	8.10 (7.99-8.33)	0.28 (0.24-0.32)
121	20.5	10	NaOH	176 (175-176)	8.15 (7.94-8.60)	0.41 (0.32-0.52)
74.8	17.4	10	NaOH	180 (179-182)	8.23 (9.07-8.63)	0.38 (0.31-0.46)
69.8	17.0	10	NaOH	182 (180-183)	8.24 (8.13-8.55)	0.39 (0.33-0.47)
341	28.4	5	NaOH	190 (189-192)	9.00 (8.97-9.05)	1.67 (1.04-2.70)

The range of other water chemistry variables (in mg/L) for all tanks at time of tests were: dissolved oxygen 7.6-9.3; NH3-N 0.00-0.11; Cl- 0.00-1.71; Ca2+ 54.9-65.7; hardness (as CaCO3) 185-208. Temperature range for all tanks was 9.0-10.7°C

Table 2: Acute toxicity of nitrite at different pH values to rainbow trout (Series II)

Avg (mean) Fish size Wt (g)	Length (cm)	Fish per tank	Acid or base	Alkalinity mg/L CaCO3 (range)	pH (range)	96h-LC50 mg/L NO2-N (95% CI)
12.8	10.4	10	H2SO4	120 (117-123)	6.99 (6.84-7.04)	0.14 (0.12-0.15)
15.3	10.2	10	H2SO4	128 (124-130)	7.02 (6.96-7.07)	0.11 (0.09-0.13)
10.4	10.0	10	H2SO4	135 (128-142)	7.24 (7.16-7.33)	0.15 (0.13-0.18)
10.0	9.4	10	H2SO4	150 (146-154)	7.37 (7.32-7.41)	0.19 -

9.3	9.2	10	H2SO4	160 (158-164)	7.59 (7.53-7.64)	0.18 (0.15-0.21)
7.0	9.1	10	NONE	188 (188-189)	7.83 (7.75-7.91)	0.40 -
12.8	10.3	10	NONE	173 (172-176)	7.86 (7.81-7.93)	0.21 (0.19-0.24)
13.2	10.3	10	NONE	177 (174-180)	7.87 (7.82-7.92)	0.22 (0.18-0.27)
8.0	8.6	10	NONE	170 (168-170)	7.88 (7.77-7.99)	0.36 (0.33-0.39)
10.0	9.3	10	NONE	172 (170-174)	7.90 (7.84-7.94)	0.21 (0.19-0.24)
8.2	8.7	10	NONE	170 (168-171)	7.91 (7.82-8.01)	0.30 (0.26-0.34)
3.1	6.3	10	NONE	165 (164-166)	7.94 (7.91-8.00)	0.25 (0.21-0.30)
10.4	9.2	10	NONE	174 (172-176)	7.95 (7.86-8.09)	0.17 (0.15-0.20)
8.4	8.9	10	NaOH	178 (177-179)	8.27 (8.21-8.36)	0.54 (0.50-0.60)
12.8	10.2	10	NaOH	185 (183-186)	8.41 (8.32-8.60)	0.46 (0.40-0.53)
15.5	10.9	10	NaOH	188 (186-190)	8.49 (8.33-8.59)	0.45 (0.39-0.52)
15.3	10.0	10	NaOH	186 (185-188)	8.61 (8.53-8.74)	0.50 (0.44-0.57)
8.2	8.8	10	NaOH	181 (180-182)	8.70 (8.64-8.78)	0.71 (0.63-0.80)
9.3	9.4	10	NaOH	188 (187-190)	8.83 (8.77-8.90)	1.17 (0.82-1.67)
10.4	9.6	10	NaOH	191 (190-193)	8.83 (8.75-8.91)	0.61 (0.52-0.71)
7.0	8.7	10	NaOH	202 (201-203)	9.03 (8.94-9.09)	1.10 -
8.0	8.8	10	NaOH	190 (188-192)	9.04 (8.97-9.12)	1.12 (0.91-1.37)

The range of other water chemistry variables (in mg/L) for all tanks at time of tests were: dissolved oxygen 6.6-9.3; NH3-N 0.00-0.06; Cl- 0.00-0.47; Ca2+ 46.8-61.3; hardness (as CaCO3) 178-209. Temperature range for all tanks was 8.9-14.7°C

Table 3: Acute toxicity of nitrite at pH 7 to rainbow trout using different acids (Series III)

Avg (mean) Fish size Wt (g)	Length (cm)	Fish per tank	Acid or base	Alkalinity mg/L CaCO3 (range)	pH (range)	96h-LC50 mg/L NO2-N (95% CI)
15.3	10.2	10	H2SO4	128 (124-130)	7.02 (6.96-7.07)	0.11 (0.09-0.13)
12.8	10.4	10	H2SO4	120 (117-123)	6.99 (6.84-7.04)	0.14 (0.12-0.15)
13.2	10.7	10	H3PO4	152 (151-153)	6.99 (6.95-7.06)	0.18 (0.13-0.26)
13.7	10.8	10	H3PO4	150 (146-155)	6.98 (6.98-7.04)	0.18 (0.16-0.21)
13.7	10.6	10	HNO3	137 (130-144)	7.01 (6.95-7.08)	0.29 (0.26-0.32)

18.2	11.8	10	HNO3	136 (131-140)	7.01 (6.95-7.16)	0.26 (0.23-0.29)	

The range of other water chemistry variables (in mg/L) for all tanks at time of tests were: dissolved oxygen 6.6-6.8; NH3-N 0.00-0.06; Cl- 0.10-0.63; Ca2+ 48.9-52.9; hardness (as CaCO3) 192-201. Temperature range for all tanks was 9.4-14.0°C

Table 4: Acute toxicity of nitrite at 10 mg/L chloride over different pH values to rainbow trout (Series IV)

Avg (mean) Fish Wt (g)	Avg (mean) Fish Length (cm)	Fish per tank	Acid or base	Alkalinity mg/L CaCO3 (range)	pH (range)	96h-LC50 mg/L NO2-N (95% CI)
28.2	12.9	10	HCl	174 (173-176)	7.50 (7.38-7.61)	3.74 (3.25-4.30)
79.0	17.6	10	NONE	177 (176-178)	7.90 (7.83-7.96)	3.54 (2.96-4.22)
147	22.0	10	NaOH	188 (186-190)	8.47 (8.39-8.58)	4.35 (3.60-5.26)
244	26.1	10	NaOH	184 -	8.59 (8.50-8.67)	5.34 (4.43-6.45)

The range of other water chemistry variables (in mg/L) for all tanks at time of tests were: dissolved oxygen 7.2-9.6; NH3-N 0.00-0.06; hardness (as CaCO3) 199-206. Temperature range for all tanks was 9.9-10.7°C

Test condition: TEST ORGANISMS:

Size: See Tables 1-4
Age: no data
Pretreatment: fish were acclimated to the test pH by gradually adjusting the dilution water pH over a 24-hour period to the desired value and maintaining that value for 48 hours before addition of sodium nitrite.
Supplier: Fish Cultural Development Center, US Fish and Wildlife Service, Bozeman, Montana, USA.

DILUTION WATER:
Source: Ground spring water
Chemistry: no data
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: See Tables 1-4
Renewal of test solution: Approximately 5 hours for 62 L tank and 1.3 hours for 350 L tank.
Exposure vessel type: 62 L plastic tanks or 350 L circular glassfibre tanks
Number of replicates, fish per replicate: See Tables 1-4
-Water parameters:

CASE A-1 to 12:
Temperature; 9.0 - 10.7 degree C
Hardness (mg/L CaC03); 185 - 208
Alkalinity (mg/L CaC03); (see Table 1)
Dissolved O2 (mg/L); 7.6 - 9.3
pH; (see Table 1)

CASE B-1 to 22:
Temperature; 8.9 - 14.7 degree C
Hardness (mg/L CaC03); 178 - 209
Alkalinity (mg/L CaC03); (see Table 2)
Dissolved O2 (mg/L); 6.6 - 9.3
pH; (see Table 2)

CASE C-1 to 6:
Temperature; 9.4 - 14.0 degree C
Hardness (mg/L CaC03); 192 - 201
Alkalinity (mg/L CaC03); (see Table 3)
Dissolved O2 (mg/L); 6.6 - 6.8
pH; (see Table 3)

CASE D-1 to 4:
Temperature; 9.9 - 10.7 degree C
Hardness (mg/L CaC03); 199 - 206
Alkalinity (mg/L CaC03); (see Table 4)

Dissolved O2 (mg/L); 7.2 - 9.6
pH; (see Table 4)
Intensity of irradiation: Room light
Photoperiod: No data
Feeding: No
Aeration: No data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured three times during the test

Test substance: Chemical Name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (2) valid with restrictions
Well-reported literature study

Flag: Critical study for SIDS endpoint

13-MAY-2005 (154)

Type: static
Species: other: Ictalurus punctatus (Channel Catfish), Tilapia aurea (Tilapia), Micropterus salmoides (Largemouth Bass)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 (Channel catf7.1) :7.1
LC50 (Largemouth B140.:140.2
LC50 (Tilapia) : 16.2

Method: other: Palachek & Tomasso method
Year: 1984

Method: METHOD FOLLOWED: Palachek & Tomasso method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: none

METHOD OF CALCULATION: Median lethal concentrations were estimated using the method of Thompson (1947)

ANALYTICAL METHODS: Nitrite concentration determined using the method described by EPA (1974).

References:
Thompson WR (1947) Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. Bacteriol. Rev. 11, 115-145

US EPA (1974) Methods for Chemical Analysis of Water and Wastes. EPA-625-/6-74-003. Methods Development and Quality Assurance Research Laboratory, National Environmental research Center, Cincinnati, OH. 215-216
Determination of methemoglobin and plasma nitrite levels:

Methemoglobin and plasma nitrite concentrations were determined using five fish per aquarium exposed to nitrite concentrations ranging from 1.5 to 194.9 mg/L. After 24 hours of exposure, fish were weighed, the caudal peduncle was severed, and blood was collected into capillary tubes from the haemal arch. Methemoglobin levels were determined spectrophotometrically (Evelyn & Malloy, 1938) after blood samples were lysed and centrifuged to remove turbidity. Total hemoglobin was determined according to Hainline (1958). Plasma nitrite levels were determined by a modification of the azo dye method (EPA 1974).

Analysis of variance followed by a student-Newman-keuls multiple range test was employed to test for difference among species.

Result: Channel catfish: LC50 (96h) = 35 mg/L $NaNO_2$
Tilapia: LC50 (96h) = 79.8 mg/L $NaNO_2$
Largemouth Bass: LC50 (96h) = 691 mg/L $NaNO_2$

The 24 hour dose-response studies showed that methemoglobin levels increased as environmental nitrite levels increased, with the exception of largemouth bass whose methemoglobin levels did not increase until nitrite concentrations reached 48.7 mg/L The channel catfish were the most susceptible and had the highest methemoglobin levels compared with the other species.

Channel catfish and tilapia exposed to 24.4 mg NO_2-N/L for 24h had levels of 76.9+/-5.0 and 61.3+/-7.9 mg plasma nitrite/L, respectively. Plasma nitrite levels in largemouth bass did not differ from control levels until environmental concentrations reached 48.7 mg NO_2-N/L or greater.

Test condition:
TEST ORGANISMS:

Largemouth Bass:
Size: 2.8+/-0.0 g
Age: no data
Supplier: National Fish Hatchery and Technology Center, San Marcos, Texas

Channel Catfish:
Size: 3.0+/-0.1 g
Age: no data
Supplier: Texas Parks and Wildlife Department, San Marcos Fish Hatchery

Tilapia:
Size: 3.4+/-0.2 g
Age: no data
Supplier: Texas A&M University Aquaculture Research Station, Texas

PRETREATMENT: All fish were acclimatized for at least four weeks prior to testing in 252 L fiberglass tanks supplied by well water at a flow rate of seven turnovers per hour. Fish were fed a commercial fish food (40% protein) ad libitum every 48 hours. Feeding was discontinued 24 hours prior to transferring fish into experimental aquaria.

DILUTION WATER:
Source: Ground spring water
Chemistry: Hardness 202.4 mg/L CaC03; Alkalinity 165.5 mg/L CaC03; Dissolved O2 8.1 mg/L; pH 7.7 (8.2 max)
Temperature: 23°C

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Measured nitrite concentrations ranged from 99.7+/-1.1% of nominal to 102.0+/-2.3% of nominal throughout the study.

REFERENCE SUBSTANCE: None

TEST SYSTEM (LC50 Determinations):
Concentrations:
Largemouth bass: 57.1-374.2 mg/L NO2-N
Channel catfish: 2.7-28.7 mg/L NO2-N
Tilapia: 11.9-77.9 mg/L NO2-N

Renewal of test solution: None, static test
Exposure vessel type: 30 L glass aquaria
Number of replicates, fish per replicate: Three or more replicates; 10 fish per dose (largemouth bass and channel catfish), 5 fish per dose (Tilapia).
Water parameters: See Table 1

Intensity of irradiation: Room light
Photoperiod: No data
Feeding: No
Aeration: Constant aeration to maintain dissolved oxygen levels near saturation.

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured every 24 hours

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Conclusion: The toxicity of nitrite varies considerably among species tested under similar water quality conditions. These differences are related to the the extent to which nitrite is concentrated in the plasma and the associated blood methemoglobin levels. Therefore, channel catfish have a lower resistance to nitrite than tilapia or largemouth bass becasue nitrite is more concentrated in catfish plasma. among the three species, largemouth bass has the greatest resistance to environmental nitrite as well as the lowest methemoglobin levels due to lower plasma nitrite concentrations. The low plasma nitrite levels may be indicative of a more selective chloride uptake cell located in the gills, resulting in exclusion of nitrite.
Reliability: (2) valid with restrictions
Well-reported literature study
Flag: Critical study for SIDS endpoint
06-MAY-2005 (141)

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 5.62

Method: other: Bortz (1977)
Year: 1977
GLP: no

Method: METHOD FOLLOWED: Based on Doudoroff et al (1951), Doudoroff (1971) and Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: none

METHOD OF CALCULATION: LC50 calculated by the Doudoroff method (1971)

ANALYTICAL METHODS: not applicable

References:
Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) Methods for Acute Toxicity Tests with fish, Macroinvertebrates and Amphibians. EPA Report 660-3-75-009. page 61

Doudoroff P (1971) In, American Public Health Association: Standard Methods for the Examination of Water and Waste Water. 13th edition. 562-580

Doudoroff P, Anderson BG, Burdick, GE, Galtsoff, PF, Hart WB, Patrick R, Strong, ER, Surber EW and VanHorn WM (1951) Bioassay Methods for the Evaluation of Acute Toxicity of Industrial Waastes to Fish. Sewage and Industrial Wastes, 23, 1380-1397
Result: Expressed as $NaNO_2$:

LC50 (96h) = 27.7 mg/L
Test condition:

TEST ORGANISMS:

Size: Weight: 40.9-199.5 g; Length 17.2-27.1 cm
Age: no data
Pretreatment: Fish were quarantined in two 150 gallon fiberglass tanks for at least ten days prior to testing. Fish were fed a diet of trout pellets. Incoming, dechlorinated city water (activated charcoal filter) with a flow rate of approximately 25 gallons per hour was chilled to 12.9+/-0.9°C.

Supplier: Eastern Fish Disease Laboratory and Hatchery, Leetown, West Virginia.

DILUTION WATER:
Source: Dechlorinated city water
Water parameters:
Hardness (mg/L CaC03); 137 (128-148)
Dissolved O2 (mg/L CaC03); 9.0 (7.3- 9.0)
Alkalinity (mg/L CaC03); 75 (66-78)
pH; 6.99 (6.90-7.10)
Temperature: 12.9 (12.0-13.8) °C

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: Stock solution concentration of 63.6 g/L sodium nitrite used. Delivered to the testing tanks using a diluter with a mean flow rate of 380 mL/min.

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: 0, 2.62, 4.52, 6.21, 9.48 and 14.87 mg/L NO2-N
Renewal of test solution:
Exposure vessel type: 54 L fiberglass tanks.
Number of replicates, fish/replicate: 6 test vessels per concentration, 10 fish/test vessel.
Test temperature: 12.0-13.8°C
Dissolved oxygen: 9.0 (7.3-9.0) mg/L O2
pH: 6.99 (6.90-7.10)
Alkalinity: 75 (66-78) mg/L CaCO3
Hardness: 137 (128-148) mg/L CaCO3
Chlorine: <100 µg/L
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no data
Aeration: no data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: No
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade
Reliability: (2) valid with restrictions
Peer reviewed Masters Thesis
13-MAY-2005 (21)

Type: semistatic

Species: other: Paralichtys orbignyanus (flatfish)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
96-h LC50 (winter) :
= 24.01
96-h LC50 (summer) :
= 30.57

Method: other: Bianchini et al method
Year: 1996
GLP: no

Method: METHOD FOLLOWED: Bianchini et al method.

According to the frequent seasonal extremes of temperature and salinity of the estuarine and coastal water where flatfishes were captured, two experimental conditions were established: the 'winter condition' (0% salinity and 12°C) and the 'summer condition' (30% salinity and 25°C).

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
LC50 values determined using the Trimmed Spearman Karber method.

METHOD OF CALCULATION:
No data

ANALYTICAL METHODS: Nitrite concentration determined using the method described by Bendschneider and Robinson (1952)

References:
Bendschneider K and Robinson RJ (1952) A New Spectrophotometric Method for Determination of Nitrite in Sea Water. J Mar Res, 11, 87-96

Result: Expressed as NaNO2:

LC50 = 118.3 - 150.7 mg/L

Test condition: TEST ORGANISMS:

Size: Weight: 88+/-17 g
Age: Juveniles
Pretreatment: Fish were acclimated in 1000 L tanks to 30% salinity and 20°C for 15 days. Water was continuously aerated and the photoperiod was 12L:12D. fish were fed ad libitum with fry of mullet Mugil platanus or silverside Odonthestes sp. and small crustaceans like the shrimps Penaeus paulensis and Artemesia longinaris. The acclimation medium was 50% renewed

daily.
Supplier: Wild fish captured at the Cassino beach or at the estuary of the Patos Lagoon in Southern Brazil.

DILUTION WATER:
Source: no data
Chemistry: no data
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:

Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: 'winter condition' 0, 15, 20, 25, 30, 40, 60, 90 or 120 mg/L NO2-N; 'summer condition' 0, 10, 20, 25, 30, 40, 50 or 60 mg/L N)2-N.
Renewal of test solution: Semi static test, 100% renewal daily.
Exposure vessel type: 30 L plastic tanks
Number of fish per dose: 10
Test temperature: 'summer' and 'winter' conditions as described in method.
Dissolved oxygen:
pH:
Alkalinity:
Hardness:
Intensity of irradiation:
Photoperiod: 12 h light, 12 h dark.
Feeding: No
Aeration: Continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Test substance: Chemical name: Sodium Nitrite (CAS No: 7632-00-0)
Purity: Pro Analytical
Supplier: Merck

Reliability: (2) valid with restrictions
16-MAY-2005 (16)

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 : = .19 - .39

Method: other: Russo et al method
Year: 1974

Method: METHOD FOLLOWED: Russo et al own method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
No data

METHOD OF CALCULATION:
No data

ANALYTICAL METHODS: Nitrite concentration determined using the method described in Strickland and Parsons (1972)

References:
Strickland JDH and Parsons TR (1972) A Practical Handbook of

Seawater Analysis. Bull. Fish Res. Board Can. 167, 77-80

Result: Table 1: Acute toxicity of nitrite to rainbow trout

Avg wt of fish (g)	Concn range tested (mg/L No2-N)	LC50 (mg/L NO2-N)	LC50 (mg/L NaNO2)
2.3	0.07 - 1.09	0.39	1.92
11.9	0.10 - 0.34	0.19	0.94
12.1	0.11 - 0.37	0.22	1.08
14	0.06 - 0.90	0.27	1.33
235	0.06 - 0.32	0.20	0.99

Test condition: TEST ORGANISMS:

Size: Weight: 2 - 235 g
Age: no data
Pretreatment: Acclimation time for the fish to their surroundings differed among the tests, but in all cases test fish were acclimated to the test dilution water for at least one week and to the test tanks for at least 2 days.
Supplier: No data - hatchery-reared.

DILUTION WATER:
Source: Ground spring water
Chemistry: no data
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: See Table 1
Renewal of test solution: Turnover time approximately 5 hours (2 hours for 235 g fish)
Exposure vessel type: 64 L plastic tanks (660 L circular glassfibre tanks for 235 g fish)
Number of replicates, fish per replicate: 1 test vessel per concentration, 10-20 fish per vessel, depending on weight.
Test temperature: 9.5 - 12.6°C
Dissolved oxygen: 5.9 - 8.6 (mg/L)
pH: 7.8 - 8.1
Alkalinity: 169 - 195 mg/L CaCO3
Hardness: 197 - 200 mg/L CaCO3
Intensity of irradiation: Room light
Photoperiod: no data
Feeding: 2 and 12 g fish not fed; 14 g fish fed 4 times daily with commercial trout feed.
Aeration: no data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 24 hour intervals

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Purity: Reagent grade
Reliability: (2) valid with restrictions
Well-reported literature study

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 (72h; lowest) :
= .22
LC50 (96h; lowest) :
= .19

Method: other: Russo & Thurston
Year: 1977

Method: METHOD FOLLOWED: Russo & Thurston method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
LC50 values and their 95% confidence limits were calcualted from the experimental data using the Spearman-Karber method.

METHOD OF CALCULATION:
No data

ANALYTICAL METHODS: Nitrite concentration determined using the method described by EPA (1974)

References:
US EPA (1974) Methods for Chemical Analysis of Water and Wastes. EPA-625-/6-74-003. Methods Development and Quality Assurance Research Laboratory, National Environmental research Center, Cincinnati, OH. 215-216
Result: Expressed as NaNO2:

LC50 (96h) = 0.94 - 1.38 mg/L

Expressed as NO2-N:

Case : Age/length; 20.6 g, 11.8 cm: Water parameters;
Temperature 10-10.2, pH = 7.94-8.12
72h LC50 = 0.29 (0.24 - 0.36) mg/L

Case : Age/length; 24.3 g, 12.3 cm: Water parameters;
Temperature 10.1-10.3, pH = 7.99-8.33
72h LC50 = 0.32 (0.27 - 0.38) mg/L
96h LC50 = 0.28 (0.24 - 0.33) mg/L

Case : Age/length; 53.1 g, 15.7 cm: Water parameters;
Temperature 9.7-10, pH = 7.58-7.79
72h LC50 = 0.35 (0.29 - 0.41) mg/L
96h LC50 = 0.27 (0.22 - 0.33) mg/L

Case : Age/length; 60.5 g, 16.6 cm: Water parameters;
Temperature 9.7-9.9, pH = 7.71-7.83

72h LC50 = 0.30 (0.25 - 0.36) mg/L
96h LC50 = 0.27 (0.23 - 0.32) mg/L

Case : Age/length; 188 g, 23.6 cm: Water parameters; Temperature 10.3-10.7, pH = 7.76-7.86
72h LC50 = 0.22 (0.17 - 0.28) mg/L
96h LC50 = 0.19 (0.15 - 0.25) mg/L

Test condition:

TEST ORGANISMS:

Size: Weight: See RESULTS
Age: no data
Pretreatment: Fish were acclimated to the test tanks for at least 2 days prior to testing.
Supplier: Bozeman (Montana) Fish Cultural Development Centre, US Fish and Wildlife Service

DILUTION WATER:
Source: Ground spring water
Chemistry: no data
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: See RESULTS
Renewal of test solution: Approximately 5-6 hours (64 L tanks) or 1.3 hours (350 L tanks)
Exposure vessel type: 64 L plastic tanks or 350 L fibreglass tanks
Number of replicates, fish per replicate: I tank per dose, 10 - 20 fish/tank.
Test temperature: See RESULTS
Dissolved oxygen: 7.9 - 10.0 mg/L
pH: See RESULTS
Alkalinity: 171 - 191 mg/L CaCO3
Hardness: 188 - 207 mg/L CaCO3
Intensity of irradiation: Room light
Photoperiod: no data
Feeding: No
Aeration: no data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (2) valid with restrictions
Well-reported literature study

25-JUL-2005 (152)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes

LC0: = .54
LC50: = .79
Limit Test: no

Method: other
Year: 2000
GLP: no

Method: METHOD: Based on ASTM (1989) Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. Annual Book of ASTM Standards, 11.04, 360-379, ASTM, Philadelphia

METHOD OF CALCULATION: Median lethal concentration and the 95% confidence intervals were calculated by the moving-average angle method (Peltier and Weber, 1985)

Result: Expressed as NaNO2:

LC50 (96h) = 3.89 mg/L
LC0 (96h) = 2.66 mg/L

Test condition: TEST ORGANISMS:

Size: Weight: 251 +/- 63 mg; Length: 33 +/- 3 mm.
Age: 40 days posthatch juveniles
Pretreatment: The fish were cultured in well water that was maintained at 12-13 degree C, as described in Buhl and Hamilton (1998). The chemical characteristics of the culture water, which were monitored weekly according to standard procedures (APHA et al. 1989), were as follows: hardness 336-1,060 mg/L as CaCO3; alkalinity 204-289 mg/L as CaCO3; pH 7.4-7.8; and conductivity 903-1,890 µS/cm at 25 degree C. Dissolved oxygen concentrations were greater than or equal to 66% of the saturation.
Supplier: Ennis National Fish Hatchery (Ennis, Montana)

DILUTION WATER:
Source: Standardized reconstituted soft water (ASTM 1989)
Chemistry: Hardness 41 (40-42) mg/L as CaCO3; alkalinity 31 (30-32) mg/L as CaCO3; PH 7.1 (7.0-7.2); conductivity 159 (154-161) µmhos/cm at 25 degree C.
Temperature: 12+/-1°C

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Measured concentrations of NO2-N at 96h were 79-89% of initial values.

REFERENCE SUBSTANCE: None

TEST SYSTEM (LC50 Determinations):
Concentrations: no data

Renewal of test solution: None, static test
Exposure vessel type: no data
Number of replicates, fish per replicate: no data
Water parameters:

Intensity of irradiation: Room light

Photoperiod: No data
Feeding: No
Aeration: no data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at start of test and at 96h
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: No data
Supplier: J. T. Baker (Phillipsburg, New Jersey)
Reliability: (2) valid with restrictions
13-MAY-2005 (28)

Type: flow through
Species: other: Oncorhynchus clarki
Exposure period: 36 day(s)
Unit: mg/l Analytical monitoring: yes
LC50(36d, lower) :
= .37
LC50(11d, lowest) :
= .39
LC50(96h, lower) :
= .48

Method: other: Thurston et al
Year: 1978

Method: METHOD FOLLOWED: Thurston et al own method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
No data

METHOD OF CALCULATION: LC50 values calculated using a computer program developed for the trimmed Spearman-Karber method.

ANALYTICAL METHODS: Nitrite concentration determined using the method described in Strickland and Parsons (1972)

References:
Strickland JDH and Parsons TR (1972) A Practical Handbook of Seawater Analysis. Bull. Fish Res. Board Can. 167, 77-80
Result: Expressed as Sodium nitrite:

Test 1:

LC50 (36 d) = 1.87 mg/L

Test 2:

LC50 (11 d) = 2.66 mg/L
LC50 (36 d) = 1.82 mg/L

Test 3:

LC50 (96h) = 2.76 mg/L
LC50 (11d) = 2.31 mg/L

Test 4:

LC50 (96h) = 2.37 mg/L
LC50 (11d) = 1.92 mg/L

There was no significant difference between the two fish sizes tested in their susceptibility to nitrite. Most of the test fish that died changed from their normal light grey colour to black prior to death. Surviving fish showed a correlation between degree of darkening and nitrite concentration.

Test condition:

TEST ORGANISMS:

Size: Tests 1 & 2 = 1g; Tests 3 & 4 = 3g
Age: Fry
Supplier: Arlee, Montana, State Fish Hatchery

PRETREATMENT: Fish were acclimated to the bioassay tanks for 2-4 days prior to testing. Fish fed a commercial salmon ration 3 times/day.

DILUTION WATER:
Source: Ground spring water
Chemistry: Hardness 199 mg/L as CaC03; Alkalinity 176 mg/L as CaC03; pH 8.0
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: no data

REFERENCE SUBSTANCE: None

TEST SYSTEM (LC50 Determinations):
Concentrations: Test 1, 0.16-0.50 mg/L NO2-N; Test 2, 0.19-0.59 mg/L NO2-N; Test 3, 0.19-2.86 mg/L NO2-N; Test 4, 0.29-1.00 mg/L NO2-N.

Renewal of test solution: Flow rate 500 mL every 3-4 minutes (equivalent to a tank change every 8 hours)
Exposure vessel type: 62 L tanks
Number of replicates, fish per replicate: One replicate, 20 fish per tank (Tests 1&2); 10 fish per tank (Tests 3&4)
Water parameters: See Table 1

Intensity of irradiation: Room light
Photoperiod: No data
Feeding: Commercial salmon ration 3 times/day
Aeration: yes

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Table 1: Test Water Parameters

Test No.	Temp (°C)	pH	Dissolved

	Mean (range)	Mean (range)	oxygen (mg/L) Mean (range)
1	12.4 (11.5-13.0)	7.85 (7.75-7.97)	8.5 (7.8-7.9)
2	12.4 (11.7-13.3)	7.88 (7.78-7.98)	8.6 (7.9-9.0)
3	11.8 (11.7-12.0)	7.88 (7.83-7.96)	8.6 (8.2-8.9)
4	12.1 (11.9-12.3)	7.80 (7.72-7.88)	8.1 (7.3-8.4)

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (2) valid with restrictions
Well reported literature study

20-MAY-2005 (177)

Type: semistatic
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 : = .3 - 31.2

Method: other
Year: 1978

Method: Effect: mortality
Fresh water (soft water; temperature; 10 degree C, hardness 25 mg/L CaC03)
Age/Weight: juvenile, 5, 10, 15 grams

Remark: Expressed as sodium nitrite:

LC50 (96h) = 1.49 - 153.8 mg/L

Result: Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness 25 mg/L (CaCO3), pH = 6.2
LC50 = 0.5 (0.4 - 0.6) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters; Hardness 25 mg/L (CaCO3), pH = 6.2
LC50 = 0.9 (0.8 - 1.1) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness 50 mg/L (CaCO3), pH = 6.8
LC50 = 0.5 (0.3 - 0.6) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters; Hardness 50 mg/L (CaCO3), pH = 6.8
LC50 = 1.9 (1.7 - 2.7) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness 150 mg/L (CaCO3), pH = 7.3
LC50 = 5.8 (5.0 - 6.7) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters; Hardness 150 mg/L (CaCO3), pH = 7.3
LC50 = 0.5 (0.4 - 0.6) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness 300 mg/L (CaCO3), pH = 7.8
LC50 = 10.3 (8.5 - 12.5) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters;
Hardness 300 mg/L (CaCO3), pH = 7.8
LC50 = 12.1 (10.7 - 13.8) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness
50 mg/L (CaCO3), pH = 6.0
LC50 = 0.3 (0.2 - 0.4) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters;
Hardness 50 mg/L (CaCO3), pH = 6.0

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LC50 = 1.5 (1.2 - 1.8) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness
50 mg/L (CaCO3), pH = 7.0
LC50 = 2.3 (1.0 - 2.9) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters;
Hardness 50 mg/L (CaCO3), pH = 7.0
LC50 = 1.9 (1.7 - 2.7) mg/L

Case : Age/weight; Juvenile, 5 g: Water parameters; Hardness
50 mg/L (CaCO3), pH = 8.0
LC50 = 2.5 (2.3 - 2.8) mg/L

Case : Age/weight; Juvenile, 10 g: Water parameters;
Hardness 50 mg/L (CaCO3), pH = 8.0
LC50 = 3.6 (3.1 - 4.2) mg/L

Case : Age/weight; Juvenile, 15 g: Water parameters;
Hardness 50 mg/L (CaCO3), pH = 6.8
Salt level; 0 - 200 mg/L
Total chloride presentation; NaCl 1.0-124 (mg/L)
LC50 = 0.54 - 1.5 mg/L
Total chloride presentation; CaCl2 1.9-130.5 (mg/L)
LC50 = 0.64 - 31.2 mg/L

Test condition: -Water parameters:
Temperature; 10 degree C
Hardness (mg/L CaC03); 25, 50, 150, 300
Dissolved O2 (mg/L); 8-10
pH; 6.0, 6.2, 6.8, 7.0, 7.3, 8.0

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade

Reliability: (2) valid with restrictions
23-MAY-2005 (193)

Type: flow through
Species: Anguilla anguilla (Fish, fresh water, marine)
Exposure period: 50 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = 15
Limit Test: no

Method: other
Year: 1996
GLP: no

Remark: 50-d LC0 = 73.9 mg/L*

* : The values were converted NO2-N into NaNO2.

Result: 50-d NOEC = 15.0 mg/L NO2-N

The sublethal effects of nitrite on growth and feed utilization were evaluated in a feeding trial lasting 77 days, divided into an acclimation period and two experimental periods. Eels of 24 g on average were divided over 20 aquaria, connected to five separate recirculation systems. In each system. the desired nitrite concentration level was maintained by water suppletion and continuous addition of NaNO2. Fish were continuously exposed to levels of 0, 1, 5, 10 or 20 g m/L NO2-N. Half of the experimental groups were fed ad libitum to study effects on feed intake, while the other half were fed a restricted ration to study effects on feed utilization. At the start and end or each experimental period. nitrite in the blood plasma, haemoglobin and methaemoglobin were measured, Fish weight and body composition were used to calculate specific growth rate and conversion efficiencies.

No significant relationship between mortality and the level of nitrite could be demonstrated. The mortality during the experiment was relativively high and highly variable between individual tanks. The mortality increased in all treatments during the experiment. Mortality resulted exclusively from agonistic behaviour between fish, a well-known phenomenon when keeping eels in relatively low densities.

Tn the range of concentrations studied, no significant effect of nitrite on maximum growth rate or feed utilization couldh be demonstrated. At the start of the experiment, low concentrations of nitrite were detected in the blood plasma, which suggests an ability of the eel to adapt to environmental nitrite. Nitrite. in the range normally encountered in intensive eel farms (max. 15 g/m3 NO2-N). can therefore be considered a factor of little significance.

Test condition: Experimental set-up
ln five independent recirculation system, each equipped with four aquaria (40 L), five different target levels of nitrite (0, 1, 5, 10 and 2O g/m3 NO2-N) were maintained. The maximum concentration maintained was aimed at approximately 15% of the 96-h LC5O. The range applied covers the concentrations normally encountered in recirculation systems.
In each system, the fish in two aquaria were fed to satiation, to detect effects of nitrite on maximum growth rate. Fish in the other two aquaria were fed an equal and restricted ration to detect effects of nitrite on feed utilization. By feeding a restricted ration, feed waste is minimized, which enables an accurate calculation of conversion efficiencies. Moreover, confounding interactions between conversion efficiency and feeding level are eliminated.
The experiment lasted 77 days, subdivided into an acclimation period of 27 days and two experimental periods of 25 days each (periods 1 and 2). These two experimental periods allow observations on time-related effects.

Sampling and measurements
Mortality was recorded daily. At the start of each

experimental period and at the end or the experiment, fish of each aquarium were weighed and counted. Blood samples were taken and fish sampled were withheld for analysis of body composition, in which approximately 15 fish per talk were killed (2 phenoxyethanol), frozen and at a later stage, ground and homogenized. Dry matter content of fish and feed was determined by feeze-drying. Protein content was measured in dried material by determination of N-Kjeldhl (protein - 6.25 X NKi). Fat Content was determined in dried material according to Bligh and Dyer (1959). The energy content of the fish and feed tvas calculated from protein, fat and carbohydrate using conversion factors of 23.64, 39.54 and 17.15 kJ/g respectively, as determined for Clarias gariepnus.

At each sampling, blood was collected from at least 10 individuals per tank. Pump failure at the end of period 2 in the systems with 0 and 10 g/m3 nitrite, prevented sampling of blood in these groups.

Fish were anaesthetized with 2-phenoxyethanol and blood was subsequently sampled with a heparinized syringe from the vena caudalis. Blood samples were pooled per aquarium.

Haemoglobin and methaemogiobin were determined according to Evelyn and Malloy (1938) with a CO-Oximeter IL482.

Nitrite in the blood plasma was determined according to Shechter, Gruner and Shuval(1972).

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
23-MAY-2005 (100)

Type: static
Species: Ctenopharyngodon idella (Fish, fresh water)
Exposure period: 120 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 1.5
LC50 (48h) : = 4.45
LC50 (72h) : = 3.04
LC50 (96h) : = 1.71

Method: other: Alcarez & Espina method
Year: 1994
GLP: no

Method: METHOD FOLLOWED: Alcarez & Espina own method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
Not applicable

METHOD OF CALCULATION:
Median lethal concentrations were calculated using the DORES program from Ramirez (1989).

ANALYTICAL METHODS: Nitrite concentration was quantified by the AZO dye colorimetric method (APHA, 1985)

References:
American Public Health Association (1985) Standard Methods for the Examination of water and Wastewater. 16th ed. APHA,

Washington
Result: Expressed as NaNO2:

LC50 (96h) = 8.43 mg/L
LC50 (120h) = 7.39 mg/L

There was no mortality in the control group. The number of deaths in each dose group was not reported in the literature.
Test condition: TEST ORGANISMS:

Size: Weight: 0.17 - 0.75 g
Age: Juveniles
Pretreatment: The test fish were held for 15 days in 60L aquaria with tap water at 24 deg C, pH 7, 6.8 mg O2/L, alkalinity 44 mg CaCO3/L and 125 mg CL-/L.
Supplier: Centro de Produccion Piscicola, Tezontepec, Estado de Hidalgo, Mexico.

DILUTION WATER:
Source: soft water
Chemistry: pH 6.7 - 6.9, alkalinity 44 mg CaCO3/L, 6.1-6.4 mg O2/L and 3 mg CL-/L.
Temperature: 24°C

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: Control (dilution water), 1, 2, 4, 8 or 10 mg N-NO2-/L
Renewal of test solution: None - static test
Exposure vessel type: 20L glass aquaria
Number of replicates, fish per replicate: 1 test vessel per concentration, 10 fish per vessel.
Test temperature: 24 °C
Dissolved oxygen: Not reported during test
pH: Not reported during test
Intensity of irradiation: Room light
Photoperiod: 12 hours light/12 hours darkness
Feeding: No
Aeration: Mild, continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 24 hour intervals
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: 99.9%
Supplier: Merck
Reliability: (2) valid with restrictions
Well-reported literature study
16-MAY-2005 (6)

Type: semistatic

Species: Anguilla anguilla (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 143.7

Method: other
Year: 1996
GLP: no data

Method: Fish, reared from common eel, were obtained from a commercial eel farm, where the fish had been exposed to nitrite in concentrations of up to 1.5 g/m3 NO2-N, according to the farmer. The eels (21.9 +/- 5.6 g) were allowed to acclimate for 2 weeks in an aerated storage tank which was continuously supplied with IJmuiden tap water (at about 25 degree C). No mortality was observed during this period. Two days before exposure to nitrite, fish were transferred to aerated 40-L aquaria filled with tap water, which was changed every morning.

Desired concentrations were obtained by addition of NaNO2 to IJmuiden tap water. Water in the aquaria was changed once every day. Water temperature, pH and concentrations of oxygen and nitrite were measured daily. PH and oxygen were measured with electrodes (WTW, Germany). Nitrite in water as measured spectrophotometrically.
Remark: 96-h LC50 = 708.2 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: Expressed as NaNO2:

LC50 (96h) = 708.2 mg/L
Test condition: The acute toxicity of nitrite to European eel was measured by determination of the 96-h LC50 in a semi-static assay.

Each aquarium was stocked with 10 fish. A series of six aquaria with increasing concentration (0, 120, 192, 307, 492 and 786 g m-3 NO2-N) was used to determine each LC50, in triplicate.

The average values of the water temperature, pH and oxygen concentration were 21.2 degree C, 8.36 and 8.7 g/m3 respectively. The average nitrite concentration, as measured in the different treatments, was 0, 121, 193, 318, 488 and 823 g/m3 NO2-N. The concentration of the major ion species in the IJmuiden tap water was determined once in a composite sample. K and Na were measured by flame-ionization, the other species spectrophotometrically. Concentrations were: Na, 69; K, 12: Ca, 76: Mg, 15: HCO3, 134: Cl,117; SO4, 106 g/m3. Dead fish were removed from the tank daily.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Pro analysis grade

Supplier: Merck
Reliability: (2) valid with restrictions
Well reported literature study.
20-MAY-2005 (100)

Type: flow through

Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring: no
LC0: > 3.09

Method: other
Year: 1994
GLP: no

Method:
METHOD FOLLOWED: Rodriguez-Moreno and Tarazona method

Fish were exposed for 8 days and observed during an additional post-exposure period of 5 days. At 72, 192, 240 and 312 hr fish were anesthetized (2-phenoxy ethanol, 0.3%) and bled from the caudal vein using insulin heparinized syringes with 21G x 1.1/2", 0.8 x 40 No.2 Luer needles, for the measurement of methenoglobin and hematocrit. Fish were identified by their head-tail length.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: One-way analysis of variance and Student's t-tests were used to analyse data.

METHOD OF CALCULATION: none

ANALYTICAL METHODS: Nitrite concentration determined daily using the spectrophotometric method (Rodier 1981)

References:
Rodier J (1981) Analisis de las Aquas Naturales, Residuales y de Mar. Omega. Barcelona, 151-152
Remark: LC0 > 15.2 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: Expressed as NaNO2:
LC0 (8 d) >15.2 mg/L

One fish at the lowest nitrite concentration died during the post exposure period. Clinical data showed infection of the bleeding area as the cause of the death.

Hematocrits did not show significant differences through the exposure and recovery periods, nor between groups; showing a negligible effect of the periodic bleeding.

Only the highest nitrite concentration produced a significant increase in methemoglobin values, with the extreme value, near 50%, after 192 h of exposure. The recovery of methemoglobin levels was very rapid; 48 h after transfer to nitrite-free water, only a slight increase could be observed and after 120 h levels had returned to pre-exposure values and showed no differences when compared to control fish.
Test condition:
TEST ORGANISMS:

Size: length = 18.9+/-0.4 cm
Age: no data
Pretreatment: Fish were acclimated to laboratory conditions

for more than 2 weeks. Fish were fed a commercial trout food (DIBAQ AE-7) once a day.
Supplier: Una fish farm, Cuenca, Spain

DILUTION WATER:
Source: Decalcified groundwater
Chemistry: hardness 74.44+/-0.40 mg/L as CaCO3; alkalinity 195.53+/-21.88 mg/L as CaCO3; dissolved oxygen 10.80+/-0.50 mg/L; pH 7.47+/-0.41; chloride 82.36+/-0.43 mg/L as NaCl
Temperature: 14.0+/-0.5°C

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: 0, 0.68+/-0.05, 1.69+/-0.05, 3.09+/-0.11 mg NO2-N/L
Renewal of test solution: flow rate = 20.45 L/h
Exposure vessel type: 30 L glass aquaria
Number of fish per dose: 3
Test temperature: See dilution water
Dissolved oxygen: See dilution water
pH: See dilution water
Alkalinity: See dilution water
Hardness: See dilution water
Intensity of irradiation:
Photoperiod: no data
Feeding: yes
Aeration: no data

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Supplier: Merck, Germany
Reliability: (2) valid with restrictions

Well reported literature study
20-MAY-2005 (147)

Type: static
Species: Anguilla anguilla (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 150

Method: other
Year: 1979

Method: Effect: mortality
Fresh water
Concentration: total
Weight: 2.4 grams
Remark: 96-h LC50 = 739.3 mg/L*

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                  * : The values were converted NO2-N into NaNO2.
Result:           24h LC50 = 351 mg/L
                  48h LC50 = 279 (255 - 306) mg/L
                  72h LC50 = 210 (188 - 262) mg/L
                  96h LC50 = 150 (128 - 172) mg/L
Test condition:   -Water parameters:
                  Temperature; 25 degree C
                  Dissolved O2 (%); >86
                  pH; 6.9 - 7.7
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: Reagent grade
Reliability:      (2)  valid with restrictions
20-MAY-2005                                                          (202)

Type:             static
Species:          Anguilla japonica
Exposure period:  96 hour(s)
Unit:             mg/l                  Analytical monitoring: yes
LC50:             = 205

Method:           other
  Year:           1979

Method:           Effect: mortality
                  Fresh water
                  Weight: 3.6 grams
Remark:           96-h LC50 = 1010.4 mg/L*

                  * : The values were converted NO2-N into NaNO2.
Result:           24h LC50 = 460 (408 - 628) mg/L
                  48h LC50 = 299 (265 - 340) mg/L
                  72h LC50 = 245 (206 - 286) mg/L
                  96h LC50 = 205 (160 - 241) mg/L
Test condition:   -Water parameters:
                  Temperature; 25 degree C
                  Dissolved O2 (%); >86
                  pH; 6.9 - 7.7

Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: Reagent grade
Reliability:      (2)  valid with restrictions
                  Well reported literature study
20-MAY-2005                                                          (202)

Type:             flow through
Species:          Oncorhynchus mykiss  (Fish, fresh water)
Exposure period:  6 day(s)
Unit:             mg/l                   Analytical monitoring: yes
LC0:              = 69.69

Method:           other
  Year:           1996
   GLP:           no

Result:           The effects of uptake into blood plasma of NO2-, NO3-, and
                  Br- from 1 mM ambient concentrations were studied in rainbow
                  trout (Oncorhynchus mykiss). Nitrite and bromide were
                  concentrated in plasma, competing for the branchial chloride
                  uptake mechanism. Nitrate appeared to be taken up passively,
                  with plasma concentrations remaining below ambient [NO3-]
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after 8 days exposure. This limited uptake appeared central to the low toxicity of NO3-, and did not measurably influence electrolyte balance or haematology. Plasma [Br-] increased to 51 mM during 14 days, which was paralleled by a 1: 1 stoichiometrical decrease in plasma [Cl-]. This was the only detected effect of Br- exposure and was tolerated without mortality. Nitrite-exposed trout fell into two distinct groups. Trout dying before 2 days of NO2- exposure quickly developed methaemoglobinaemia; high plasma [NO2-], [lactate], and [K+]; and low [Cl-]. In trout surviving up to 4 days, plasma [NO2-] and methaemoglobin rose more slowly, plasma [Cl-] decreased less, and extracellular lactate and potassium levels were not significantly elevated. In both groups, plasma [NO3-] rose to values comparable with plasma [NO2-] (about 3 mM), reflecting an internal conversion of nitrite to nitrate. Nitrite-exposure significantly decreased skeletal muscle [K+], whereas no significant changes were observed in cardiac muscle.

Test condition: Rainbow trout were obtained from a local hatchery. Two different size-classes of fish were used in the experiments. Larger fish (weight 336 +/- 58 g, mean +/- SD, N = 33) were used in cannulation experiments, and smaller ones (weight 119 +/- 27 g, N = 93) were used uncannulated. The fish were acclimated to aerated Odense tap water for at least one month before experiments.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions

23-MAY-2005 (171)

Type: static
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 7.55

Method: other
Year: 1975

Method: Effect: mortality
Fresh water
Age/Weight: 40 grams

Result: 24h LC50 = 10.3 (8.58 - 12.3) mg/L
48h LC50 = 8.77 (7.55 - 10.2) mg/L
72h LC50 = 8.31 (7.03 - 9.83) mg/L
96h LC50 = 7.55 (6.85 - 8.37) mg/L

Test condition: -Water parameters:
Temperature; 21 degree C
Dissolved O2 (mg/L); >7.4
Alkalinity (mg/L CaC03); 50-70
pH; 7.2-7.8

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions

23-MAY-2005 (107)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 (72h; lower) :
= 3.94

LC50 (96h; lower) :
= 2.3

Method: other
Year: 1977

Method: Effect: mortality
Fresh water
Age/Weight: variable (see RESULT)
Remark: 96-h LC50 =11.3 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: Case : Age/length; 2.3 g, 6.2 cm: Water parameters;
Temperature 12.7-13.2, pH = 8.03-8.09
72h LC50 = 5.54 (3.86 - 7.95) mg/L
96h LC50 = 2.99 (2.35 - 3.81) mg/L

Case : Age/length; 2.3 g, 6.4 cm: Water parameters;
Temperature 12.5-12.8, pH = 7.96-8.10
72h LC50 = 3.94 (2.37 - 6.55) mg/L
96h LC50 = 2.3 mg/L
Test condition: -Water parameters:
Temperature; (see RESULT)
Hardness (mg/L CaC03); 199 (188 - 207)
Alkalinity (mg/L CaC03); 177 (171 - 191)
Dissolved O2 (mg/L); 7.9 - 10.0
pH; (see RESULT)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (2) valid with restrictions
23-MAY-2005 (152)

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring: yes
LC50 (lower val.) :
= .14

Method: other
Year: 1974

Method: Effect: mortality
Fresh water
Age/Weight: 12 grams
Remark: 8-d LC50 = 0.69 - 1.92 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: 8d LC50 = 0.14 mg/L
8d LC50 = 0.15 mg/L
Test condition: -Water parameters:
Hardness (mg/L CaC03); 199 (197-200)
Alkalinity (mg/L CaC03); 176 (169-195)
pH; 7.9 (7.8-9.1)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (2) valid with restrictions
23-MAY-2005 (153)

Type: static

Species: Lepomis cyanellus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 160

Year: 1986

Method: METHOD FOLLOWED: Tomasso own method

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: no data

METHOD OF CALCULATION: No data

ANALYTICAL METHODS: not applicable
Result: Expressed as $NaNO_2$:

LC50 (96h) = 788.6 mg/L
Test condition: TEST ORGANISMS:

Size: Weight: 7.3+/-0.3 g
Age: no data
Pretreatment: Fish were acclimated to laboratory conditions for at least one month prior to testing (temperature = 23°C; pH=7.2-7.4; hardness=300 mg/L as ca CO_3; dissolved oxygen >6.0 mg/L; alkalinity = 255 mg/L as $CaCO_3$; chloride = 22 mg/L; nitrite <0.01 mg/L; sulfate = 31 mg/L; nitrate-nitrogen = 7.4 mg/L; total phosphorous <0.1 mg/L).
Supplier: US Fish and Wildlife Service National Fish Hatchery and Technology Center (San Marcos)

DILUTION WATER:
Source: no data
Chemistry:
Temperature:

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: no data

REFERENCE SUBSTANCE: None

TEST SYSTEM (LC50 Determinations):
Concentrations: no data

Renewal of test solution: None, static test
Exposure vessel type: Glass aquaria containing either 15 or 30 L of test solution.
Number of replicates, fish per replicate: 2-8 replicates, 5 fish per replicate.
Water parameters: Oxygen near saturation; Temperature 23°C; pH 7.9-8.4; alkalinity 236+/-5 mg/L as $CaCO_3$; hardness 240+/-5 mg/L as $CaCO_3$
Intensity of irradiation: Room light
Photoperiod: No data
Feeding: No
Aeration: yes

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at start of test and at 96h

Reliability: (4) not assignable
Insufficient experimental detail

23-MAY-2005 (179)

Type: semistatic
Species: other: Dicentrarchus labrax (Sea Bass)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 90 - 100

Method: other: Scarano et al
Year: 1984

Method: METHOD FOLLOWED: Scarano et al method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: no data

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: Nitrite concentration determined using the sulfanilimide naphthalenediamine method described by Strickland and Parsons (1972)

References:
Strickland JDH and Parsons TR (1972) A Practical Handbook of Seawater Analysis, 2nd edn. Fisheries research Board of Canada Buletin, 167

Result: Expressed as $NaNO_2$:

LC50 = 443 - 492 mg/L

Test condition: TEST ORGANISMS:

Size: 12-14 cm
Age:
Pretreatment: Fish were reared in water from the same source as the test water.
Supplier: no data

DILUTION WATER:
Source: Sea water
Chemistry: As test system
Temperature: As test system

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:

Concentrations: 0, 25, 50, 75, 100 or 150 mg/L NO2-N
Renewal of test solution: Semi-static test - Seawater changed daily
Exposure vessel type: Glass aquaria
Number of fish per dose: 6
Test temperature: 26°C
Dissolved oxygen: >95% saturation
pH: 8.1
Salinity: 36%
Alkalinity:
Hardness:
Intensity of irradiation:
Photoperiod:
Feeding: No
Aeration: Continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (4) not assignable
Insufficient experimental detail

23-MAY-2005 (158)

Type: flow through
Species: Rutilus rutilus (Fish, fresh water)
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 10.1

Method: other: Solbe et al
Year: 1985

Method: METHOD FOLLOWED: Solbe et al method

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
Not applicable

METHOD OF CALCULATION:
Median lethal concentrations were calculated using methods based on probit analysis.

ANALYTICAL METHODS: none

Remark: 14-d LC50 = 49.8 mg/L*

* : The values were converted NO2-N into NaNO2.

Result: LC50 = 10.1 (8.98 - 11.2) mg/L

Test condition: -Water parameters:
Temperature; 16.1 degree C
Hardness (mg/L CaC03); 268
Alkalinity (mg/L CaC03); 186
Dissolved O2 (%); >80.0
pH; 7.40

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade

Reliability: (4) not assignable
Insufficient experimental detail

16-MAY-2005 (167)

Type: flow through
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 10 day(s)
Unit: mg/l Analytical monitoring: no
LC50: = 15.6

Method: other
Year: 1985

Method: METHOD FOLLOWED: Solbe et al method

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:
Not applicable

METHOD OF CALCULATION:
Median lethal concentrations were calculated using methods based on probit analysis.

ANALYTICAL METHODS: none
Remark: 10-d LC50 = 76.9 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: LC50 = 15.6 (13.3 - 17.9) mg/L
Test condition: -Water parameters:
Temperature; 14.2 degree C
Hardness (mg/L CaC03); 281
Alkalinity (mg/L CaC03); 195
Dissolved O2 (%); 98.8
pH; 7.62
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade
Reliability: (4) not assignable
Insufficient experimental detail
16-MAY-2005 (167)

Type: static
Species: other: Micropterus treculi
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 187.6

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: 6.5 grams
Remark: 96-h LC50 = 924.6 mg/L*

* : The values were converted NO2-N into NaNO2.
Test condition: -Water parameters:
Temperature; 22 degree C
Hardness (mg/L CaC03); 203-222
Alkalinity (mg/L CaC03); 163-183
pH; 7.9-8.4
Reliability: (4) not assignable

Insufficient experimental detail
20-MAY-2005 (180)

Type: static
Species: Gambusia affinis (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 7.5

Method: other: Wallen et al
Year: 1957

Test condition: TEST ORGANISMS:

Size: no data
Age: Adult females
Pretreatment: Fish kept in the laboratory for 2-3 weeks prior to tests. Terramycin added to water to eliminate tail rot. fish fed on plankton and detritus collected locally, along with various artificial foods.
Supplier: fish collected from stillwater Creek, Payne County, OK

DILUTION WATER:
Source: Collected from local farm pond.
Chemistry: pH 7.8-8.3
Temperature: room temperature

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: No data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: 0, 10, 18, 32, 56 100 ppm
Renewal of test solution: Static test
Exposure vessel type: Pyrex jars, 12 inches high by 12 inches in diameter containing 15L of water
Number of fish per dose: 10
Test temperature: 21-24°C
Dissolved oxygen:
pH: 7.1-7.5
Alkalinity: <100 mg/L CaCO3
Hardness:
Intensity of irradiation:
Photoperiod:
Feeding: No
Aeration: Continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: no
Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Purity: stated as chemically pure
Supplier: no data
Reliability: (4) not assignable
Insuficient experimental detail

20-MAY-2005 (192)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 9.8

Method: other:Eddy et al
Year: 1983

Method: Effect: mortality
Fresh water
Age/Weight: 10-25 grams
Test condition: -Water parameters:
Temperature; 10 degree C
pH; 7.0
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (3) invalid
19-MAY-2005 (51)

Type: static
Species: Salmo salar (Fish, fresh water, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (fresh water) :
= 10.08
LC50 (salt water) :
= 315

Method: other: Eddy et al
Year: 1983

Method: Effect: mortality
Salt water, Fresh water
Test condition: Fresh water:
-Water parameters:
Temperature; 10 degree C

Salt water:
-Water parameters:
Temperature; 10 degree C
Salinity; 16 ppt
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (3) invalid
19-MAY-2005 (51)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (lower) : = 20

Method: other: Ewell et al
Year: 1986

Method: Effect: mortality
Fresh water

Age/Weight: juvenile, 0.2-0.5 gram
Result: LC50 > 20 mg/L
LC50 = 20 mg/L

The lower value was = 20 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
Non standard method involving simultaneous exposure of a number of species

19-MAY-2005 (57)

Type: static
Species: Perca fluviatilis (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 82.8

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: 20-40 grams
Remark: Purpose of this study;
This study using whole animals was conducted to explore the relationship between chloride and nitrite uptake and to relate this to the toxicity of nitrite for a variety of fresh water teleosts, using rainbow trout and perch as the principal examples.
Therefore, there were only 24h LC50 values.
Test condition: -Water parameters:
pH; 6.9-7.4
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (3) invalid
20-MAY-2005 (198)

Type: static
Species: Tinca tinca (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 3450

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: 113-168 grams
Remark: Purpose of this study;
This study using whole animals was conducted to explore the relationship between chloride and nitrite uptake and to

relate this to the toxicity of nitrite for a variety of fresh water teleosts, using rainbow trout and perch as the principal examples.
Therefore, there were only 24h LC50 values.
Test condition: -Water parameters:
pH; 6.9-7.4
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (3) invalid
20-MAY-2005 (198)

Type: static
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 2415

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: 2-78 grams
Remark: Purpose of this study;
This study using whole animals was conducted to explore the relationship between chloride and nitrite uptake and to relate this to the toxicity of nitrite for a variety of fresh water teleosts, using rainbow trout and perch as the principal examples.
Therefore, there were only 24h LC50 values.
Test condition: -Water parameters:
pH; 6.9-7.4
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (3) invalid
20-MAY-2005 (198)

Type: static
Species: Anguilla anguilla (Fish, fresh water, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 5520

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: 59-138 grams
Remark: Purpose of this study;
This study using whole animals was conducted to explore the relationship between chloride and nitrite uptake and to relate this to the toxicity of nitrite for a variety of fresh water teleosts, using rainbow trout and perch as the principal examples.
Therefore, there were only 24h LC50 values.
Test condition: -Water parameters:
pH; 6.9-7.4
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade

Reliability: (3) invalid
20-MAY-2005 (198)

Type: static
Species: other: Diplodus sargus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 1360
EC50 : = 330

Method: other
Year: 1980

Method: Effect: feeding behavior for EC50, mortality for LC50
Salt water
Age/Weight: larvae
Result: EC50 = 330 (270 - 410) mg/L
LC50 = 1360 (1170 - 1590) mg/L
Test condition: -Water parameters:
Temperature; 15 degree C
Dissolved O2 (mg/L); 7.9-8.1
Alkalinity (mg/L CaCO3); 108-123
pH; 7.79-7.85
Salinity; 34.4-35.7 ppt
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: No data
Reliability: (3) invalid
20-MAY-2005 (27)

Type: static
Species: other: Gaidropsarus capensis
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 2210
EC50 : = 450

Method: other
Year: 1980

Method: Effect: feeding behavior for EC50, mortality for LC50
Salt water
Age/Weight: larvae
Result: EC50 = 450 (380 - 530) mg/L
LC50 = 2210 (1900 - 2560) mg/L
Test condition: -Water parameters:
Temperature; 15 degree C
Dissolved O2 (mg/L); 7.9-8.1
Alkalinity (mg/L CaCO3); 108-123
pH; 7.79-7.85
Salinity; 34.4-35.7 ppt
Test substance: Chemical name: Sodium nitrite (CAS No.. 7632-00-0)
Purity: no data
Reliability: (3) invalid
20-MAY-2005 (27)

Type: static
Species: other: Heteromycteris capensis
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 2440

EC50 : = 340

Method: other
Year: 1980

Method: Effect: feeding behavior for EC50, mortality for LC50
Salt water
Age/Weight: larvae
Result: EC50 = 340 (270 - 440) mg/L
LC50 = 2440 (2200 - 2690) mg/L
Test condition: -Water parameters:
Temperature; 15 degree C
Dissolved O2 (mg/L); 7.9-8.1
Alkalinity (mg/L CaCO3); 108-123
pH; 7.79-7.85
Salinity; 34.4-35.7 ppt
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: no data
Reliability: (3) invalid
20-MAY-2005 (27)

Type: static
Species: other: Lithognathus mormyrus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 1230
EC50 : = 360

Method: other
Year: 1980

Method: Effect: feeding behavior for EC50, mortality for LC50
Salt water
Age/Weight: larvae
Result: EC50 = 360 (260 - 480) mg/L
LC50 = 1230 (1020 - 1470) mg/L
Test condition: -Water parameters:
Temperature; 15 degree C
Dissolved O2 (mg/L); 7.9-8.1
Alkalinity (mg/L CaC03); 108-123
pH; 7.79-7.85
Salinity; 34.4-35.7 ppt
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: no data
Reliability: (3) invalid
20-MAY-2005 (27)
date: 04-JAN-2006

Type: static
Species: other: Synaptura kleini
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 2110
EC50 : = 350

Method: other
Year: 1980

Method: Effect: feeding behavior for EC50, mortality for LC50
Salt water

	Age/Weight: larvae
Result:	EC50 = 350 (250 - 500) mg/L
	LC50 = 2110 (1670 - 2670) mg/L
Test condition:	-Water parameters:
	Temperature; 15 degree C
	Dissolved O2 (mg/L); 7.9-8.1
	Alkalinity (mg/L CaC03); 108-123
	pH; 7.79-7.85
	Salinity; 34.4-35.7 ppt
Test substance:	Chemical name: Sodium nitrite (CAS No. 7632-00-0)
	Purity: no data
Reliability:	(3) invalid

20-MAY-2005 (27)

Type:	static
Species:	Ictalurus punctatus (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l Analytical monitoring: no
LC50:	= 1.3
Method:	other
Year:	1976
Method:	Effect: mortality
	Fresh water
	Age/length: fingerling, 50-76 mm
Result:	Water temperature 22 and 30 degree C: both LC50s were 1.3 mg/L.
	The 0.048 and 0.083 values as LC50 were reported in this report. These data vary widely. This repot was assigned as (3) invalid.
Test condition:	-Water parameters:
	Temperature; 22, 30 degree C
	Hardness (mg/L CaC03); 102
	Alkalinity (mg/L CaC03); 220
	pH; 8.6-8.8
Test substance:	Chemical name: Sodium nitrite (CAS No. 7632-00-0)
	Purity: Reagent grade
Reliability:	(3) invalid

20-MAY-2005 (42)

Type:	semistatic
Species:	Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period:	10 day(s)
Unit:	mg/l Analytical monitoring: yes
Method:	other: APHA 1965
Year:	1974
Method:	Effect: Tolerance
	Fresh water
	Life stage/length/weight: fingerling, 1.50-10.55 grams, 51-103 mm
Result:	TLm (96h) = 2.9 ppm NO2
	TLm (7d) = 2.4 ppm NO2
Test condition:	-Water parameters:
	Temperature; 13.6 - 15.6 degree C
	Dissolved O2 (mg/L); 7
	pH; 6.8 - 7.2

Reliability: (3) invalid
20-MAY-2005 (195)

Type: static
Species: Morone saxatilis (Fish, estuary, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 163 measured/nominal

Method: other
Year: 1991
GLP: no

Result: Striped bass mortality showed a clear dose-response relation to NO2- concentration.
The 24-h LC50 value was 163.0 +/-8.8 mg NO2-/L.
As NO2- in the water was increased from 150 mg/L to 175 mg/L, mortality increased sharply from 20 % to 80%.
Test condition: Striped bass averaging 27 +/-2 cm (SE) in standard length and 250 +/-4.4 g in live weight were maintained at the Southeastern Fish Cultural Laboratory, Marion, Alabama, for at least 4 weeks in 1,200-L fiberglass tanks supplied with aerated well water at a flow rate of two turnovers per hour. Physicochemical characteristics of the water were temperature 23 degree C, pH 7.5, hardness 106 mg/L as calcium carbonate, alkalinity 108 mg/L as calcium carbonate, dissolved oxygen >6.0 mg/L, Cl- 38.6 mg/L, NO2- and ammonia <0.01 mg/L. Fish were fed commercial food (40% protein) daily; feeding was discontinued 48 h before the fish were transferred to experimental tanks.
Survival of striped bass during exposure to NO2- (added as sodium nitrite) was determined in triplicate in a series of nine treatments in which the NO2- concentration ranged from 50 to 250 mg/L increments of 25 mg/L. In an experiment to evaluate the protective effects of Cl-, either CaCl2 (62.5-5,000 mg/L as Cl-) or NaCl (500-2,500 mg/ L as Cl-) was added to tanks containing 250 mg NO2-/L, a concentration normally lethal to striped bass in fresh water. Nitrite (250 mg/L) and freshwater controls were included in triplicat
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: no data
Reliability: (4) not assignable
20-MAY-2005 (117)

Species: other: Seriola quinqueradiata
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: > 147

Method: other: APHA/AWWA/WPCF 1981
Year: 1991
GLP: no

Result: Mortality
40 % at 147 mg/L for 96
Therefore, 96-h LC50 is greater than 147 mg/L.
Test condition: Temperature 25.5 +/- 1 degree C
juvenile 20g, 10/group
pH; 8.0 - 8.2
Salinity 32.2 - 33.3

Test substance: Substance name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Supplier: WAKO Chemical, Japan
Reliability: (4) not assignable
20-MAY-2005 (172)

Type: static
Species: Morone sp.
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (a case as water condition: accumated salinity; 1g/L) :
= 35 measured/nominal
LC50 (a case as water condition: accumated salinity; 8g/L) :
> 100 measured/nominal
LC50 (overall all calcium conc. tested) :
= 12.8 measured/nominal

Method: other
Year: 1993
GLP: no

Result: Environmental calcium did not affect nitrite toxicity, and the 96-h LC50 of nitrite-nitrogen (nitrite-N) was 12.8 +/- 1.6 mg/L (mean +/- SE) over all calcium concentrations tested. The 96-h LC50 of nitrite-N for fish acclimated to a salinity or 1 g/L was 35.0 +/- 2.3 mg/L (mean +/- SE), whereas LC50s of nitrite-N for fish acclimated to salinities of or higher than 8 g/L were greater than 100 mg/L (the highest exposure level).
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (4) not assignable
20-MAY-2005 (194)

Species: Leuciscus idus (Fish, fresh water)
Unit: mg/l Analytical monitoring:
LC0: = 165
LC50: = 360
LC100: = 462

Method: other
Year: 1978

Test substance: Chemical name: Sodium nitrite (CAS No 7632-00-0)
Reliability: (4) not assignable
20-MAY-2005 (99)

Species: Leuciscus idus (Fish, fresh water)
Unit: mg/l Analytical monitoring:
LC0: = 300
LC50: = 565
LC100: = 700

Method: other
Year: 1978

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
20-MAY-2005 (99)

Type: static
Species: Cynoscion nebulosus (Fish, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 980

Method: other
Year: 1987

Method: Effect: mortality
Salt water
Life stage: egg-larvae
Test condition: -Water parameters:
Temperature; 26-27 degree C
Salinity; 25-30 ppt
pH; 7.45-8.0
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (3) invalid
20-MAY-2005 (44)

Type: static
Species: other: Chanos chanos
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (salt water) :
= 675
LC50 (fresh water) :
= 12

Method: other
Year: 1987

Method: Effect: mortality
Salt water, Fresh water
Age/Weight: juvenile, 31 grams
Result: Salt water:
LC50 = 675 (435.8 - 1045.4) mg/L

Fresh water:
LC50 = 12 (7.4 - 19.6) mg/L
Test condition: -Water parameters:
Temperature; 27.4-27.8 degree C
Hardness (mg/L CaC03); 203-222
Salinity; 16 ppt
pH; 8.0-8.5
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (3) invalid
20-MAY-2005 (8)

Type: static
Species: Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 5.8

Method: other
Year: 1977

Method: Effect: mortality

```
                  Fresh water
                  Age/Weight: fingerling, 13.4 grams
Result:           LC50 = 5.8 (3.0 - 11.0) mg/L
Test condition:   -Water parameters:
                  Temperature; 9.1 (9.0-9.4) degree C
                  pH; 7.8 (7.8-7.9)
Test substance:   Chemical name: Sodium nitrite
                  Purity: Reagent grade
Reliability:      (3)  invalid
20-MAY-2005                                                          (43)

Type:             static
Species:          Semolitus atromaculatus  (Fish, fresh water)
Exposure period:  24 hour(s)
Unit:             mg/l                   Analytical monitoring: no
LC0:              = 81
LC100:            = 400

Method:           other
  Year:           1952

Method:           Effect: mortality
                  Fresh water
                  Concentration: total
                  Length: 8-10 cm
Test condition:   -Water parameters:
                  Temperature; 15-21 degree C
                  Hardness (mg/L CaC03); 98.0
                  pH; 8.3
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: Commercial grade
Reliability:      (3)  invalid
20-MAY-2005                                                          (64)

Type:             flow through
Species:          Rasbora heteromorpha  (Fish, marine)
Exposure period:  48 hour(s)
Unit:             mg/l                   Analytical monitoring: no
LC50:             = 43
LC50 (24h) :      = 77

Method:           other
  Year:           1969

Method:           Effect: mortality
                  Fresh water
                  Length: 1.3-3 cm
Test condition:   -Water parameters:
                  Temperature; 20 degree C
                  Hardness (mg/L CaC03); 250
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: 98%
Reliability:      (3)  invalid
20-MAY-2005                                                           (4)

Type:             static
Species:          Sciaenops ocellata  (Fish, marine)
Exposure period:  48 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = 85.7
```

Method: other
Year: 1989
GLP: no data

Method: Effect: mortality
Salt water
Age/Weight: fingerling, 5.6 grams
Test condition: -Water parameters:
Temperature; 24-25 degree C
Salinity; 36.0 ppt
pH; 7.4-7.7
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (3) invalid
20-MAY-2005 (199)

Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 49 day(s)
Unit: mg/l Analytical monitoring:
NOEC: = .5
Limit Test: no

Method: other
Year: 1978

Remark: Exposure to 0.1 mg NO2-/l for 6 months in freshwater caused no lethality, growth reduction, gill histological changes orhaematological dyscrasions after 7 weeks. Although 0.05 mg NO2-/l caused significant increase in methaemoglobin levels, the change was slight and not of biological significance.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
25-JUL-2005 (193)

Type: static
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 1.52

Method: other
Year: 1980

Method: Effect: mortality
Fresh water
Life stage/length: fingerling, 7-13 cm
Test condition: -Water parameters:
Temperature; 21 - 24 degree C
Hardness (mg/L CaC03); 40
Alkalinity (mg/L CaC03); 47
pH; 7.0
Test substance: Chemical name: Sodium nitrite
Purity: Reagent grade
Reliability: (3) invalid
23-MAY-2005 (181)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: semistatic
Species: other: Penaeus paulensis
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 109.4

Method: other
Year: 1996

Method: METHOD FOLLOWED: Cavalli et al method.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: LC50 calculated with the Trimmed Spearman Karber method

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: none

Result: Expressed as sodium nitrite:

LC50 (96h) = 539.2 mg/L

No mortality was observed in the control units.

-LC50 (mg NO2-N/L) and their 95% confidence limits of nitrite for
Penaeus paulensis broodstock over time.

- 77/297 -
date: 04-JAN-2006

24-h LC50 = 291.3 (279.4 - 303.7)
48-h LC50 = 146.7 (139.5 - 154.3)
72-h LC50 = 127.6 (116.7 - 139.5)
96-h LC50 = 109.4 (90.1 - 132.7)

Test condition:

TEST ORGANISMS:

Size: females 53.1+/-7.7 g, carapace length 7.05+/-0.53 mm; males 28.0/-2.7 g, carapace length 5.80+/-0.26 mm
Age: no data, but individuals in the intermoult period selected for testing.
Pretreatment: After capture in the wild, shrimp were maintained in 10-ton tanks for 3-4 months.
Supplier: Wild-caught

DILUTION WATER:
Source: 1.0 µm filtered water
Chemistry:

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: not measured

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: 0, 50, 100, 150, 200, 300 or 400 mg/L NO2-N
Renewal of test solution: 70%/day exchange
Exposure vessel type: 15L tank
Number of replicates: 2 replicates, 2 females and one male per tank.
Water parameters: temperature 27+/-0.2°C; salinity 32 ppt; pH 7.7+/-0.1
Intensity of irradiation: no data
Photoperiod: 15 h light/9 h dark
Feeding: no
Aeration: moderate, continuous

TEST PARAMETER: Mortality (shrimp were considered dead when presenting no response to mechanical stimuli)

MONITORING OF TEST SUBSTANCE CONCENTRATION: no

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (2) valid with restrictions
Well reported literature study

Flag: Critical study for SIDS endpoint

23-MAY-2005 (31)

Type: static
Species: other: Procambarus clarkii
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes

LC50(96h, low chloride) :
= 28

LC50 (96h, high chloride) :
= 75

Method: other
Year: 1985

Method: METHOD FOLLOWED: Gutzmer & Tomasso method.

26 trials were conducted. 19 trials tested the effect of crayfish weight on tolerance to nitrite; 14 trials tested effect of gender on tolerance to nitrite (conducted simultaneously with size trials) and seven trials tested the effect of the addition of 100 mg/L environmental chloride on tolerance to nitrite toxicity.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: A two-tailed t-test, one way analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) multiple range test were used where appropriate.

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: Nitrite concentration analysed usingazo-dye method (EPA 1974)

References: US EPA (1974) Methods for Chemical Analysis of Water and Wastes. Office of Technology Transfer, Washington, DC.

Result: Expressed as sodium nitrite:

LC50 (96 h) = 42 mg/L (22 mg/L chloride)
LC50 (96 h) = 112 mg/L (100 mg/L chloride)

Neither gender nor weight significantly affected LC50 of nitrite to crayfish size.

Environmental chloride significantly increased the LC50 values at all time periods tested. These results indicate that chloride inhibits nitrite toxicity in crayfish, probably by competitively excluding nitrite from active transport sites on gill cells that normally transport chloride.

Test condition:

TEST ORGANISMS:

Size: Gender/size trials 0.5+/-0.03g to 12.4+/-1.76g; chloride inhibition tests 1.57+/-0.22g to 17+/-1.65g
Age:
Pretreatment: Animals were held at least 48-h prior to testing in 252 L fiberglass tanks supplied with well water at the San Marcos National Fish Hatchery and Technology Center. Flow rate 6-7 turnovers/hour. Animals were fed a formulated fish food (40% protein) when first placed in the tanks. Feeding was suspended 24 h before crayfish were placed in the experimental aquaria for testing.
Supplier: Southwest Texas State University, San Marcos, Texas, USA

DILUTION WATER:
Source: Well water
Chemistry: temperature 23°C; pH 7.2; dissolved oxygen > 6.0 mg/L; hardness 310 mg/L as calcium carbonate; chloride 22 mg/L.

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Nitrite concentration between 144 and 114% of nominal.
REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: 10 to 83.5 mg/L for gender and weight tests; 29 to 242 mg/L for chloride inhibition tests.
Renewal of test solution: none, static test
Exposure vessel type: 15 L glass aquaria
Number of replicates: One replicate, 5 crayfish per dose.
Water parameters: pH 7.9-8.3; dissolved oxygen 6.9-7.5 mg/L; alkalinity 153-196 mg/L as CaCO3; hardness 198-232 mg/L as CaCO3.
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no
Aeration: constant to maintain dissolved oxygen close to saturation.

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (2) valid with restrictions
Well reported literature study
Flag: Critical study for SIDS endpoint
23-MAY-2005 (73)

Type: static
Species: other: Cherax quadricarinatus
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 1

Method: other: APHA 1980
Year: 1995

Method:
METHOD FOLLOWED: APHA (1980)

DEVIATIONS FROM GUIDELINE: None

STATISTICAL METHODS: Finney (1971)

METHOD OF CALCULATION: LC50 values calculated from a basic microcomputer program

ANALYTICAL METHODS: Nitrite concentration determined by the diazotisatin method (APHA, 1980)

References: APHA, American Water Works Association and Water Pollution Control Federation (1980) Standard Methods for the Examination of Water and Wastewater. 15th edn. APHA, Washington, DC

Finney DJ (1971) Probit analysis. Cambridge University Press. London/New York, NY 333pp

Result: Expressed as sodium nitrite:

LC50 (96h) = 4.93 mg/L

LC50 (mg/L NO2-N)
values in mg/L for hatchling reclaw crayfish. pH 8.3, temperature 28 degree C

24-h LC50 = 1.4
48-h LC50 = 1.1
72-h LC50 = 1.1
96-h LC50 = 1.0

Test condition: TEST ORGANISMS:

Size:
Age: Hatchlings
Pretreatment: Feed withheld 24h prior to transfer to treatment tanks
Supplier: redclaw broodstock produced at the Aubum University Agricultural Experiment Station, Auburn Alabama

DILUTION WATER:
Source:
Chemistry:

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Final nitrite concentrations varied less than 7% from the original concentration.

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: 0, 0.5-3.5 mg/L NO2-N at 0.5 mg/L intervals
Renewal of test solution: Static test, but nitrite concentrations adjusted to initial levels if necessary every 24h
Exposure vessel type: 4 L plastic jars filled with 3L of culture water
Number of replicates: Triplicate, 4 crayfish per concentration
Water parameters: temperature 28+/-1°C; pH 8.3; dissolved oxygen 7.4 mg/L; alkalinity 20 mg/L; hardness 54 mg/L
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no
Aeration: gentle to maintain dissolved oxygen levels at saturation

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
26-MAY-2005 (150)

Type: semistatic
Species: other: Macrobrachium rosenbergii
Exposure period: 192 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 : = 4.5
LC50 (96h) : = 8.6

Method: other
Year: 1976

Method:

METHOD FOLLOWED: Armstrong et al method

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: 2-Way ANOVA

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: Nitrite concentration analysed using a sulfanilamide-based colourimetric reaction (Federal Water Pollution Control Administration, 1969)

References: Federal Water Pollution Control Administration (1969) FWPCA Methods for Chemical Analysis of Water and Waste.

US Dep. Inter. Washington DC, 181-187
Result: Expressed as sodium nitrite:

LC50 (96h)= 42.4 mg/L
LC50 (192h) = 22.2 mg/L
Test condition:
TEST ORGANISMS:

Size: 80-140 µg
Age: 10-14 day-old larvae
Pretreatment:
Supplier: in-house, University of California, Davis

DILUTION WATER:
Source:
Chemistry:

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Nitrite concentration never less than 94% of nominal at 24 hour change

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: Broods 1&2 0.3-970 mg/L NO2-N; Brood 3 1.8 -30 mg/L NO2-N
Renewal of test solution: Every 24 hours
Exposure vessel type: 250 mL glass beakers immersed in a constant temperature bath
Number of replicates: triplicate, 15 larval shrimp per concentration
Water parameters: temperature 28+/-0.5°C; salinity 12+/-0.5 ppt; dissolved oxygen 5.7+/-0.7 mg/L; pH 7.98+/-0.05 (t=0h) and 8.22+/-0.03 (t=24h)
Intensity of irradiation: no data
Photoperiod: no data
Feeding: newly hatched brine shrimp naupii every 24 h when solutions were changed.
Aeration: no

TEST PARAMETER: Mortality (cessation of heart beat in the first 24 h and development of opaqueness in immobile animals after 24 h).

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes, every 24 hours

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Well reported literature study
24-MAY-2005 (11)

Type: semistatic
Species: other: Penaeus chinensis
Exposure period: 192 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 22.95

LC50 (96h) : = 37.1

Method: other
Year: 1990
GLP: no

Method: METHOD FOLLOWED: Hubert (1980) and the American Public Health Association (1985)

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:

METHOD OF CALCULATION:

ANALYTICAL METHODS: none

References:
American Public Health Association (1985). Standard Methods for the Examination of Water and Wastewater. 16th ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington DC.

Hubbert JJ (1980) Bioassay. Kendall Hunt Publishing Co. Toronto

Result: Expressed as sodium nitrite:

LC50 (96h) = 182.9 mg/L
LC50 (192h) = 113.1 mg/L

LC50 values of Nitrite-N mg/L
24-h LC50 = 339
48-h LC50 = 286
72-h LC50 = 117
96-h LC50 = 37.1
120-h LC50 = 26.98
144-h LC50 = 26.98
168-h LC50 = 24.36
192-h LC50 = 22.95

Test condition:

TEST ORGANISMS:

Size: mean body length 3.96+/- 0.18 cm; weight 0.36 +/- 0.06 g
Age: juveniles
Pretreatment: Acclimated for about 1 week in 500 L holding tanks
Supplier: Tainan Branch, Taiwan Fisheries Research Institute

DILUTION WATER:
Source: Seawater pumped from the Keelung coast adjacent to the University was filtered through a sand and gravel bed by air-lifting, and aerated for 3 d before use.
Chemistry:

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: Distilled water
Preparation: Nitrite test solutions were prepared by dissolving 4.93 g of sodium nitrite with distilled water to make 1000 mg/L "nitrite-N" (nitrite as nitrogen), and then

diluted to desired concentrations with seawater.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: The nominal concentrations of nitrite-N ranged in 10 mg/L increments from 20 to 80 mg/L, in 40 mg/L increments from 80 to 200 mg/L, and in 50mg/L increments from 200 to 400 mg/L.
Renewal of test solution: daily
Exposure vessel type: 30 L polyethylene tanks containing 10 L test solution
Number of replicates: Triplicate, 8 prawns/test concentration/tank
Water parameters: temperature 26+/-2°C; dissolved oxygen 5.6+/-0.2 mg/L; pH 7.94+/-0.06.
Intensity of irradiation: no data
Photoperiod: no data
Feeding: Diet (protein 42.9%) designed for Penaeus monodon by Tairon Feedstuff Co. (Taipei, Taiwan) three times a day (10:00, 16:00, 22:00 hrs) at 15% body wt/d.
Aeration: yes

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: no

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Supplier: Merck

Reliability: (2) valid with restrictions
Well reported literature study

24-MAY-2005 (39)

Type: semistatic
Species: other: Metapeaeus ensis
Exposure period: 120 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = .71
LC50 : = 7.06

Method: other
Year: 1991
GLP: no

Method: Effect: immobilisation, intoxicant
Salt water
Remark: Expressed as sodium nitrite:

120-h LC50 = 34.8 mg/L
120-h NOEC = 3.5 mg/L

Result: Percentage mortalities of shrimp exposed to various concentrations of nitrite are presented in Table. Only one N3 larvae and one M2 larva died in the control solution; no Z2 or PL1 larvae died in control solutions.
The probit of mortality of the larvae exposed to nitrite-N had a linear relationship with log nitrite-N at various times of exposure.
The LC50s and associated 95% confidence limits of nitrite-N

for different stages of M. ensis larvae are shown below. The 24 h LC50 of nitrite-N was 31.29, 16.05, 47.60, and 70.06 mg/L on N3, Z2, M2, and PL1, respectively. PL1 larvae were the most tolerant and Z2 the least tolerant to nitrite.
The LC50 decreased with increase of exposure time for all stages of M. ensis larvae tested.
The LC50 declined sharply during the first 12-36 h for M2 and PL1. The "threshold time" (a time at which responses cease) for PL1 was 108 h. The "incipient LC50" (the LC50 for an exposure time at the asymptotic point of the toxicity curve) was determined to be 7.06 mg/L nitrite-N for PL1 in a salinity of 33 ppt at a pH of 8.20 and a water temperature of 30 degree C.
Relation between probit of mortality and nitrite-N concentrations as mg/L at various time, and LC50 and 95% confidence limits.

Time (h)	LC50 Nitrite-N (mg/L)
Nauplius third substage (N3)	
12	40.36 (21.91 - 75.90)
24	31.29 (16.16 - 61.25)
Zoea second substage (Z2)	
12	33.66 (25.10 - 45.53)
24	16.05 (10.77 - 23.95)
Mysis second substage (M2)	
12	110.77 (48.17 - 262.59)
24	47.60 (36.68 - 57.12)
36	34.59 (28.93 - 41.36)
48	20.67 (16.14 - 26.61)
Postlarva first substage (PL1)	
12	132.60 (80.33 - 226.68)
24	70.06 (57.17 - 85.85)
36	40.48 (30.88 - 54.43)
48	27.10 (18.16 - 40.81)
60	18.11 (11.43 - 28.91)
72	12.76 (7.14 - 23.02)
84	11.64 (6.73 - 20.15)
96	9.13 (5.16 - 16.30)
108	7.06 (4.39 - 11.47)
120	7.06 (4.39 - 11.47)

Test condition: Fertilized eggs released from a single brood were hatched and reared to different stages in the laboratory. The larvae used in the study were third nauplius substage (N3), second zoea substage (Z2), second mysis substage (M2), and first postlarva substage (PL1).
Seawater (33 ppt) pumped from the Keelung coast adjacent to the National Taiwan Ocean University was filtered through sand and gravel filters and aerated three days before use in toxicity tests.
Nitrite test solutions were prepared by dissolving 4.93 g of reagent grade sodium nitrite with 1.00 L of distilled water to make a stock solution of 1,000 mg/L nitrite-N.
The stock solution was then diluted to desired concentrations with seawater The nominal nitrite-N concentration ranged in geometric progression (factor of 2)

from 1 to 64 mg/L (plus a 96 mg/L treatment) for N3, from 2 to 64 mg/L (plus a 12, 24 and 96 mg/L treatment) for Z2, from 2 to 64 mg/L (plus a 24 and 96 mg/L treatment) for M2, and from 2 to 128 mg/L (plus a 24 and 96 mg/L) for PL1. Concentrations of nitrite-N were measured spectrophotometrically by the diazotization method (Strickland and Parsons 1972).
Toxicity tests were conducted according to Hubert (1980) and the American Public Health Association et al. (1985). Shrimp were collected randomly from the holding tank and exposed to test and control solutions in triplicate 1.00 L polyethylene beakers containing 1.00 L of the test solution. Each beaker contained 15 test larvae for N3, Z2 or M2, or 1O test larvae for PL1. All breakers were aerated by an air stone. During the experiments, shrimp were fed artificial plankton BP (Nippai Co. Ltd, Tokyo, Japan) for zoea, mysis and postlarvae three times a day. However, nauplius larvae were not fed (American Public Health Association et al. 1985).
Toxicity tests were conducted using the "static renewal method" (Hubert 1980; Buikema et al. 1982; American Public Health Association et al. 1985) with test solutions renewed daily. In all test solutions, dissolved oxygen (DO) maintained 5.4-5.6 mg/L, pH 8.10-8.30 and water temperature 29-31 degree C. Observations were usually made at 12 h intervals up to 24 h for N3 and Z2; to 48 h for M2; and to 132 h for PL1. Death was assumed when shrimps were immobile and showed no response when the beaker was shaken gently. The dose response of test organisms was determined by plotting probit transformed percent mortality against log concentration (Finney 1971 ; Buikema et al.1982). The median lethal concentration (LC50) of nitrite-N and associated 95% confidence limits were calculated from a microcomputer program (Trevors and Lusty 1985).

Chemical analysis of saltwater water
Salinity; 33 ppt
Total alkalinity; 108 mg/L as CaCO3
Total hardness; 6,214 mg/L as CaCO

Test substance: Chemical name: Sodium Nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Conclusion: Metapenaeus ensis larvae at different stages were exposed to a series or nitrite-N (nitrite as nitrogen) concentrations in static renewal toxicity tests. Larvae at zoea stage were the most susceptible, and postlarvae were the most tolerant to nitrite among the larvae tested. The 24 h LC 50s were 31.29, 16.05, 47.60 and 70.06 mg/L nitrite for third nauplius substage (N3), second zoeasubstage (Z2), second mysis stage (M2), and first postlarvae substage (PL1). respectively, in 33 ppt seawater at a pH of 8.20 and a water temperature of 30 C. The 48 h LC50S for M2 and PL1 were 20.67 and 27.10 mg/L nitrite-N. respectively. The ''threshold time" was 108 h, and "incipient LC50" for M. ensis PL1 was 7.06 mg/L nitrite-N. A "safe level" for rearing M. ensis larvae was estimated to be 0.71mg/L nitrite-N in the hatche

Reliability: (2) valid with restrictions
24-MAY-2005 (37)

Type: static

Species: other: Crassostrea virginica
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50 (adult) : = 660
EC50 (juvenile) : = 800

Method: other
Year: 1975

Method: METHOD FOLLOWED: Epifanio and Srna method.

After 96-h exposure to a toxin, surviving individuals were transferred to uncontaminated seawater in which they underwent a 24-h recovery period. The number surviving after this recovery period was used in calculation of 96-h mean lethal tolerance limits.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: no data

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: none

Test condition:

TEST ORGANISMS:

Size: (a) 13-17 mm (b) 46-62 mm
Age: (a) Juveniles, (b) Adults
Pretreatment: Held in a recirculating sea-water system and fed a diet of cultured algae for at least 3 weeks prior to use in an experiment. Test individuals were starved during the 24 h immediately preceeding a test.
Supplier: Juveniles hatchery reared by test lab, adults collected locally.

DILUTION WATER:
Source: Seawater pumped from Breakwater Harbour at the mouth of Delaware Bay, USA
Chemistry: Temperature 20/-2°C; Salinity 27+/-2%

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: not measured

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: 1.0E-02-10.0E-02 M/L
Renewal of test solution: none, static test
Exposure vessel type: Glass aquaria containing 30 L sea water
Number of replicates: duplicate, 10 specimens per aquarium
Water parameters: Temperature 20/-2°C; Salinity 27+/-2%; oxygen 7-8.2 ppm; pH 7.7-8.23
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no
Aeration: Continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: no
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (2) valid with restrictions
23-MAY-2005 (55)

Type: static
Species: other: Mercenaria mercenia
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50 (adult) : = 1200
EC50 (juvenile) : = 1100

Method: other
Year: 1975

Method: METHOD FOLLOWED: Epifanio and Srna method.

After 96-h exposure to a toxin, surviving individuals were transferred to uncontaminated seawater in which they underwent a 24-h recovery period. The number surviving after this recovery period was used in calculation of 96-h mean lethal tolerance limits.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS: no data

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: none
Test condition:
TEST ORGANISMS:

Size: (a) 4.7-5.2 mm (b) 28-32 mm
Age: (a) Juveniles, (b) Adults
Pretreatment: Held in a recirculating sea-water system and fed a diet of cultured algae for at least 3 weeks prior to use in an experiment. Test individuals were starved during the 24 h immediately preceeding a test.
Supplier: Juveniles hatchery reared by test lab, adults collected locally.

DILUTION WATER:
Source: Seawater pumped from Breakwater Harbour at the mouth of Delaware Bay, USA
Chemistry: Temperature 20/-2°C; Salinity 27+/-2%

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: none
Preparation: not applicable

STABILITY OF THE TEST CHEMICAL SOLUTIONS: not measured

REFERENCE SUBSTANCE: none

TEST SYSTEM:

Concentrations: 1.0E-02-10.0E-02 M/L
Renewal of test solution: none, static test
Exposure vessel type: Glass aquaria containing 30 L sea water
Number of replicates: duplicate, 10 specimens per aquarium
Water parameters: Temperature 20/-2°C; Salinity 27+/-2%;
oxygen 7-8.2 ppm; pH 7.7-8.23
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no
Aeration: Continuous

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: no
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (2) valid with restrictions
23-MAY-2005 (55)

Type: semistatic
Species: other: Callinectes sapidus
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50 (pre-molt juvenile) :
= 71.3
LC50 (intermolt juvenile) :
= 93.4

Method: other
Year: 1989

Method: METHOD FOLLOWED: Ary & Poirrier method.

Two nitrite bioassay experiments were conducted: one with premolt crabs near ecdysis and one with intemolt crabs.

DEVIATIONS FROM GUIDELINE: Not applicable

STATISTICAL METHODS:

METHOD OF CALCULATION: LC50 calculated using trimmed Spearman-Karber method.

ANALYTICAL METHODS: Nitrite concentration was measured by the diazotisation method (APHA et al 1980)

References:
APHA, American Water Works Association and Water Pollution Control Federation (1980) Standard Methods for the Examination of Water and Wastewater. 15th edn. APHA, New York
Result: Based on Sodium nitrite:

LC50 (96h) = 351.4 - 460.3 mg/L
Test condition:
TEST ORGANISMS:

Size:
Age: premolt and intermolt
Pretreatment: Held in closed, recirculating-seawater systems. Intermolt crabs acclimatised for 72-168 h. Premolt held until

within 4 days of ecdysis
Supplier: Collected from Clermont Harbor, Mississippi

DILUTION WATER:
Source: 3-5 day old aerated New Orleans city tap water dechlorinated with sodium thiosulfate. Artificial sea salts (Rila Marine Mix) were added.
Chemistry: salinites averaged 17.6 ppt and ranged from 14.0 - 19.0 ppt.

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration:
Preparation:

STABILITY OF THE TEST CHEMICAL SOLUTIONS: no data

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: 0, 10, 25, 50, 75, 100, 150, 200 mg/L NO2-N
Renewal of test solution: daily
Exposure vessel type: Plastic box, 50.8 x 30.5 x 15.2 cm
Number of replicates: 22 premolt crabs, 10 intermolt crabs per test concentration, all individually housed.
Water parameters: temperature 21-24°C; pH 7.8 (7.5-7.9)
Intensity of irradiation: no data
Photoperiod: no data
Feeding: no
Aeration: yes

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes, daily prior to test solution renewal

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Purity: Reagent grade

Reliability: (2) valid with restrictions

26-MAY-2005 (12)

Type: semistatic
Species: other: Cherax quadricarinatus
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 25.9

Method: other
Year: 1995

Method: Effect: mortality, increasing
Fresh water
Remark: 96-h LC50 = 126.7 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: Expressed as sodium nitrite:

LC50 (96h) = 126.7 mg/L

No mortalities were observed in control individual throughout the experiments. Survival was substantially

reduced when juvenile crayfish were exposed to nitrite. At 0 and 10 mg/L total NO2-N, no mortalities were observed through 120 hr of exposure. At 25, 50, and 100 mg/L total NO2-N, average LT50s were 96, 22, and 5 hr, respectively. The calculated LC50 values for 24, 48, and 96 hr were 42.9 +/-O.22, 37.1 +/- 0.16 and 25.9 +/- 0.35 mg/L NO2-N, respectively.

Test condition: -Animal
Juveniles were obtained from broodstock females maintained in Campbell Hall at the University of Alabama at Birmingham (UAB). Broodstock individuals were held in raceways (28 degree C) with associated recirculating biofilters to ensure water quality and the health of broodstock animals. Juveniles used ranged in size from 9 - 13 mm total length (10 - 25 mg wet weight) and were fed AB crayfish feed (UAB Research Foundation). Juveniles lacking appendages were excluded for use in experiments.

-Toxicity Assays
Preliminary experiments determined the range of concentrations of nitrite to be examined (data not shown). For each test solution examined, 10 siblings were placed individually in polystyrene bowls containing 100 mL of the desired test solution. In many cases, the variability in survival of siblings among females was high, thus numerous other groups of siblings from other females were exposed to the toxicants to increase the statistical value of mean lethal times and concentrations. All juveniles used for toxicity experiments were not fed during exposure nitrite.
Stock solutions were made by mixing 4.92 g sodium nitrite with 1 L of conditioned freshwater (28 degree C, pH 7.5 +/- 0.2, alkalinity 70 +/- 5 mg/L, hardness 300 +/- 10 mg/L, and chloride 450 mg/L).
Test toxicant solutions were all maintained at 28 degree C and, if necessary, were adjusted to pH 7.5 +/- 0.2 using the appropriate amounts of 1 M NaOH or HCl. Other water quality parameters of the test toxicant solutions were maintained. Survival of juveniles was determined at concentrations of 0 (control), 10, 25, 50, and 100 mg/L total nitrite-nitrogen (NO2-N). The dose response of crayfish was determined by plotting the probit of mortality transformed from percent mortality against log concentration (Buikema et al. 1982). Moving averages and interpolation were used to determine LC50 (+/-SD) (Btlikema et al, 1982).

Reliability: (2) valid with restrictions
26-MAY-2005 (118)

Type: static
Species: other: Penaeus setiferus
Exposure period: 72 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = 172.8

Method: other
Year: 1997

Method: Effect: mortality, increasing
Salt water
Life stage: post larvae
Remark: Expressed as sodium nitrite:

EC50 (72h) = 851.7 mg/L
Test condition: -Water parameters:
Temperature; 28 degree
Salinity; 30 +/- 1 o/oo
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: 99%
Reliability: (2) valid with restrictions
26-MAY-2005 (7)

Type: semistatic
Species: other: Penaeus monodon
Exposure period: 240 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 106

Method: other
Year: 1990
GLP: no

Method: Effect: length increase
Salt water
Remark: 240-h EC50 = 522.4 mg/L*

* : The values were converted NO2-N into NaNO2.
Result: All the prawns were killed by exposure to nitrite-N at concentrations of 280 mg/L nitrite-N for 12 h, 260 mg/L for 24 h, 240 mg/L for 72 h, 220 mg/L for 132 h, 200 mg/L for 192 h, or 180 mg/L for 216 h, while those exposed to 160 mg/L for 12 h, to 140 mg/L for 24 h and to 120 mg/L nitrite-N for 60 h survived.
The LT50 was 201 h and 14.4 h for prawns exposed to 120 and 200 mg/L nitrite-N, respectively.

24-h EC50 = 218
48-h EC50 = 193
96-h EC50 = 171
144-h EC50 = 140
192-h EC50 = 128
240-h EC50 = 106
(mg/L)
(salinity 20ppt, pH 7.57 and water temperature 24.5 degree C)

The EC50 values decreased with increasing exposure times.
Test condition: The P. monodon adolescents were shipped to the laboratory from a private nursery located at Pingtung, Taiwan, and acclimated for 1 week before use.
The prawns had an average total length of 91.0 +/- 8.0 mm, an average carapace length of 21.4 +/- 2.8 mm, and weighed 4.87 +/- 1.4 g. These sizes are categorized as adolescents by Motoh (1985). Seawater pumped from the Keelung coast was filtered through a sand and gravel filter and salinity was adjusted to 20 ppt with municipal water, which had been dechlorinated with sodium thiosulfate and aerated 3 days before use. The chemical characteristics of the water used are shown below.

Nitrite test solutions were prepared by dissolving requisite amounts of sodium nitrite (Merck GR grade) in 20

ppt of seawater. The nominal concentrations of nitrite-N were prepared in 10 mg/L increments from 120 to 280 mg/L.

Chemical analysis of the water used in bioassays
Salinity; 20 ppt
Total alkalinity; 1.6 me/L
Total hardness;3180mg/L as CaCO3
pH; 7.57

Prawns were sampled randomly from the stocking tanks and exposed to each test solution and control in triplicate tarlks. Bioassay experiments to establish tolerance limits were conducted in 15-L circular glass tanks containing 101 of the test solution (Franson, 1980; Hubert, 1980). Each tank was placed in a water bath (24-25 degree C) and contained ten test animals. All tanks were aerated by an air-stone with a blower. Each test solution was renewed daily, in accordance with a static renewal method for toxicity tests (Buikema et al., 1982). During the experiment, the prawns were fed commercial prawn feed twice a day at a rate of 10% of body weight per day. In all test solutions, dissolved oxygen was 5.6-6.2 mg/L; pH varied from 7.49 to 7.67 during the experiment.
Observations were usually made at 12-h intervals up to 264 h for the nitrite test. Death was assumed when prawns were immobile and showed no response when touched with a glass rod. The dose response of test organisms combined from triplicate tanks of each solution was determined by plotting probit transformed percent mortality against log concentration (Finney, 1971; Buikema et al., 1982). The LC50 value of nitrate and their 95% confidence limits were calculated from a microcomputer program (Hubert, 1980; Trevors and Lusty, 1985).

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: GR grade
Supplier: Merck

Reliability: (2) valid with restrictions
26-MAY-2005 (38)

Type: static
Species: Asellus intermedius (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (lower) : = 20

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: juvenile, 0.012 grams
Result: LC50 > 20 mg/L
LC50 = 20 mg/L

The lower value was = 20 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
Non standard method involving simultaneous exposure of a number of species
23-MAY-2005 (57)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (lower) : = 8.3

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Life stage: 1st and 2nd instar larvae
Result: LC50 = 9.7 mg/L
LC50 = 8.3 mg/L

The lowest value was = 8.3 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable

Non standard method involving simultaneous exposure of a number of species
23-MAY-2005 (57)

Type: static
Species: other: Dugesia tigrina
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 (lower) : = 20

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: juvenile, 0.006 grams
Result: LC50 > 20 mg/L
LC50 = 20 mg/L

The lower value was = 20 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
Non standard method involving simultaneous exposure of a number of species
23-MAY-2005 (57)

Type: static
Species: Gammarus fasciatus (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 6.5

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: juvenile, 0.007 grams
Result: LC50 = 6.5 mg/L
LC50 = 6.5 mg/L

The both values were 6.5 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
Non standard method involving simultaneous exposure of a number of species
23-MAY-2005 (57)

Type: static
Species: other: Ramshorn snail (Helisoma trivolvis)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC50 (lower) : = 12

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: juvenile, 0.1806 grams
Result: LC50 = 12.0 mg/L
LC50 = 20.0 mg/L

The lower value was 12.0 mg/L.
Test condition: -Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (4) not assignable
Non standard method involving simultaneous exposure of a number of species
23-MAY-2005 (57)

Type: semistatic
Species: other: Penaeus monodon
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 13.55

Method: other
Year: 1988

Method: Effect: mortality
Salt water
Life stage: nauplius, zoea, mysis, post-larvae
Result: Expressed as sodium nitrite:

LC50 (96h) = 66.8 mg/L

Case : Life stage; nauplius: Water parameters; pH = 8.10-8.30
24h LC50 = 5.0 (4.0 - 6.24) mg/L NO2-N

Case : Life stage; zoea: Water parameters; pH = 8.05-8.30
24h LC50 = 13.2 (8.99 - 19.38) mg/L NO2-N

Case : Life stage; mysis: Water parameters; pH = 7.92-8.30
24h LC50 = 20.65 (12.02 - 35.45) mg/L NO2-N
48h LC50 = 8.3 (5.81 - 11.85) mg/L NO2-N

Case : Life stage; post-larvae: Water parameters; pH = 7.85-8.30
24h LC50 = 61.87 (51.61 - 74.16) mg/L NO2-N
48h LC50 = 33.17 (26.79 - 41.06) mg/L NO2-N
72h LC50 = 20.53 (16.20 - 26.00) mg/L NO2-N
96h LC50 = 13.55 (11.21 - 16.38) mg/L NO2-N
Test condition: -Water parameters:
Dissolved O2 (mg/L); 5.1-5.4
pH; (see RESULT)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: GR grade
Reliability: (4) not assignable
Insufficient experimental detail
24-MAY-2005 (40)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 43.6

Method: other
Year: 1977

Method: Effect: mortality
Fresh water
Test condition: -Water parameters:

Temperature; 20-22 degree C
Hardness (mg/L CaC03); 70
pH; 7.6-7.7
Reliability: (4) not assignable
Insufficient experimental detail
26-MAY-2005 (25)

Type: static
Species: other: Thamnocephalus platyurus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 3.9

Method: other
Year: 1995

Method: Effect: mortality, increasing
Fresh water
Result: Mean 24 h LC50 (in mg/L) +/- SD = 3.90 +/- 0.24
Test condition: Thammocephalus platyurus (n=3)
-Cyst Hatching and Larval Molting
The T. plalyurus used in this study was originally collected from temporary desert pools west of Los Angeles, California. Laboratory populations were established that have been inbred and selected for cyst production. Subsequently, all T, plalyurus resting eggs used in this study are laboratory-produced cysts from controlled continuous cultures.
The hatching rate of T. plalyurus cysts Was determined by hydrating 100 +/- 50 cysts in a moderately hard freshwater medium [U.S. Environmental Protection Agency (EPA), 1985], referred to as EPA water hereafter. Cyst incubation was performed in polystyrene multiwell plates (6 wells, 10 mL contents. water depth +/- 1 cm) at 25 degree C under continuous illumination (white fluorescenl lamp: 1000-2000 lux). Unless otherwise specified, this temperature and light conditions will be referred to as the standard incubation conditions for all further experiments. Each experiment was set up in 3 replicates. Hatching results were determined by counting and removing the nauplii at regular intervals during a period of 5 days. To determine the effect of a medium with lower water hardness (10 instead of 80-100 mg CaCO3/L. ion composition, and a lower pH (6.5-7.0 instead of 7.6-7.9) on the hatching success, the EPA water was diluted with deionized water in a ratio 1:8. This dilution factor was rested based on previous satisfactory results with S. proboscideus cyst hatching.
To study the molting time, 25 mg of T. plalyurus cysts were hydrated in 100 mL EPA water in an Erlenmeyer flask with gentle bottom aeration, and incubated in standard conditions. After 12 h, a single random harvest of early hatchers was obtained by gentle suction using a Pasteur pipette. Subsequently, each hatched larva (instar 1-nauplius or E2 phase hatcher) was placed in one well of a 96-well polystyrene multiwell plate in 300 micro-L EPA water, Incubation was continued under standard conditions allowing the un fed nauplii to molt further. To determine the percentage of larvae in a particular instar stage, observations were made every hour fora local period of 10 h. A total of 72 observations were made. Distinction of the

first larval stages under the dissection microscope (50 x magnihcation) was done on the basis of the body size and the number of body segments as described by Bernice (1972).

-Toxicity Test Procedure
static acute toxicity tests were conducted in conventional 24-well polystyrene multiwell plates (6 columns with 4 rows) according to the standard operational procedure of the Streptoxkit F test (Centeno et al., 1993a).
Each test comprised one control and five toxicant concentrations, with three replicates per treatment. Each replicate consisted of one well receiving 10 nauplii.
The plates were incubated in darkness at 25 +/- 1 degree C for 24 h, after which the number of dead larvae in each well was counted under a dissection microscope at 1o-12 x magnification. Larvae were considered dead when no movement of the appendages was noted within 10s of observation.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (3) invalid
23-MAY-2005 (32)

Type: static
Species: other: Streptocephalus proboscideus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : = 4.1

Method: other
Year: 1995

Method: Effect: mortality, increasing
Fresh water
Result: Streptocephalus proboscideus (n=9)
Mean 24 h LC50 (in mg/L) +/- SD = 4.10 +/- 1.30
Test condition: See: Test Condition of the adjacent test with Thamnocephalus platyurus
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (3) invalid
23-MAY-2005 (32)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: other: growth rate and biomass
Exposure period: 72 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: 100
EC50 (growth rate) :
> 100
EC50 (biomass) : > 100
Limit Test: yes

Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 2005
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Analysis of the test concentrations at 0 and 72 hours showed measured test concentrations to be near nominal, hence the

EC50 values were estimated beased on the nominal test concentrations only.

Additional analysis of the test samples was conducted for the presence of sodium nitrate at both 0 and 72 hours in order to determine whether conversion of nitrite to nitrate occurred. The results obtained showed measured test concentrations of less than 1% of nominal for sodium nitrite at 0 hours and 5% of nominal at 72 hours thereby indicating that no conversion occurred.

Table 1: Measured test concentrations

Sample	Nominal Concentration (mg/L)	Measured Concentration (mg/L)	Percent of Nominal (%)
0 Hours	Control	<LOQ	-
	100 R1-R3	98.7	99
	100 R4-R6	99.6	100
72 Hours	Control	<LOQ	-
	100 R1-R3	93.3	93
	100 R4-R6	94.8	95

LOQ = Limit of quantitation
R1-R6 = Replicates 1-6

Table 2: Cell densities and pH values in the definitive test

Nominal Concentration (mg/L)	Cell Densiities* (cells/mL) 0h	24h	48h	72h
Control				
R1	9.80E+03	2.63E+04	5.33E+04	2.86E+05
R2	8.48E+03	2.92E+04	5.92E+04	3.18E+05
R3	8.76E+03	2.68E+04	5.88E+04	2.97E+05
Mean	9.01E+03	2.74E+04	5.71E+04	3.00E+05
100				
R1	8.28E+03	2.59E+04	6.34E+04	3.50E+05
R2	7.35E+03	2.95E+04	6.59E+04	3.05E+05
R3	7.07E+03	2.32E+04	6.23E+04	3.69E+05
R4	8.01E+03	2.39E+04	5.26E+04	3.88E+05
R5	7.63E+03	3.08E+04	7.37E+04	3.57E+05
R6	7.99E+03	2.96E+04	8.32E+04	4.70E+05
Mean	7.72E+03	2.72E+04	6.68E+04	3.71E+05

* cell densities represent the mean number of cells/mL calculated from the mean of the cell counts from 3 counts for each of the replicate flasks.

R1-R6 = Replicates 1 - 6

Table 3: Inhibition of growth rate and biomass

Nominal Concentration	Area under Curve at 72 h	% Inhibition (0-72h)	Growth Rate	% Inhibition
Control	5.09E+06	-	0.049	-
100	6.25E+06	[23]	0.054	[10]

[] Increase in growth as compared to the controls
Based on nominal concentrations:

EbC50 (72h) >100 mg/L
ErC50 (0-72h) >100 mg/L

Test condition: - Test Organisms: Scenedesmus subspicatus strain CCAP 276/20
Supplier: Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, Scotland
Method of Cultivation: Cultures were maintained in the laboratory by the periodic replenishment of the culture medium
Pretreatment: The culture was maintained in the laboratory at a temperature of 21+/-1 deg C under conditions of continuous illumination (7000 lux) and constant aeration

- Test Conditions:
Medium:

NaNO3	25.5 mg/L
MgCl2.6H2O	12.164 mg/L
CaCl2.2H2O	4.41 mg/L
MgSO4.7H2O	14.7 mg/L
K2HPO4	1.044 mg/L
NaHCO3	15 mg/L
H3BO3	0.1855 mg/L
MnCl2.4H2O	0.415 mg/L
ZnCl2	0.00327 mg/L
FeCl3.6H2O	0.159 mg/L
CoCl2.6H2O	0.00143 mg/L
Na2MoO4.2H2O	0.00726 mg/L
CuCl2.2H2O	0.000012 mg/L
Na2EDTA.2H2O	0.30 mg/L
Na2SeO3.5H2O	0.000010 mg/L

Exposure Vessel Type: 250 mL glass conical flasks fitted with foam bungs containing 100 mL of solution
Nominal concentrations: 0 (control), 100 mg/L
Vehicle: Test medium
Stock solution: 100 mg of test material was dissolved in culture medium and the volume adjusted to 500 mL to give a 200 mg/L stock solution. This stock solution was mixed with algal suspension (500 mL) to give the required test concentration of 100 mg/L.
Number of replicates: 6 for treatment group, 3 for controls.
Initial cell density: 10,000 cells/mL
Temperature: 24+/-1 deg C
pH: 7.3 (0 hours) - 7.8 (72 hours)
Light condition: Continuous illumination (7000 lux)
Shaking: Constant saking at 150 rpm

-Methods of analysis: Samples were taken from the control (replicates R1-R3 pooled) and the 100 mg/L test group (replicates R1-R3 and R4-R6 pooled) at 0 and 72 hours for

quantitiative analysis. Duplicate samples were taken at 0 and 72 hours and stored at approximately -20 deg C for further analysis if necessary.

-Statistical analysis: A Student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the area under the growth curve data at 72 hours for the control and the 100 mg/L test concentration to determine statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package.

Reliability: (1) valid without restriction
Study conducted to OECD TG

Flag: Critical study for SIDS endpoint

04-JAN-2006 (94)

Species: other algae
Endpoint: other: CO2 Fixation

Year: 1978

Method: METHOD FOLLOWED: Wodzinski et al method

Algae were grown at 25°C on a rotary shaker (170 rpm) with constant illumination in 500 mL Erlenmeyer flasks containing 100 mL of Bristol solution at pH 6.0. When the optical density at 580 nm had reached about 0.2, the cells were collected by centrifugation and resuspended in Bristol solution to an optical density of 0.01 to 0.05, depending on the activity of the culture. Sodium nitrite solution was prepared in Bristol solution of the desired pH immediately before use.

The reaction mixture consisted of 1.0 mL each of resuspended cells, Bristol solution and the sodium nitrite solution in test tubes (13 x 120 mm). Test tubes containing the same ingredients were wrapped in aluminium foil to measure activity in the dark. All tubes were incubated at 25°C for 40 min under 450 µEinsteins of light/m2/s, after which 0.25 mL of Bristol solution containing 0.05 µCi of H[14]CO3- with a specific activity of 59 mCi/mmol was added to each tube. the tubes were then sealed with serum stoppers and incubated for an additional 30 minutes.

The uptake of [14]CO2 was terminated by injection of 0.4 mL of 37% formaldehyde into each tube. 1 mL portions from each tube were then filtered through 0.45 µm filters and the cells thus retained were washed with 10 mL of 1.0 MM H2SO4 to remove residual [14]CO2. after drying, the filters were placed in scintillation vials containing 10 mL of Bray liquid scintillation cocktail and the radioactivity was counted. The activity of sodium nitrite-free cells was determined simultaneously and the results expressed as a percent activity compared with these controls.

The same procedure was used to determine the effects of nitrite on [14]CO2 uptake by algae grown and tested at a higher pH. In these instances the concentration of phosphate in the Bristol solution was increased to 0.01 M and the pH was adjusted to 7.7

Result: Table: Sensitivity of algal photosynthesis to nitrite at pH 6

Microbial group	Genus	% inhibition by 1.0 mM NO2-
Blue-green algae		
	Anacystis nidulans	60
	Lyngbya sp.	97
	Anabaena flos-aquae	95
	Oscillatoria sp.	97
	Schizothrix sp.	91
	Synechococcus cedrorum	77
	Calothrix anomala	100
	Fischerella muscicola	99
	Cylindrospermum sp.	98
Green algae		
	Scenedesmus quadricauda	0
	Ulothrix fimbriata	5
	Chlamydomonas reinhardtii	1
	Ankistrodesmus falcatus	0
	Schizomeris leibleinii	12
	Oedogonium foeolarum	0
	Staurastrum sp.	0
	Draparnaldia pulmosa	19
	Gloeocystis vesiculosa	0

The nine genera of blue-green algae tested were strongly inhibited by 1.0 mM nitrite, the rate of CO2 fixation being reduced by 60 - 100%. On the other hand, the activity of nine genera of green algae was little or not at all affected at this nitrite concentration.

The IC50 ([14]CO2 uptake) by Anabaena flos-aquae was calculated to be 100 uM nitrite (6.9 mg/L as NaNO2)

Test condition: TEST ORGANISMS:

Blue-green algae

Anacystis nidulans
Lyngbya sp.
Anabaena flos-aquae
Oscillatoria sp.
Schizothrix sp.
Synechococcus cedrorum
Calothrix anomala
Fischerella muscicola
Cylindrospermum sp.
Green algae
Scenedesmus quadricauda
Ulothrix fimbriata
Chlamydomonas reinhardtii
Ankistrodesmus falcatus
Schizomeris leibleinii
Oedogonium foeolarum
Staurastrum sp.

Draparnaldia pulmosa
Gloeocystis vesiculosa
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
17-OCT-2005 (200)

Species: other aquatic plant: chlorococcales
Endpoint: other: Assimilation-Depletion test (A-D test)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC10: > 670

Method: other
Year: 1991

Remark: The Assimilation-Depletion test (A-D test) is in principle the modelling of the two basic reactions of the biological self-purification of waters in form of two parall in-vitro tests for the determination of the ecophysiological effects of wastewaters and their constituents.
The two basic reactions of biological self-purification in natural waters are:
a) the bacterial decomposition of organic pollutants and their oxidative recycling as simple inorganic compounds (like CO2 and NO2)
b) the incorporation of the resulting mineralization products by algae and other plants by producing organic substances through photosynthesis ("assimilation").
The bacterial decomposition processes are oxygen-consuming (depletion) white the bioproduction of plants through photosynthesis releases molecular oxygen which - in turn - is again available for bacterial decomposition of organics. Thus. the two processes are directly interconnected and establish - provided the material budget is balanced - a biological equilibrium in the surface water.
Aquatic bacteria and algae consequently constitute in this sense a genuine part of an ecosystem which is suitable to serve in form of the A-D test as a model of the metabolic dynamics in the water body and as an indicator of anthropogenic impacts through sewage, industrial and municipal wastewaters or through specific micro pollutants.
The measured parameters of the A-D test are the 02 depletion of mixed bacterial populations (Depletion test) and the photosynthetic oxygen production of planktonic algae (Assimilation test). They are measured in dilution series depending on the concentration of the wastewater or pollutant. Any inhibition or promotion of microbial growth are reflected in the result because of the test duration of 24 hours.
The significance of the A-D test for water management purposes lies in the possibility of being able to quantify harmful effects on the oxygen budget of water bodies. Therefore this method has a central position in water-toxicological studies of the Federal Institute of Hydrology.
The test shows the "effective concentrations" (EC-values) which produce inhibition effects of 10% (24 h EC 10 value) at a temperature of 20 degree C after a test period of 24 h. The concentrations were computed from the weighed samples (nominal concentration). In the case of inorganic substances

the concentrations are related to the effective ion, in case of organics to the indicated molecular compound.
The pH of the original solution has not been standardized; exceptions have been marked accordingly. Substances that are difficult to dissolve in water have been emulsified by means of a high-speed disperser.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
26-MAY-2005 (109)

Species: other algae: See Test Condition
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: no
EC100 (median for 13 species) :
= 4600
EC100 (range for 13 species) :
= 580 - 9200

Method: other
Year: 1984
GLP: no
Test substance: other TS

Method: -Growth experiments.
Growth experiments were performed in 250-uL cultures on microtitration plates (Flow Laboratories) using a procedure similar to those of Heldal et al. (1978) and Blanck et al. (1983). A plate contains 8 X 12 wells (250 uL). Chemicals in distilled water (100 uL) were spread into the wells by successive twofold dilutions using a 12-channel pipet. The resulting geometric concentration series (factor 0.5) covered 4.2 orders of magnitude (14 concentrations on two plates). Algal suspension (150 uL) was inoculated into each well giving an initial chlorophyll a concentration of 10 ng/mL. Each culture contained nutrients corresponding to Z8 at 10% strength.
Four replicates were used for each concentration. Thus, two plates were used to test one compound on three algal species. All handling of the microtitration plates was done under aseptic conditions. Test cultures were incubated under the same growth conditions as the precultures. Growth inhibition was estimated by visual inspection (cf. Blanck et al., 1983) after 14 days by recording the lowest concentration of the tested compounds giving no detectable growth (EC100).
It was suspected that adsorption of chemicals to the wells or to the pipet tips during the dilution sequence could distort the concentration series. No such effect was evident for any of the chemical as checked by comparing EC values obtained by successive dilutions on the plates and from premade solutions. Furthermore, repeated testing with one of the algae, Monoraphidium pusillum, showed that replicate EC100's only exceptionally deviated more than a factor of 2 (i.e., one row on the plates which corresponds to one class). Thus experimental errors contribute no more than one class to the recorded variation in sensitivity.

-Chlorophyll a, carbon, and nitrogen determinations.
Algal chlorophylls were extracted with dimethyl sulfoxide at

65 degree C for 30 min (Hiscox and Israelstam, 1979) and measured in 1:1 dimethyl sulfoxide and 90% acetone (Shoaf and Lium, 1976). The chlorophyll a concentrations were calculated using the equations of Jeqrey and Humphrey (1975).
Samples for carbon and nitrogen analyses were prepared by filtering aliquots of the algal suspensions onto a Whatman glass micro fiber filter (Type GF/F, 14 mm in diameter) precombusted at 450 degree C. Filters were washed in distilled water and any residual (bi)carbonate was driven off by exposing the filters to HCl vapor after which they were dried in a vacuum desiccator. Dried filters were packed into tin capsules for analysis of carbon and nitrogen, performed on a Carlo-Erba Model 1106 elemental analyzer using benzimidazole as standard. Results were used to calculate chlorophyll a/carbon and chlorophyll a/nitrogen ratios for all inocula used in the growth experiments. From these data the amounts of carbon and nitrogen initially present in the test cultures were calculated.

-Calculations.
Rank correlations between EC100 and initial carbon content of algal cultures were estimated using a nonparametric test calculating Spearman's rank correlation coeffcient. A correction for ties was made. A least-squares linear regression line was fitted to the data.

Result: -Median and range of EC100 values obtained for Sodium nitrite tested on 13 algal species
EC100 (mg/L) = 4600 (median), 580 - 9200 (Range)
Log(EC100 max/ EC100 min) = 1.2

-Variation algal sensitivity as corrected for differences in carbon content initially present in test culture
EC100 max/ EC100 min (Chlorophyll a basis) = 16
EC100 max/ EC100 min (Carbon basis) = 29

Test condition: - Cultivation of algae.
Axenic strains of 13 algal species were kept on agar slants (CCAP, 1976). None of the strains were, to our knowledge, isolated from an environment known to be severely polluted. Precultures were grown in an inorganic medium, Z8 (Kotai, 1972), modified by the addition of Si (0.16 mM) and vitamins (thiamine, 200 ug/liter; biotin, 1 ug/L; B12 , 1 ug/L). Cultures were continuously illuminated by Cool White fluorescent tubes (General Electric F96 PG 17CWX Power Groove de Luxe) at an irradiance of 10 +/- 1 W/m2 at 400-700 nm. The temperature was 20 +/- 1 degree C. Log phase cells were used as inoculum for the growth experiments.
Prior to inoculation of the test cultures the precultures were tested for bacterial contamination by streaking onto agar plates (CCAP, 1976).

-Test compounds.
Stock solutions of the chemicals in sterile distilled water were freshly prepared under aseptic conditions prior to each experiment. pH was adjusted to 6.5-7.5 using HCl or NaOH. Whenever a cosolvent (ethanol) was used, the anal concentration never exceeded 0.2%.

SOURCE AND TAXONOMIC IDENTITY OF THE ALGAL STRAINS
-Chlorophyta

--Volvocales
Chlamydomonas dysosmos Moewus; CCAP 11/36a
--Chlorococcales
Chlorella emersonii Emerson; CCAP 211/8h
Kirchneriella contorta (Schmidle) Bohlin; Isolated from Lake Skarvlangen, 1981, by the authors.
Monoraphidium pusillum (Printz) Komarkava -Legn; Isolated from Lake Lilla Stockelidsvatten, 1978, by Hans Blanck
Scenedesmus obtusiusculus Chod.; Dr. C-M. Larsson, University of Stockholm, originally obtained from Agricult. Univ. Wageningen
Selenastrum capricornutum Printz; CCAP 278/4
-Ulotrichales
Klebsormidium marinum Deason; UTEX 1706
--Chaetophorales
Raphidonema longiseta Vischer; UTEX 339
-Xanthophyta
--Heterococcales
Bumilleriopsis jillformis Vischer; CCAP 809/2
Monodus subterraneus Petersen; CCAP 848/1
--Heterotrichales
Tribonema aequale Paxher; CCAP 880/1
-Cyanophyta
--Oscillatoriales
"LPP sp."; PCC 6402
--Chrobcoccales
Synechococcus leopoliensis (Racib.) Komarek; UTEX 625

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Pro analysis 98.5%
Manufacturer: Merck
Reliability: (4) not assignable
26-MAY-2005 (18)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring:
NOEC: = 1230

Method: other
Year: 1978

Reliability: (4) not assignable
26-MAY-2005 (26)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring:
TGK : = 1233

Method: other
Year: 1977

Reliability: (4) not assignable
26-MAY-2005 (24)

Species: Scenedesmus quadricauda (Algae)
Unit: mg/l Analytical monitoring:
TGK : > 1000

Method: other
Year: 1960

Reliability: (4) not assignable
26-MAY-2005 (23)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: other
Species: other protozoa
Unit: Analytical monitoring: no
See result :

Method: other
Year: 1998
GLP: no

Method: Test Procedure
Preliminary Bioassay: The aim of the preliminary test was to find a level of toxicity of the sample tested. The test was carried out in conventional 24-well (6 X 4) polystyrene multiwell plate. Each test had one control and 11 toxicant's concentrations with two duplicates.
Two mL of tested sample was placed in A1 and C1. All other welts were filled with 1 mL Tyrod solution (diluent). Serial dilution (2X) of the tested sample was prepared by transferring of 1 mL of the sample from A1 to A2, after mixing from A2 to A3 up to B5. The same dilution was prepared in rows C and D. B6 and D6 contained only diluent as a control. One drop of Spirostomum ambiguum suspension (containing 1O-20 cells) was added to each well. After 24 h incubation in darkness at 25 degree C, toxicity was estimated with the aid of binocular. Concentrations to the definitive test were then chosen: between 0 and 100% lethality of S. ambiguum.

Definitive Test: The test was carried out in a conventional 24-well (6 X 4) polystyrene multiwell plate. Each test was one control and five toxicant's concentrations with three duplicates per concentration.
Dilution of the sample (logarithmic progression) was made directly in the plate. For this purpose, the following amount of diluent and sample was added to all four cells in each column: 0, 0.44, 0.68, 0.82, 0.90, 1.00 mL of diluent and 1.00, 0.56, 0.32, 0.18, 0.10, 0 mL of sample. To minimize dilution of the test solutions, the protozoa were first transferred (35 per well) with the aid of a micropipette into the bottom row (D). which served as rinsing wells. Then, under a binocular microscope (8X magnification), protozoa were subsequently transferred into the three wells of the same column (10 cells per well). The plates were incubated in darkness at 25 degree C. Two kinds of test responses were observed: (1) different deformations (E), which means morphological changes such as shortening, bending of the cell, and so forth; and (2) lethal response (L), spherical deformation and autolysis. On this basis two values were calculated for each row of the microplate: EC50: the concentration producing different deformations of 50% of the test organisms, and LC50: the concentration producing

lethal response of 50% of the test organisms. The EC50 and LC50 values were determined by graphical interpolation of test response versus toxicant concentration (log scale). Mean values (EC50 and LC50) +/- SD were then calculated for each microplate.

Result: Toxicity in Spirotox test, unit; ppm (mg of anion/L)
24-h EC50 = 285 +/- 135
24-h LC50 = 430 +/- 92
48-h EC50 = 281 +/- 137
48-h LC50 = 355 +/- 127

Results (mean of three tests +/- SD) are expressed in ppm (mg of anion/L). EC50 is the concentration producing different deformations of 50% of the test organisms after 24 h (48 h) of incubation. LC50 is the concentration producing lethal response of 50% of the test organisms after 24 h (48 h) of incubation

Test condition: Protozoan Spirostomum ambiguum

Spirostomum ambiguum is one of the biggest protozoans, 2-3 mm long (Raabe 1970). The strain used in the work was originally collected in Kampinos National Park near Warsaw and has been cultured in laboratory for more than 20 years.

Culturing the Spirostomum ambiguum
S. ambiguum was routinely cultured in 5-1 aquariums containing 4 L of natural, unpolluted water originating from a very deep source (pH = 7.5; total hardness 150 mg CaCO3/L). Cultures were maintained at 20-25 degree C in darkness. Protozoa were fed once a week with a diet of flaked oats and dried alder leaves (50:1). Every 4 weeks, two-thirds of the water in the aquarium was replaced with fresh water.

Preparation of the Protozoa for the Test
In order to prepare the protozoa for the test, it was necessary to rinse them from the culturing medium. Several hundred cells were taken from the culture and placed in a 50-mL cylinder. After the cells had fallen to the bottom, water was poured out and organisms were rinsed three times with diluent Diluted Tyrod solution, 1:64 was used; it was made upof 125 mg NaCl, 3.1 mg KCl, 3.1 mgCaCl2, 1.55 mg MgCl2, 15.6 mg NaHCO3, and 0.78 mg NaH2PO4 per Liter of deionized water (Milli-Q quality)L Total hardness was 2.8 mg CaCO3/L and pH 7.4 +/- O.2.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
27-MAY-2005 (128)

Type: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: = 757.9
EC50: = 1600

Method: other
Year: 1993
GLP: no

Remark: (Mobility)
96-h NOEC = 3735 mg/L*

* : The values were converted NO2-N into NaNO2.
organism : Tetraselmis chuii
endpoint : mortality reduction
Result: EC50 (concentration that cause lost of mobility on 50% of the population)
24-h EC50 = 2,200 mg/L NO2-N
48-h EC50 = 1,510 mg/L NO2-N
72-h EC50 = 1,590 mg/L NO2-N
96-h CC50 = 1,600 mg/L NO2-N

24-h NOEC = 67.4 mg/L NO2-N
48-h NOEC = 222.8 mg/L NO2-N
72-h NOEC = 413.8 mg/L NO2-N
96-h NOEC = 757.9 mg/L NO2-N
Test condition: -Effects of nitrite on growth of the marine micoralgae (Tetraselmis chuii)

Toxicity of nitrite on the microalgae T. chuii was studied in static bioassay.
Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
27-MAY-2005 (140)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: other: Penaeus monodon (salt water shrimp)
Endpoint: other: Mortality and Growth
Exposure period: 80 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = 2
EC50: = 23.31
LC50 : > 20

Method: other
Year: 1992
GLP: no

Method: METHOD FOLLOWED: APHA (1985), Buikema et al (1982)

DEVIATIONS FROM GUIDELINE:

STATISTICAL METHODS: LC50 calculated with the Trimmed Spearman Karber method

METHOD OF CALCULATION: no data

ANALYTICAL METHODS: Nitrite determined using the method of Strickland and Parsons (1972)

References:

APHA (1985) Standard Methods for the Examination of Water and Wastewater. 16th edn. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington DC

Buikema AL jr., Niedertehner RR and CairnsJ Jr. (1982) Biological Monitoring - IV. Toxicity Testing. Water Res. 16, 239-262

Strickland JDH and Parsons TR (1972) A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottowa, Canada.

Remark: -Mortality
80-d LC50 > 95.6 mg/L*

-EC50 for weight gain
80-d EC50 = 114.9 mg/L*
80-d NOEC = 9.86 mg/L*
* : The values were converted NO2-N into NaNO2.

Result:

Nominal	nitrite-N (mg/L)	Survival
Control	0.118 +/- 0.004	30
2	1.955 +/- 0.020	30
4	3.920 +/- 0.140	29
8	7.735 +/- 0.200	26
20	19.789 +/- 0.300	21

-Mortality
80-d LC50 > 20 mg/L (Nitrite-N)

-EC50 for length increase (Nitrite-N)
20-d EC50 = 16.14 mg/L
30-d EC50 = 18.20 mg/L
40-d EC50 = 19.96 mg/L
50-d EC50 = 22.50 mg/L
60-d EC50 = 26.20 mg/L

-EC50 for weight gain (Nitrite-N)
10-d EC50 = 16.99 mg/L
20-d EC50 = 17.41 mg/L
30-d EC50 = 18.03 mg/L
40-d EC50 = 20.12 mg/L
50-d EC50 = 21.85 mg/L
60-d EC50 = 22.45 mg/L
70-d EC50 = 24.85 mg/L
80-d EC50 = 23.31 mg/L

-NOEC[MATC (maximum acceptable toxicant concentration)]= 2 mg/L (Nitrite-N)

Survival of the shrimps exposed to different test solutions at various periods is shown below. All shrimps exposed to lower than 8 mg/L nitrite-N after 30 days, and those exposed to 2 mg/I nitrite-N and the control survived after 80 days.

At growth in weight and length of the P. monodon juveniles reared in each test solution, a one-way analysis of variance indicated that weights of shrimps exposed to 4, 8 and 20 mg/L were significantly lower (P < 0.05) than those exposed to 2mg/L nitrite-N and controls after 20 days.
Analysis also indicated that weights of shrimps exposed to 2 mg/L were significantly lower (P < 0.05) than those exposed as controls after 60 days. The MATC (maximum acceptable toxicant concentration) was 4, 2, and less than 2 mg/L after 10, 30, and 60 days of exposure.

At the relationship between weight and length, statistical analysis indicated that no significant difference (P > 0.05) in growth factor was observed among the shrimps exposed as controls, or to 2, 4 and a mg/L. A significant difference (P <0.05) or growth factor was found between those exposed to 20 mg/I nitrite-N and control. The MATC for P. monodon juveniles was 8 mg/L nitrite-N calculated from the growth factor.

The linear regression of mean individual weight gain vs concentration or nitrite-N was significant (p<0.05) after various periods. The mean individual final weight (5.71 g) of the control animals was 4.11 times their initial weight (1,39 g). However, the mean individual final weight (4.00 g) of the shrimps exposed to 20 mg/L nitrite-N was 2.72 times their initial weight (1.47 g). The EC50 (concentration that reduced growth by 50% of that of the controls) for weight gain was 17.41, 20.12 and 22.45 mg/L nitrite-N after 20, 40 and 60 days of exposure.

Linear regression of mean individual length increase vs concentration of nitrite-N was significant (P < 0.05) after various periods except at 10, 70 and 80 days. The mean individual final length (9.44 cm) of the control animals was 1.57 times their initial length (6.00 cm), However, the mean individual final length (8.42cm) of the shrimps exposed to 20 mg/L nitrite-N was 1.37 times their initial length (6.16 cm). The EC50 for length increase was 16.14, 19.96 and 26.20mg/L nitrite-N after 20, 40 and 60 days of exposure.

The ratio or carapace length to total length of the P. monodon juveniles exposed to different test solutions is given. Statistical analysis indicated that this ratio for shrimps exposed to 2 mg/L was significantly lower (P < 0.05) than for those of the controls.

Nitrite increased molting frequency of P. monodon juveniles during the 80 days of bioassay. In the control solution, 7 molted 6 times, 11 molted 7 times and 4 shrimps molted 8 times. In the 20 mg/1 nitrite-N, 4 molted 6 times. 5 molted 7 times and 11 shrimps molted 8 times. A one-way analysis of variance indicated that there was no significant difference (P > 0.05) in molting frequency between the shrimps exposed to 2 mg/L nitrite-N and the controls.
Mean intermolt period of the shrimps exposed to 8 mg/L nitrite-N was significantly less (P < 0.05) than those exposed as controls for 2nd-3rd molting. The MATC was 2 mg/L nitrite-N from the molting frequency and intermolt period.

MATC means NOEC.

Test condition: TEST ORGANISMS:

Size: The shrimps used were 6.03+/-0.07 cm average body length and 1.40+/-0.05 g wet weight
Age: Juveniles
Pretreatment: Aclimated for one week
Supplier: Private nursery located in Iilan, Taiwan

DILUTION WATER:
Source: Seawater pumped from the Keelung coast adjacent to the University was diluted with municipal water to 25 ppt and filtered through sand and gravel filters by air-lift pumping.
Chemistry: no data
Temperature: no data

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: Nitrate test solutions were prepared using 43.3 g of sodium nitrite in 1 litre of distilled water to make 10,000 mg/L nitrite-N (nitrite as nitrogen) and then diluted with salt-water

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No data

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: 2, 4, 8 and 20 mg/L
Renewal of test solution: Daily (semi-static test)
Exposure vessel type: 60 x 30 x 36 cm tank containing 30 L of solution. Each tank contained 10 cylindrical plastic cages (10 cm diameter, 30 cm high, 2x3 mm net size).
Number of replicates, fish per replicate: Triplicate, 10 fish/tank/concentration
Water parameters: water temperature, pH and dissolved oxygen was 29 +/- 1.4 degree C, 8.14 +/- 0.13 and 5.91 +/- 0.23mg/L, respectively.
Intensity of irradiation:
Photoperiod:
Feeding: Shrimps were offered commercial shrimp ration (Trairoun Product Co., Taipei) three times a day (9:00, 15:00 and 21:00) at a rate of 9% of body weight per day.
Aeration:

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION:

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Supplier: Merck

Conclusion: 1. Survival or Penaeus monodon juveniles (1.40 +/- 0.05 g; 6.30 +/- 0.07 cm) reared in control, 2, 4, 8 and 20 mg/L nitrite-N by a static renewal method after 80 days was 100, 100, 96.7, 86.7 and 70%, respectively.

2. Growth of the shrimps reared at 4, 8 and 20 mg/L nitrite-N was significantly lower ($P < 0.05$) than control animals and those reared at 2 mg/L nitrite-N after 20 days.

3. EC50 (concentration that reduced growth by 50% or that of the controls) rot weight gain was 17.41 and 22.45 mg/L nitrite-N. and EC50 for length increase was 16.14 and 26.20mg/L nitrite-N after 20, and 60 days, respectively.

4. Nitrite decreased the ratio of carapace length to total length, and enhanced molting frequency of the shrimps. Average molting frequency of shrimps reared as control and at 2, 4, 8 and 20 mg/L nitrite-N was 6.27, 6.30, 6.34, 6.92 and 7.14 times, respectively.

5. The NOEC [MATC (maximum acceptable toxicant concentration)] was estimated to be 2 mg/L nitrite-N from the growth and molting.

NOEC : = 2 (Growth Inhibition)
EC50 : = 23.31 (Growth inhibition)
LC50 : > 20 (mortality)

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
26-MAY-2005 (36)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

Species: Lumbriculus
Endpoint: Mortality
Expos. period: 96 other:hour(s)
Unit: other: mg/L
LC50 (lower) : = 20

Method: other
Year: 1986

Method: Effect: mortality
Fresh water
Age/Weight: juvenile, 0.006 grams
Result: LC50 > 20 mg/L
LC50 = 20 mg/L

The lower value was = 20 mg/L.
Test condition: Latin name: Lumbriculus variegatus

-Water parameters:
Temperature; 20 degree C
Dissolved O2; >40%
pH; 6.5-8.5

Food was withheld for the 24 preceding start of the test.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
27-MAY-2005 (57)

Species: other: Corbicula manilensis
Endpoint: Mortality
Expos. period: 96 other: hours

Unit: other: mg/L
LC50: = 51

Method: other
Year: 1979

Method: Static
Effect: mortality, increasing
Fresh water
Weight: 1.0-2.7 grams
Test condition: -Water parameters:
Temperature; 16 degree C
Hardness (mg/L CaC03); 16-26
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Technical grade
Reliability: (4) not assignable
27-MAY-2005 (34)

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

Species: other: Eisenia fetida (earthworm)
Endpoint: mortality
Exposure period: 48 hour(s)
Unit: other: micro-g/cm2
LC50: = 100 - 1000

Method: OECD Guide-line 207 "Earthworm, Acute Toxcity Test"
Year: 1984

Method: Filter paper method
Result: 48-h LC50 range 100 - 1,000 micro-gram/cm2 (moderately toxic)
Test condition: -Test animals
E. foetida were purchased as needed from Bert's Bait Farm, Irvine, KY. The earthworms were housed in Nalgene boxes filled with moist peat moss and rabbit manure, and stored at 18 degree C. Cornmeal was added as an additional food source and calcium carbonate was added as necessary to maintain a soil pH, above 5.5. Mature worms showing a developed clitellum and weighing 370 to 450 mg each were used for the toxicity experiments.

-Contact toxicity test
The testing protocol used for evaluating chemical toxicity against earthworms involves exposing individual E. foetida to concentrations of chemicals in paper-lined glass vials for 48 h in the dark.

Glass shell vials (22 mm x 85 mm) (Research Products International, Mt. Prospect, IL) were lined with Whatman No, 1 filter paper strips (9.5 cm x 6.8 cm; surface area, 65 cm2) and placed in cardboard scintillation vial trays. Chemical concentrations used in the contact toxicity tests were expressed in ug/cm2. After the vials had been under the stream of warm air for 3 min, they were air-dried for an additional 2 h to remove all remaining solvent. Then, 1 mL

of water was added to each vial to moisten the paper and one earthworm was placed into each vial.
After the earthworms were placed in the vials, the containers were capped and kept stored in the dark in the horizontal position for 48 h. Death was recorded if the worm did not respond to gentle probing of its anterior end. Worms that were severely morbid, even having lost their posterior half but still responding to the probe, were considered alive. The approximate range of acute toxicity was determined with five exposure concentrations, using 10 worms per exposure and chemical concentrations that increased logarithmically from 0.1 to 1,000 fig/cm2.
The approximate lethal concentration was considered to be the lowest exposure that killed 50% or more of the earthworms. LC50 values were then determined for certain chemicals by exposing earthworms to a geometric series of five to seven exposure concentrations, including the approximate lethal dose. This series was replicated so that a minimum of 100 earthworms were used for determining each LC50 Value. The dose-lethality data provided a slope and LC50 values with 95% confidence intervals when calculated by the Litchfield-Wilcoxon log dose-effect probit transformation method.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Technical or analytical grade

Reliability: (4) not assignable

27-MAY-2005 (146)

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Species: other: Ambystoma texanum
Endpoint: other: equilibrium, behavior
Expos. period: 96 hour(s)
Unit: other: mg/L
LC50: = .33

Method: other
Year: 1980

Method: Static
Fresh water
Life stage/weight: larvae, 0.45 grams

Loss of equilibrium, i.e. ecological death, was the criterion for lethality.

Result: LC50 = 0.33 (0.15 - 0.76) mg/L
Test condition: -Water parameters:
Temperature; 25 degree C
Hardness (mg/L CaC03); 140
pH; 7.0

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (4) not assignable

27-MAY-2005 (80)

Species: other: Ramshorn snail (Helisoma trivolvis)
Endpoint: mortality
Expos. period: 24 hour(s)
Unit: other: mg/L
LC50: = 552

Method: other
Year: 1986
Test substance: other TS

Method: Static
Effect: mortality
Fresh water
Age/Weight: 0.07-0.11 grams, 3 wk tadpole
Test condition: -Water parameters:
pH; 6.9-7.4
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analar grade
Reliability: (2) valid with restrictions
27-MAY-2005 (198)

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vivo
Type: Absorption
Species: rat
Route of administration: gavage

Year: 1980
GLP: no

Method: Aqueous solutions of sodium nitrite were administered orally by gavage to rats which were fasted overnight.

Blood samples were obtained either by decapitation of from caudal veins.

Methaemoglobin and total haemoglobin were determined by the Evelyn and Malloy's (Evelyn et al, 1938) and cyanomethaemoglobin (Van Assendelft, 1970) methods, respectively.

The nitrosyl haemoglobin content was estimated from the peak heights of electron spin resonance spectra obtained at 77K with a Varian E-12 X-band ESR spectrometer.

Evelyn KA & Malloy HT (1938) Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood. J. Biol. Chem. 126, 655-662

Van Assendelft OW (1970) Spectrophotometry of Haemoglobin Derivatives. Assen: Royal Vangorcum.

Result: The methemoglobin level increased to 45-80% one hour after the administration of the LD50 dose and returned to the normal level after 24 hours if the animals survived. The dose-maximum methemoglobin concentration curve was found to be S-shaped. Formation of nitrosyl hemoglobin preceded that of methemoglobin, its maximum concentration being a quarter that of the latter derivative.

The concentration of nitrite anion reached a maximum (about 1mM) 20-30 min after administration, and disappeared with half-life period of approximately 70 min, while the concentration of nitrate anion remained at a high level (1.0 - 1.5mM) for more than 5 hours.

Test condition: Test Species: Sprague-Dawley rat
Age/weight: 4 months/200 g
Dosage: 0, 10, 25, 50, 100, 150 mg/kg bw
Animals/dose: 5

Test substance: Chemical Name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

26-APR-2005 (85)

In Vitro/in vivo: In vivo
Type: Absorption
Species: mouse

Year: 1972

Method: Time course of in vivo disappearance of sodium nitrite from mouse stomach:

Sodium nitrite (150 µg) was administered to each mouse by gavage in 0.1 mL aqueous solution. Animals, in groups of 13-18, were then killed by cervical dislocation within a minute and at 10, 20 and 30 minutes after administration. Stomachs, together with attached 5 mm segments of the esophagus and duodenum, were removed and assayed individually for sodium nitrite.

Effect of ligation of gastroduodenal junction on rate of in vivo disappearance of sodium nitrite from mouse stomach:

Groups of 5-8 mice were anesthetised by intraperitoneal injection of 150 mg/kg sodium hexobarbital. The gastroduodenal junction was ligated in some groups, while in controls the ligature was left loose. The stomachs were then injected intraluminally with 150 µg sodium nitrite in 0.1 mL of water. The abdominal wall was then sutured. Mice were killed by cervical dislocation 10 or 30 min later and stomachs were removed and assayed individually for sodium nitrite.

Nitrite determination:

Individual stomachs were placed in 20 mL distilled water, buffered with 0.25 mL of 0.67M NH_4Cl/NH_4OH at pH 9.6-9.7 and sliced open. Activated charcoal was added and the flasks were agitated for 15 min at room temperature. Then 0.2 mL of 1.04 M $ZnSO_4$ was added and the flasks were agitated for a further 5 min. Contents of each flask were centrifuged at 2000 ppm for 15 min. Aliquots were taken for colorimetric determination of sodium nitrite.

Result: Following oral administration, sodium nitrite diasappeared rapidly from the mouse stomach. 85 and 95% losses were seen at 10 and 30 minutes, respectively.

The rate of sodium nitrite disappearance from the mouse stomach in vivo was not significantly reduced by ligature at the gastroduodenal junction. Although there was consistently more gastric sodium nitrite lost in mice not having the gastroduodenal junction ligated, this difference was not statistically significant and did not represent a major pathway of nitrite loss.

Test condition: Test animals: Swiss ICR/Ha mice
Sex: Male
Weight: 20-25 g

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: No data

Conclusion: The authors concluded that the major pathway of loss available gastric nitrite is absorption directly from the stomach into the bloodstream.

Reliability: (2) valid with restrictions
26-APR-2005 (62)

Type: Metabolism
Species: other: cat, rabbit, rat

Year: 1988

Method: In vivo study:
Animals were dosed by intravenous injection with 0.30 mmol/kg sodium nitrite. Prior to dosing, animals were anaesthetised with either 30 mg/kg i.v. pentobarbitol (rabbits), 60 mg/kg chloralose by gastric tube (cats) or ether in a glass tube (rats). All animals were allowed to breath spontaneously. The anaesthetics were repeatedly administered as required. Heparin was always give to the anaesthetised animals (500 I.U./kg i.v.). Blood samples were taken via a catheter from the femoral vein.

In vitro study:
Heparinised blood samples were taken from the animals via a catheter from the femoral vein. Human PPL erythrocyte concentrate was delivered from a blood bank.

For the preparation of the erythrocyte suspensions, the human and animal samples were centrifuged at 4000 rpm for 10 minutes. After removing the supernatant, the cellular sediment was washed three times with 0.15 M saline and centrifuged before resuspension in 0.2 M phosphate buffer, pH 7.4, with 5 mM glucose.

Purified human haemoglobin was prepared by chromatography.

Ferrihaemoglobin formation was followed in tightly capped HPLC vessels containing 1.5 mL of the red blood cell suspension. Sodium nitrite, diluted in 0.15 M saline, was added in microquantities (<= 5 µL) from a Hamilton syringe through the rubber membrane of the vessel cap. The incubates were shaken intensively in a water bath at 37°C.

Ferrihaemoglobin was determined photometrically at 550 nm after addition of KCN to haemolysate samples. Blood or washed erythrocytes (0.1mL) were haemolysed in ice-cold deionised water (10 mL) during 10 minutes and adjusted to pH 6.6 with 0.2 M phosphate buffer (1.0 mL) before centrifugation at 4000 rpm for 5 minutes.

For ferrihaemoglobin determinations in solutions of purified haemoglobin, 0.1 mL of the solution was likewise diluted in a total volume of 11.0 mL. Total haemoglobin was assayed after oxidation of ferrohaemoglobin to ferrihaemoglobin with potassium hexacyanoferrate (III) (10 g/dL).

Result: In vivo study:
Sodium nitrite induced ferrihaemoglobin formation with maxima of 47.7+/-1.3% at 90 min in cats, 7.5+/-1.0% at 10 min in rabbits and 18.4+/-0.0% at 30 min in rats. Despite the five times greater ferrihaemoglobin maxima due to treatment with sodium nitrite in cats compared to rabbits, respiratory rate increased three times less. Total haemoglobin was not influenced by nitrite.

In vitro study:
Human erythrocytes: The haemoglobin content in the sealed test tubes averaged 12.2+/-0.2 g/dL (n=17). In the presence of 1.4 mM sodium nitrite the gain in FE3+ was 1.97=/-0.06 mmol/mol NaNO2.

Erythrocytes from different animal species: in the presence of 2.5 mM sodium nitrite, ferrihaemoglobin maxima were not reached by 60 minutes except for rabbit erythrocytes. The courses of the rate of formation curves were similar for the erythrocytes of cats, oxen, dogs and humans. The initial rate of ferrihaemoglobin production in canine red blood cells was higher than in the cells of the other species. Half the maxima were attained within less that five minutes in the erythrocytes from dogs or rabbits and within approximately 20 minutes or greater in human, bovine and cat erythrocytes. In the rabbit cells, the ferrihaemoglobin content did not vary significantly between 30 and 60 minutes.

Test condition: In vivo study:

Test animals:

Chinchilla rabbits
Weight: 4.4+/-0.2 kg
No. of animals: 6

Cats
Weight: 2.5+/-0.2 kg
No. of animals: 4

Sprague-Dawley rats
Weight: 295+/-8.7 g
No. of animals: 3

Test substance: Chemical Name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Purest grade
Supplier: Merck (Darmstadt, Germany)

Reliability: (2) valid with restrictions

29-APR-2005 (104)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: other: human, rat, sheep

Year: 1983

Method: Preliminary testing established a concentration of sodium nitrite (3 mM) which produced evidence of an approximately 50% increase in methaemoglobin levels in human blood cells. This amount of sodium nitrite was administered in 10 µL aliquots/mL of whole blood. This amount of blood and oxidant was then incubated with increasing levels of ascorbic acid (1.0 - 9.0 mM) for a 2-hour exposure at 37°C. An incubated control with no ascorbic acid addition was employed. Additional controls employing ascorbic acid (1.0 - 9.0 mM) were run to assess the potential effects of ascorbic acid acting by itself.

Blood samples were taken from six normal humans obtained via venipuncture of the brachial vessels, six female adult sheep of the Dorset strain obtained via puncture of the jugular vein, and six male Sprague-Dawley rats aged approximately 3 months obtained via cardiac puncture. The blood was collected in heparinised tubes on the morning of testing, held in an ice bath until use in the experiment that day. Methaemoglobin was measured according to the method of Brown (1973) using potassium ferricyanide and potassium cyanide as reagents and measuring changes in optical density at 630 nm.

Brown BP (1973) Hematology: Principles and Procedures, 1st ed, Lea & febiger, Philadelphia, USA

Result: Rats: Treatment of rat erythrocytes with 3 mM sodium nitrite produced 14.2% methaemoglobin compared to 1.7% in the control. This level of methaemoglobin was reduced in a dose-dependant manner when incubated in the presence of ascorbic acid additions.

Sheep: The formation of methaemoglobin in sheep erythrocytes increased substantially (48.7% compared with control mean of 2.2%) upon incubation with 3 mM sodium nitrite. Ascorbic acid treatment, however, did not alter this level of methaemoglobin formation significantly.

Humans: Incubation of normal human erythrocytes with 3 mM sodium nitrite produced a methaemoglobin level of 48.6% compared to 1.8% in the incubated controls. Ascorbic acid treatment significantly ($p<0.001$) reduced methaemoglobin levels in a dose-dependant manner.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: The 2-hour incubation of whole blood with 3mM of sodium nitrite produced an approximately 50% methaemoglobin level for both sheep and normal humans. However, rats were considerably less susceptible to nitrite induced methaemoglobin formation, with the same dose producing only 14% methaemoglobin. The difference in sensitivity is probably due to the fivefold difference in erythrocyte methaemoglobin reductase activity between humans and rats [Smith and Beutler, 1966].

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

26-APR-2005 (30)

In Vitro/in vivo: In vitro

Type: Toxicokinetics

Species: other: human, goats, sheep, horse, cattle, pig

Year: 1966

Method: All blood samples were collected from mature individuals of the various species into ACD solution to which sodium chloride had been added to the extent of 0.25% to increase the osmotic strentgh to a level more nearly isotonic with RBCs. The blood samples were stored at 4°C for up to 7 days prior to use. The rate of oxidation of oxyhaemoglobin to methaemoglobin was determined by the method of Betke et al. Erythrocytes from the various species were washed three times with 1.5% sodium chloride solution and hemolysed by dilution with distilled water. The hemolysate was mixed with 4-5 vol colloidal aluminium hydroxide and filtered. The haemoglobin

concentration was determined by the cyanmethaemoglobin method, adjusted to 105 mg/100 mL, and 1/20 vol of 2.8 M phosphate buffer, pH 6.8 was added. The reaction was started by the addition of 0.02 mL freshly prepared 0.073 M sodium nitrite solution to 2.5 mL haemoglobin solution. The reaction rate was followed at 630 mμ in a Gilford model 2000 multiple absorbance recorder.

Methaemoglobin reduction was carried out in a system previously described except that the volume of all reactants was reduced by one third (Beutler & Beluda). Blood from the various species was centrifuged and the plasma and buffy coat removed. The haemoglobin was converted to methaemoglobin by incubating the erythrocytes with 2 vol 0.145 M sodium nitrite solution for 20 min. The cells were then separated by centrifugation and washed with 7-10 vol isotonic saline. The reaction mixture contained: nitrite-washed erythrocytes, 29%; potassium phosphate buffer, pH 7.4, 65 mM; glucose, 28 mM; and sodium chloride, 45 mM. Methylene blue, 0.017 mN, was used to activate the triphosphopyridine nucleotide-linked system. Methaemoglobin percentage was determined by the method of Evelyn and Malloy at two hour intervals for a total of six hours.

Betke K, Greinacher I and Hecker F (1956) Oxidation of Human and Animal Oxyhemoglobin by Sodium Nitrite. arch. Exptl. Pathol. Pharmakol. 229, 207-219

Beutler E and Baluda MC (1963) Methemoglobin Reduction. Studies of the Interaction Between Cell Populations and of the Role of Methylene Blue. Blood, 22, 323-333

Evelyn KA & Malloy HT (1938) Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood. J. Biol. Chem. 126, 655-662

Result: Methaemoglobin formation: The rate of oxidation of haemoglobin to methaemoglobin occurred most rapidly in ruminants (sheep, goat and cow) and was slower in nonruminants (man, horse and pig).

Methaemoglobin reduction with glucose: When nitrite-treated, washed erythrocytes were incubated with glucose, the reduction of methaemoglobin was linear for the first six hours of incubation. There was a marked difference between each of the species studied. With the exception of man, the animals with the most rapid reduction rate were ruminants.

Methaemoglobin reduction with glucose and methylene blue: The rates of methaemoglobin reduction with methylene blue and glucose again showed marked differences between the various species. Methylene blue produced an acceleration in the reduction rate. This acceleration was most pronounced in man (six-fold) and cattle (four-fold).

Test substance: Chemical name: Sodium nitrate (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
26-APR-2005 (166)

In Vitro/in vivo:	In vivo
Type:	Metabolism
Species:	rat

No. of animals, males: 22
No. of animals, females: 0
Doses, males: 0, 10, 30, 100, 300, 1000 µmol/kg bw
Route of administration: infusion
Exposure time: 5 minute(s)

Year: 1997

Method: Male Wistar rats were anaesthetised with urethane (1.3 g/kg bw ip). The trachea was canulated to avoid obstruction of the airways by mucus secretion. The right femoral artery was cannulated for measurement of arterial blood pressure and heart rate, the left femoral vein was cannulated for taking blood samples and the left jugular vein was prepared for infusion of nitrite.

A solution of sodium nitrite (1.25 mL) was infused over 5 minutes into the left jugular vein. For each dose four animals were used except for the control and high dose groups for which three animals were used.

Blood pressure and heart rate were recorded at time -5, 0, 5, 15, 30, 45 and 60 minutes. For measurement of plasma nitrate and nitrite, blood sampls (0.4 mL) were taken immediately after infusion of sodium nitrite and collected in EDTA vials. Body temperature was kept between 36 and 39°C by a heating pad.

Result: In urethane-anaesthetised rats infusion of NaNO2 [0 (n=3), 10 (n=4), 30 (n=3), 100 (n=4), 300 (n=4) or 1000 (n=3) µmol/kg bw] over 5 minutes, resulted in a dose-dependent increase in plasma levels of nitrite and a rapid conversion of nitrite into nitrate. Mean arterial pressure (MAP) and heart rate (HR) just prior to infusion of NaNO2 were 92.6+/-3.2 mmHg and 396+/-6.9 beats/min, respectively. NaNO2 decreased MAP dose dependently but no marked effects on HR were observed.

Test condition: Test animals: Wister male rats
Weight: 275-350g

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade
Source: E Merck AG (Darmstadt, Germany)

Reliability: (2) valid with restrictions

26-APR-2005 (188)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, females: 21
Route of administration: gavage

Year: 1984

Method: 21 rats were dosed with 30 mg/kg bw sodium nitrite by gavage. Groups of three animals were exsanguinated at intervals from 2.5 to 360 minutes.

Plasma nitrite was determined photometrically according to the method of Grau and Mirna with minor modification. Methaemoglobin was determined photometrically using the method of Evelyn and Malloy.

Grau R and Mirna A (1957) Z. Anal. Chem. 158, 182

Evelyn KA & Malloy HT (1938) Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood. J. Biol. Chem. 126, 655-662

Result: Oral treatment with NaNO2:

When starved rats received a single dose of 30 mg/kg bw of NaNO2 in aqueous solution by gavage (10 - 15% of LD50), plasma nitrite and methaemoglobin levels were already increased after 2.8 minutes and maximum effects were observed after 22.5 minutes. After three hours both parameters had returned to the physiological range.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
26-APR-2005 (76)

In Vitro/in vivo:	In vivo
Type:	Absorption
Species:	rat
Route of administration:	dermal

Year: 1997

Method: Male Wistar rats weighing about 480 - 500g were anesthetized by intraperitoneal injection of 60 mg/kg pentobarbital. normal and abraded skin were prepared as follows: Dorsal hair was shaved with a razor (normal skin). About 10 cm2 of epidermis was scraped with a razor from this shaved area (abraded skin). Indwelling cannulae were implanted in the left and right femoral artery. One catheter was used to monitor arterial blood pressure. A 7 cm2 skin area was delimited by an open circular cell fixed to the skin using cyanoacrylate glue. Each liniment solution (120 µL) was applied to six animals for both normal and abraded skin. Heparinized blood samples were placed in a hematocrit tube from another cannula. The arterial blood gas sample was analyzed for MetHb (OSM3 Hemoximeter, Radiometer, Copenhagen, Denmark). Samples were taken before application and at 5 and 10 min after application and every 10 min thereafter, up to 180 min.

Result: Application of liniment solutions containing 30 g nitrite/L to abraded skin caused a gradual increase of MetHb which reached a peak within 40-50 minutes and then decreased relatively contantly throughout the observation period of 180 minutes.

Application of liniment solutions containing 140 g nitrite/L to abraded skin caused a marked increase in MetHb concentration. regardless of the application time, the concentration of MetHb was consistently significantly higher in abraded skin than in normal skin. rats with abraded skin to which liniment solution B2 was applied died after about 170 minutes.

MetHb formation was significantly correlated with the skin condition at the same nitrite concentration.

Application of liniment solutions containing 30 g nitrite/L to normal skin resulted in an immediate decline of arterial blood pressure, followed by an increase. Application of liniment

solutions containing 140 g nitrite/L to normal and abraded skin significantly decreased the blood pressure.

Within 10 min after the application of liniment solutions containing 140 g nitrite/L to abraded skin, cutaneous blood flow dropped significantly to between 50.2+/-3.2% and 41.6+/-6.0% of the pretreatment values. This decrease continued to the end of the experiment. Application of liniment solutions containing 30 g nitrite/L to abraded skin also significantly decreased the subcutaneous blood flow. In normal skin, the subcutaneous blood flow was significantly increased compared to the abraded skin.

Test substance: Liniment solutions containing sodium nitrite, as follows:

Liniment A: Base of nonionic surfactant/oil containing 30 g nitrite/L

Liniment A2: Base of nonionic surfactant/water containing 30 g nitrite/L

Liniment A3: Base of water containing 30 g nitrite/L

Liniment B: Base of nonionic surfactant/water containing 140 g nitrite/L

Liniment B2: Base of water containing 140 g nitrite/L

Reliability: (2) valid with restrictions
Not a standard test, but closely related to human data which is an important hazard warning.

29-APR-2005 (155)

In Vitro/in vivo: In vivo
Species: rat

Year: 1975

Remark: Adaptation of rats following sodium nitrite induced methemoglobinemia. The effect of repeated intraperitoneal injections of sodium nitrite on methemoglobin, hemoglobin and blood sugar level, on leucine aminopeptidase activity in plasma and methemoglobin reductase activity in red blood cells was investigated in rats.

Repeated methemoglobinemia produced gradual disappearance of hyperglycemia, changes of hemoglobin content in blood and increase of methemoglobin reductase activity in red blood cells.

The enzyme methemoglobin reductase catalyzes the reduction of methemoglobin to hemoglobin and protects red cells against oxidative damage. Along with methemoglobin concentrations, methemoglobin reductase activities increased after nitrite administration to rats.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions

29-APR-2005 (142)

Type: Metabolism
Year: 1996

Remark: Reduction of nitrate to nitrite in vivo may be effected by both enteric bacteria and mammalian nitrate reductase activity. Many species of micro-organisms resident in the oro-gastrointestinal tract possess nitrate reductase activity and this enzyme has been detected in rat liver and intestinal mucosa.

From comparitive studies in germ-free and conventional rats the author concluded that of the 40-50% of a dose of nitrate reduced to nitrite in conventional animals, approximately half was effected by mammalian nitrate reductase. The major site of conversion of nitrate to nitrite varies with species and is dependent on the sites of microbial colonisation and absorption of nitrate.

The presence of nitrite in the oral cavity of humans is attributed to a stable population of nitrate reducing bacteria established at the base of the tongue. On th ebasis of the (highly variable) salivary levels of nitrate and nitrite after oral ingestion of nitrate by humans, it has been estimated that, of the 25% of ingested ntirate secreted in saliva, 20% is reduced to nitrite (i.e. about 5% of the oral dose). and it appears that oral reduction of nitrate is the most important source of nitrite for man and most species that possess an active salivary secretory mechanism.

Reliability: (2) valid with restrictions
22-JUL-2005 (191)

Type: Metabolism

Year: 2001

Remark: Infants under 3 months old are particularly sensitive to nitrite. A large proportion of haemoglobin in these infants is in the foetal haemoglobin form, which is more readily oxidised to methaemoglobin than adult haemoglobin. Further, reduced nicotinamide-adenine dinucleotide (NADH)-dependent methaemoglobin reductase, the enzyme responsible for reduction of methaemoglobin back to normal haemoglobin, has only about half the activity present in adults

22-JUL-2005 (3)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: mouse
Strain: other: White
Sex: male/female
No. of Animals: 100
Doses: 100, 150, 200, 250, 300 mg/kg
Value: = 214 - 216 mg/kg bw

Year: 1950

Remark: Route; oral

LD50(mg/kg) = 214 (male)
LD50(mg/kg) = 216 (female)
Result: -Male
Dose (mg/kg) - Proportion killed
300 - 10/10
250 - 8/10
200 - 7/10
150 - 2/10
100 - 0/10
LD50 = 214 mg/kg

-Female
-Male
Dose (mg/kg) - Proportion killed
300 - 10/10
250 - 8/10
200 - 8/10
150 - 1/10
100 - 0/10
LD50 = 216 mg/kg

All animals that died were found to have methaemoglobin in their blood, although the levels are not reported. Mice receiving the larger doses died within a few minutes and all other mice (except one) that died did so within 24 hours.
Test condition: Animal: about 20 g
Substance was given in a 0.5-2% aqueous solution.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-MAY-2005 (144)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Vehicle: no data
Doses: 10, 50, 100, 150 mg/kg bw
Value: = 150 mg/kg bw

Year: 1980

Remark: fasted before dosing
Result: The oral LD50 of NaNO2 for rats was found to be 150 mg/kg bw. The methemoglobin level increased to 45-80%, 1h after the administration of the LD50 dose and returned to the normal level after 24 h.
Test substance: Chemical name: Sodium nitrite (CAS No 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
22-JUL-2005 (85)

Type: LD50
Species: rabbit
Strain: other: New Zealand
No. of Animals: 24
Value: = 124 mg/kg bw

Year: 1974

Result: Oral LD50 = 124 mg/kg

(95% C. I. = 114- 134 mg/kg)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
31-MAY-2005 (49)

Type: LD50
Species: rat
Strain: other: BD
Value: = 77 - 130 mg/kg bw

Year: 1963

Remark: Route; gavage

LD50(mg/kg) = 130
LD50(mg/kg) = 77 (fasted)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
22-JUL-2005 (50)

5.1.2 Acute Inhalation Toxicity

Type: LC0
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 10
Vehicle: water
Doses: 10 and 100 mg/m3
Exposure time: 4 hour(s)
Value: = .0951 mg/l

Method: other
Year: 1985
GLP: yes

Remark: Original report: McLean-Head L and Mould AP (1985). ICI Unpublished Report CLT/T/2461
Result: During exposure signs typically seen in restrained animals were somewhat more severe in treated animals. Methaemoglobin was significantly increased above concurrent control values only in females exposed to 10 mg/m3. However,the increase was judged to be not haematologically significant as the value was within the range seen for control animals of this age. Further there was no significant increase in males. There were no toxicologically significant effects on animals maintained for 14 days post-exposure.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Conclusion: Current controls of exposure of workers to sodium nitrite (ie at 10 mg/m3) was more than adequately protective for acute hazards.
Test condition: Groups of 10 male and 10 female Alpk:AP (Wistar) rats were exposed nose only for 4 hours to Sodium nitrite aerosols, generated from solutions in deionised water, at target concentrations of 10 or 100 mg/m3. Aerosols dried rapidly so animals were exposed to dry particulate test material. Air humidity was approximately 60%. Mass mean aerodynamic diameter was 1.7 and 2.0 µm for the low and high groups respectively. Controls killed after exposure and blood

taken for measurement of methaemoglobin, the remaining animals were maintained for 14 days, and then subject to a full post-mortem examination.
Reliability: (4) not assignable
Flag: Critical study for SIDS endpoint
31-MAY-2005 (56)

5.1.3 Acute Dermal Toxicity

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Strain: other: SB
Route of admin.: i.v.
Value: = 65 mg/kg bw

Year: 1963

Result: Oral toxicity was conducted concurrently.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
22-JUL-2005 (50)

Type: LD50
Species: mouse
Strain: Swiss
Sex: male
Route of admin.: i.p.
Value: = 159.7 mg/kg bw

Year: 1963

Test condition: Animal: Swiss, Albino, male, 20-25g (BW)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
31-MAY-2005 (132)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 65 mg/kg bw

Reliability: (3) invalid
31-MAY-2005 (143)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Result: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1985

GLP: yes

Method: Approximately 500 mg of sodium nitrite was applied to the shaved backs of 6 male New Zealand White rabbits and covered with a semi-occlusive dressing for four hours. The animals were examined one hour, one, two and three days after removal of the chemical.
Result: Some slight irritation was observed one hour after removal of the substance, but all signs had disappeared by the one day observation and the substance is not considered to be a skin irritant.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-MAY-2005 (168)

5.2.2 Eye Irritation

Species: rabbit
Result: moderately irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1985
GLP: yes

Method: 100 mg of substance was applied into the conjunctival sac of the left eye of six female New Zealand White Rabbits. The eyes of three of the rabbits were irrigated with water for two minutes 30 - 60 seconds after application of the substance.
Result: Conjunctival effects were seen in all animals and consisted of moderate redness, mild chemosis and severe discharge. All signs of irritation had disappeared by twelve days. No corneal effects were observed
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-MAY-2005 (168)

Species: rabbit
Concentration: .1 other: mol/L
Result: not irritating

Method: other: see "test condition"
Year: 1948
Test substance: as prescribed by 1.1 - 1.4

Result: Test Substance/Concentration/No. Eyes Tested/Severity of Reaction

NaNO2/0.08M/1/0
Cf:
H2O/0/-/0
NaCl/0.9%(0.16M)/-/0
H2O2/0.25%/1/95
Test condition: In the course of the studies reported in the preceding papers a great number of substances were injected into the corneas of rabbits and the resulting reactions observed. These experiments were performed, in part, as controls for a wide variety of special studies, in part as a preliminary

survey of the general toxicology of the corneal tissue. The results are compiled.

Unless otherwise noted, 0.1 mL. of the solution of test substance was injected intracorneally, using a # 25-27 gauge needle and tuberculin syringe. Occasionally the anterior chamber was entered accidently,in some cases resulting in a persistent edematous bulging of the cornea which could be identified clinically and the false positive corneal reaction discarded. The exact quantity injected within limits of 0.05 cc. -0.2 cc. was of less importance than the concentration of the injected material. Secondary infection was uncommon. Accidental injection of air into the cornea did not increase the severity of the reaction produced.
The reactions following intracorneal injection were similar in severity to those following mechanical removal of the corneal epithelium with a cotton toothpick swab, followed by irrigation for 10 minutes with the test solution.
The single numerical value represents the sum of the maximum values of each symptom observed over a period of 7-14 days, expressed in percentage of the maximum possible total.

Reliability: (3) invalid
Method is too unique to used for evaluation.

31-MAY-2005 (81)

5.3 Sensitization

Type: other

Remark: No studies are available in animals investigating the sensitising potential of sodium nitrite. As this substance is endogenously generated, sensitisation potential is not expected. No evidence of sensitisation in humans has been reported.

31-MAY-2005

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 14 weeks
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 375, 750, 1500, 3000, or 5000 ppm in drinking water available ad lib.
Control Group: yes, concurrent vehicle

Method: other: FDA (21 CFR, Part 58)
Year: 2001
GLP: yes

Result: Exposure to 0, 375, 750, 1,500, 3,000 or 5,000 ppm in drinking water was equivalent to approximate daily doses of 0, 30, 55,

115, 200 or 310 mg/kg bw/day in males and 0, 40, 80, 130, 225 or 345 mg/kg bw/day in females.

NOAEL : not obtained (all doses showed methaemoglobin formation)

LOAEL: Males = 115 mg/kg bw/day; Females = 225 mg/kg bw/day

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
Clinical signs: Brown discoloration in the eyes and cyanosis of the mouth, tongue, ears, and feet of 200 and 310 mg/kg bw/day males and of 130 mg/kg bw/day and higher females.

Bodyweight and food consumption: Body weights of 200 and 310 mg/kg bw/day males and 345 mg/kg bw/day females were significantly less than those of the controls. Water consumption by 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females was less than that by the controls at weeks 2 and 14.

Mortality and time to death: One 225 mg/kg bw/day female died before the end of the study.

Urinary examination:

Clinical Pathology: Methaemoglobin levels were significantly elevated in all treated groups compared to the controls by the end of the treatment period. For males, mean methaemoglobin levels after 14 weeks were 0.03±0.01, 0.08±0.01, 0.12±0.02, 0.25±0.07, 0.71±0.20 and 3.38±0.80 g/dL at doses of 0, 30, 55, 115, 200, and 310 mg/kg bw/day. For females, mean methaemoglobin levels after 14 weeks were 0.06±0.02, 0.14±0.02, 0.16±0.02, 0.48±0.05, 0.99±0.20 and 2.27±0.54 g/dL at doses of 0, 40, 80, 130, 225 and 345 mg/kg bw/day.

Haematology: Reticulocyte counts were increased in 200 and 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females. The erythron was decreased on day 19 but increased by week 14 in 310 mg/kg bw/day males and 345 mg/kg bw/day females.

Gross pathology incidence and severity: The incidences of squamous cell hyperplasia of the forestomach in 310 mg/kg bw/day males and 345 mg/kg bw/day females were significantly increased.

Organ weight changes: The relative kidney and spleen weights of 200 and 310 mg/kg bw/day males and 225 and 345 mg/kg bw/day females were significantly greater than those of the controls.

Histopathology: Increased erythropoietic activity in the bone marrow of exposed males and females was observed.

Sperm Motility and Vaginal Cytology: Sperm motility in 115 and 310 mg/kg bw/day males was significantly decreased.

Test condition: Study Laboratory:Microbiological Associates, Inc. (Bethesda, MD)

Strain and Species: Rat F344/N

Animal Source: Taconic Farms (Gemantown, NY)

Time Held Before Studies:14 days (males) or 15 days (females)

Average Age When Studies Began:7 weeks

Duration of Exposure:14 weeks

Average Age at Necropsy:20 weeks

Size of Study Groups: core study, 10 males and 10 females; clinical pathology study, 15 males and 15 females

Method of Distribution: Animals were distributed randomly

into groups of approximately equal initial mean body weights

Animals per Cage: 5

Method of Animal Identification: Tail tattoo

Diet:NIH-07 open formula powdered diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed weekly

Water: Charcoal-filtered deionized water via amber glass bottles with stainless steel sipper tubes, available ad libitum and changed twice weekly

Cages: Solid-bottom polycarbonate

Bedding: Sani-Chips

Cage Filters: DuPont 2024 spun-bonded polyester filter (Snow Filtration Co., Cincinnati, OH)

Racks: Stainless steel

Animal Room Environment:
Temperature: 72 degree +/-3 degree F
Relative humidity: 50% +/-15%
Room fluorescent light: 12 hours/day
Room air changes: Minimum 10/hour

Exposure Concentrations:0, 375, 750, 1,500, 3,000, or 5,000 ppm in drinking water, available ad libitum

Type and Frequency of Observation: twice daily. Core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Drinking water consumption was measured daily.

Method of Sacrifice: CO2 asphyxiation

Necropsy: Necropsy was performed on all core study. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.

Clinical Pathology:
Blood for hematology and clinical chemistry was collected

from the retroorbital sinus of anesthetized clinical pathology study rats on days 5 and 19 and from core study rats at the end of the study. Two blood samples each were collected from the abdominal aorta of 15 male and 15 female clinical pathology study rats on day 70 (2000 or 2200 hours) or 71 (0900 hours) for hemoglobin, methemoglobin and nitrosamine concentrations; stomach contents were also collected for nitrosamine concentrations. Blood and stomach contents were collected from five males and five females at each time point.

Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; erythrocyte and platelet morphologic assessments; methemoglobin concentration; reduced glutathione concentration in erythrocytes; and Heinz body count.

Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids

Nitrosamine concentrations: serum and gastric nitrosamine

Histopathology: Complete histopathology was performed on 0 and 5,000 ppm core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, muscle, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, skin, stomach (forestomach and glandular), testis (and epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. The forestomach of 750 (males), 1,500, and 3,000 ppm animals were also examined.

Sperm Motility and Vaginal Cytology: At the end of the studies, samples were collected for sperm motility or vaginal cytology evaluations from male rats in the 0, 375, 1,500, and 5,000 ppm groups and female rats in the 0, 375, 750, and 3,000 ppm groups. The left cauda, epididymis, and testis were weighed. The following parameters were evaluated: spermatid heads per gram testis, spermatid heads per testis, spermatid count, motility, and concentration. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies for vaginal cytology evaluations. The length of the estrous cycle and the length of time spent in each stage of the cycle were evaluated.Statistical analyses were conducted about survival rate, neoplasm and non-neoplastic lesion incidences.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Purity: >99%
Lot No: A42340 and H05714

Conclusion: NOAEL : not obtained (all doses showed methaemoglobin

formation)

LOAEL: Males = 115 mg/kg bw/day; Females = 225 mg/kg bw/day
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint

18-JUL-2005 (134)

Type: Sub-chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 14 weeks
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 375, 750, 1500, 3000, or 5000 ppm in drinking water available ad lib.
Control Group: yes, concurrent vehicle

Method: other: FDA (21 CFR, Part 58)
Year: 1989
GLP: yes

Result: Exposure to 0, 375, 750, 1,500, 3,000 or 5,000 ppm in drinking water was equivalent to approximate daily doses of 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1,230 mg/kg bw/day in females.

NOAEL : not obtained (methaemoglobin levels not reported)

LOAEL: Males = 750 mg/kg bw/day; Females = 445 mg/kg bw/day

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

Clinical signs: No clinical signs of toxicity

Bodyweight and food consumption: Body weights of 990 mg/kg bw/day males were significantly less than those of the controls. Water consumption by males exposed to 1,500 ppm or greater was slightly less than that by the controls at week 13.

Mortality and time to death: All mice survived until the end of the study

Urinary examination:

Clinical Pathology: Methaemoglobin levels were not reported.

Haematology:

Gross pathology incidence and severity: There were increased incidences of squamous cell hyperplasia of the forestomach in 990 mg/kg bw/day males and 1230 mg/kg bw/day females.

Organ weight changes: Relative spleen weights of 750 and 990 mg/kg bw/day males and absolute and relative heart, kidney, liver, and spleen weights of 840 and 1230 mg/kg bw/day females females were greater than those of the control groups.

Histopathology: There were increased incidences of extramedullary hematopoiesis of the spleen in 750 and 990 mg/kg bw/day males and 445 mg/kg bw/day or greater females, and degeneration of the testis in 750 and 990 mg/kg bw/day males.

Sperm Motility and Vaginal Cytology: Sperm motility was decreased in 990 mg/kg bw/day males, and the estrous cycles of 445 and 1230 mg/kg bw/day females were significantly longer than in the controls.

Test condition: Study Laboratory:Microbiological Associates, Inc. (Bethesda, MD)

Strain and Species:Mice B6C3F1

Animal Source:Taconic Farms (Gemantown, NY)

Time Held Before Studies:11 days

Average Age When Studies Began:6 weeks

Duration of Exposure:14 weeks

Average Age at Necropsy:19 weeks (males) and 20 weeks (females)

Size of Study Groups:Mice: 10 males and 10 females

Method of Distribution:Animals were distributed randomly into groups of approximately equal initial mean body weights

Animals per Cage:1

Method of Animal Identification:Tail tattoo

Diet:NIH-07 open formula powdered diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed weekly

Water:Charcoal-filtered deionized water via amber glass bottles with stainless steel sipper tubes, available ad libitum and changed twice weekly

Cages:Solid-bottom polycarbonate

Bedding:Sani-Chips

Cage Filters:DuPont 2024 spun-bonded polyester filter (Snow Filtration Co., Cincinnati, OH)

Racks:Stainless steel

Animal Room Environment:
Temperature: 72 degree +/-3 degree F
Relative humidity: 50% +/-15%
Room fluorescent light: 12 hours/day
Room air changes: equal or more than 10/hour

Exposure Concentrations:0, 375, 750, 1,500, 3,000, or 5,000 ppm in drinking water, available ad libitum

Type and Frequency of Observation:twice daily. Core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Drinking water consumption was measured daily.

Method of Sacrifice:CO2 asphyxiation

Necropsy:Necropsy was performed on all core study. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.

Clinical Pathology:Blood for hematology and clinical chemistry was collected from the retroorbital sinus of anesthetized clinical pathology study rats on days 5 and 19 and from core study rats at the end of the study. Two blood samples each were collected from the abdominal aorta of 15 male and 15 female clinical pathology study rats on day 70 (2000 or 2200 hours) or 71 (0900 hours) for hemoglobin, methemoglobin and nitrosamine concentrations; stomach contents were also collected for nitrosamine concentrations. Blood and stomach contents were collected from five males and five females at each time point.

Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; erythrocyte and platelet morphologic assessments; methemoglobin concentration; reduced glutathione concentration in erythrocytes; and Heinz body count.

Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids

Nitrosamine concentrations: serum and gastric nitrosamine

Histopathology:Complete histopathology was performed on 0 and 5,000 ppm core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, gallbladder, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (female), muscle, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, skin, stomach (forestomach and glandular), testis (and epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. The forestomach, testis, and spleen of all remaining mice were also examined.

Sperm Motility and Vaginal Cytology: At the end of the studies, samples were collected for sperm motility or vaginal cytology evaluations from male and female mice in the 0, 375, 1,500, and 5,000 ppm groups. The left cauda, epididymis, and testis were weighed. The following parameters were evaluated: spermatid heads per gram testis, spermatid heads per testis, spermatid count, motility, and

concentration. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies for vaginal cytology evaluations. The length of the estrous cycle and the length of time spent in each stage of the cycle were evaluated.Statistical analyses were conducted about survival rate, neoplasm and non-neoplastic lesion incidences.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Purity: >99%
Lot No: A42340 and H05714
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2005 (134)

Type: Chronic
Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 750, 1500, or 3000 ppm in drinking water available ad lib.
Control Group: yes, concurrent vehicle

Method: other: NTP Protocol
Year: 2001

Result: Exposure to 0, 750, 1500 or 3000 ppm sodium nitrite in drinking water was equivalent to average daily doses of approximately 0, 35, 70 or 130 mg/kg bw/day for males and 0, 40, 80 or 150 mg/kg bw/day for females.

NOAEL = 130 mg/kg bw/day (males), 150 mg/kg bw/day (females)

Clinical signs: None

Mortality: Survival of exposed groups was similar to that of the controls (29/50, 38/50, 36/50 and 36/50 for males at doses of 0, 35, 70 and 130 mg/kg bw/day, respectively and 33/50, 31/50, 36/50 and 33/50 for females at 0, 40, 80 or 150 mg/kg bw/day, respectively).

Bodyweight/Food consumption: Mean body weights of 130 mg/kg bw/day males and 150 mg/kg bw/day females were less than those of the controls throughout the study. Water consumption by high dose males and females was less than that by the controls throughout the study and that by the other exposed groups was generally less after week 14.

Clinical pathology: Methaemoglobin levels were measured at two weeks and three months. At both 2 weeks and three months, methaemoglobin levels were high at night when the rats were actively feeding and drinking and low during the day when the rats were less active. Methaemoglobin levels tended to increase with increasing dosage.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Purity: >99%
Lot No: A42340 and H05714

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-MAY-2005 (134)

Type: Chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 750, 1500, or 3000 ppm in drinking water available ad lib.
Control Group: yes, concurrent vehicle

Method: other: NTP protocol
Year: 1997

Result: Exposure to 0, 750, 1500 or 3000 ppm sodium nitrite in drinking water was equivalent to average daily doses of approximately 0, 60, 120 or 220 mg/kg bw/day for males and 0, 45, 90 or 165 mg/kg bw/day for females.

NOEL = 220 mg/kg bw/day (males), 165 mg/kg bw/day (females)

Clinical signs: none

Mortality: Survival of exposed groups was similar to that of the controls (39/50, 45/50, 42/50 and 39/50 for males at doses of 0, 60, 120 or 220 mg/kg bw/day, respectively and 40/50, 34/50, 37/50 and 41/50 for females at doses of 0, 45, 90 or 165 mg/kg bw/day, respectively).

Body weight/Food consumption: Mean body weights of 165 mg/kg bw/day females were less than those of the controls throughout the study. Exposed groups generally consumed less water than the control groups.

Clinical pathology: At 12 months, no significant increase in methaemoglobin level was observed in either sex at any dose.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Purity: >99%
Lot No: A42340 and H05714
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-MAY-2005 (134)

Type: Chronic
Species: rat Sex: male
Route of administration: drinking water
Exposure period: 24 months
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 100, 1000, 2000, 3000 mg/L
Control Group: yes, concurrent vehicle

Year: 1972

Remark: NOEL = 10 mg/kg bw/day (Focal degeneration and fibrosis of the

heart, dilatation of the bronchi with infiltration of lymphocytes and alveolar hyperinflation in lungs) equivalent to 6.7 mg NO2/kg bw/day.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily nitrite intake of 0 to 0.07 mg NO2/kg bw/day by applying a safety factor of 100 to this NOEL.

Result: Exposure to 0, 100, 1000, 2000 or 3000 mg sodium nitrite/L in drinking water was equivalent to approximately 0, 10, 100, 250 or 350 mg/kg bw/day, respectively

There were no significant differences in growth, development, mortality or total haemoglobin levels between the control and treated groups. However, the methaemoglobin levels in the groups receiving 100, 250 and 350 mg/kg bw/day sodium nitrite were raised significantly throughout the study and averaged 5, 12 and 22% of total haemoglobin, respectively.

The main histopathological changes occurred in the lungs and heart. Focal degeneration and fibrosis of the heart muscle were observed in animals receiving the highest dose of nitrite. The coronary arteries were thin and dilated in these aged animals, instead of thickened and narrow as is usually seen at that age. Changes in the lungs consisted of dilatation of the bronchi with infiltration of lymphocytes and alveolar hyperinflation. Such changes were observed in rats receiving 100, 250 and 350 mg/kg bw/day sodium nitrite.

Test condition: number of test animals per group: 8
Parameters measured:
Body weight; 1ce a month
Mortality;
Methemoglobin;
Blood chemistry; glucose, pyruvate, lactate
Pathology; heart, lungs, kidneys, liver, spleen, pancreas, adrenals and some brains.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
02-JUN-2005 (163)

Type: Sub-chronic
Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 6 weeks
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 500, 1250, 2500, 5000, 10000 ppm in drinking water
Control Group: yes, concurrent vehicle

Year: 1982

Result: Four female rats in the 10000 ppm group and one male and one female in the 5000 ppm group died. None of the other control or treated group rats died.

In all the experimental groups except the 10000 ppm group, depression of body weight gain compared to the control group was less than 10%.

At autopsy, the abnormal colour of the blood and the spleen due to methaemoglobin was marked in rats of the two highest dose groups.

On the basis of these results it was determined that the maximum tolerated dose of sodium nitrite in F-344 rats was 2500 ppm in drinkin water.

Test condition: Test Animals:
Age: 5 wk old
Source: Charles river Japan Inc. (Kanawaga)

Groups of 10 male and 10 female rats were given 20 mL of water each day containing the appropriate concentration of sodium nitrite. Controls received 20mL water only.

All animals were observed daily; signs of toxicity and mortality were recorded and body weights were determined every other week. Dead animals were completely autopsied.

At the end of the study, all surviving animals were killed for gross and microscopic examinations.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: 98.5%
Supplier: Koso Chemical Co. Ltd (Tokyo)

Reliability: (2) valid with restrictions
22-JUL-2005 (116)

Type: Chronic
Species: rat Sex: male
Strain: Sprague-Dawley
Route of administration: drinking water
Exposure period: 14 months, 16 weeks
Frequency of treatment: continuous
Post exposure period: no
Doses: 200 ppm (16 weeks) 2000 ppm (14 months)
Control Group: yes, concurrent no treatment

Year: 1980

Result: Experiment 1 (14 months):

during the 14 month treatment period, the blood of animals receiving 2000 ppm sodium nitrite in drinking water had 1-35% Methaemoglobin as compared with less than 2% in that of animals receiving water only. The methaemoglobin values of the nitrite-treated animlas fluctuated from time to time. the red cells of controls and treated animals had less than 5% haemolysis throughout the entire experimental period.

Seven of 12 nitrite-treated and four of nine control animals died of respiratory infection during the first six months of the experimental period. the difference was not statistically significant.

At the time of sacrifice, the body weights of surviving animals averaged 457 g for treated animals and 556 g for controls.

The nitrite-treated group had the lightest livers and heaviest lungs; all the lungs in thsi group exhibited severe lesions.

GSH level was significantly increased in the red cells and plasma vitamin E was significantly decreased in the nitrite-treated group compared with controls.

Experiment 2 (16 weeks):

No animals died during this study.

The methaemoglobin content in the nitrite-treated group averaged from 0.5-3.1% and the values also fluctuated from time to time. The control group had less than 1.2% methaemoglobin in the blood during the entire experimental period.

At the end of the treatment period, the lungs of nitrite-treated rats averaged 2.0 g compared with 2.5 g for the controls. The weight of other tissues (liver, spleen, heart and kidney) and the body weight were not altered by treatment.

The levels of GSH in the red cells, of vitamin E in plasma and of GSH peroxidase in the red cells and in plasma were not significantly changed as a result of nitrite treatment.

Test condition: Experiment 1 (14 months):

2 month old male rats maintained on a commercial diet (Purina rat chow) were given water containing either none or 2000 ppm sodium nitrite in drinking water.

Tail blood samples were collected every other week to measure the level of methaemoglobin. Haemoglobin content was determined using cyano-MHb reagent at 540 nm. The degree of red cell haemolysis was also measured.

At the end of 14 months, surviving animals from each group were killed and examined for tissue abnormalities. Hemolysate from heparinized blood samples were analysed for levels of GSH, GSH peroxidase and haemoglobin. Vitamin E content and GSH peroxidase activity were also measured in plasma.

Experiment 2 (16 weeks):

Groups of 1-month old male rats (8/group) were given drinking water that contained either none or 200 ppm sodium nitrite for 16 weeks.

During the treatment period blood samples were taken periodically to measure the level of methaemoglobin as in experiment 1.

At the end of 16 weeks animals were killed, blood samples collected and the animals examined for any tissue abnormalities.

Blood samples were assayed for levels of GSH and GSH peroxidase in red cells and of GSH peroxidase and Vitamin E in plasma.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions

02-JUN-2005 (41)

Type: Sub-chronic
Species: rat Sex: male
Strain: Wistar
Route of administration: drinking water
Exposure period: 90 days
Frequency of treatment: daily ad libitum
Post exposure period: up to 8 weeks
Control Group: yes

Year: 1996
GLP: yes
Test substance: other TS

Result: LOEL = 12 mmol KNO2, equivalent to 54 mg NO2-/kg bw/day.

All rats survived the experimental period. Rats receiveing nitrite appeared cyanotic during the first month of the treatment period.

In both feed groups the body weights of the rats exposed to the low and medium dose of nitrite did not differ statistically significantly from the concurrent control rats. In feed group A the body weight gain the the high dose group was statistically significantly retarded compared to the control rats. During the recovery period the body weights of these rats remained lower than those in the concurrent control group. The retardation of body weight gain in the dose group fed with diet B was less prominent than of the rats on feed A and recovery of weight gain was observed after withdrawal of nitrite.

In both feed groups the liquid intake of the rats exposed to the low and medium dose of nitrite did not differ statistically from the concurrent control rats. The average daily drinking water consumption per kg body weight of the high dose rats was, during the exposure period, always statistically significantly decreased compared to the control rats. The reduction was generally more pronounced in the rats fed with diet A.

After 4 and 8 weeks of exposure to the high nitrite dose the haemoglobin concentration in the blood of rats on feed A was statistically significantly lower than that of concurrent controls. The haemoglobin concentration was lowest after 4 weeks, rose gradually afterwards and from week 13 no difference between treatment groups could be seen. This decrease of haemoglobin concentration was not seen in rats fed on diet B.

The concentration (and fraction) of methaemoglobin in blood of the control rats fed on diet A were statistically significantly slightly higher than those of the control rats on feed B. Exposure to the low or medium nitrite dose for 13 weeks caused no effects on theses parameters. In the high dose groups, the methaemoglobin concentration (and fraction) in blood of rats of both feed groups was statistically significantly increased ($p<0.01$, rank sum test) compared to the control groups.

Pathology:

Gross examination revealed no remarkable observation in most of the rats.

Absolute and relative weights of the adrenal glands of the nitrite dosed rats did not differ significantly from controls.

All high nitrite dosed rats of both feed groups showed slight hypertrophy of the adrenal zona glomerulosa. This hypertrophy manifested from the first examination (after 4 weeks of exposure) and on all subsequent examinations during the exposure period (after 8 and 13 weeks). After 90 days of exposure, the incidence and intensity of the hypertrophy of the adrenal zona glomerulosa was dose dependent. The low dose group showed minimal hypertrophy in 3/10 rats on feed A and 0/10 on feed B. In the medium dose groups very slight hypertrophy was observed in the adrenals of 8/10 feed A and 9/10 feed B rats.

Test condition:

Animal Source: National Institute of Public Health and Environmental Protection

Time Held Before Studies: 2 weeks

Average Age When Studies Began: no data

Size of Study Groups: Controls - 100 rats; low and medium dose - 20 rats each; high dose - 100 rats

Method of Distribution: The animals were weighed and allocated to 4 test groups (including the control group) matched by weight by a computer randomisation program.

Animals per Cage: 2/cage

Method of Animal Identification: no data

Diet: Feed A - semisynthetic diet prepared with purified components (SSP-TOX, Hope Farms BV, Woerden, NL); Feed B - Diet used in Til study (van Eck, Cothen, NL obtained from TNO, Zeist)

Water: ad libitum

Cages: Macrolon cages (Type III)

Bedding: no data

Cage Filters: no data

Racks: no data

Animal Room Environment:
Temperature: 19-24°C
Relative humidity: 37-83%
Room fluorescent light: 12 hr light/dark cycle
Room air changes: about 10/hr

Exposure Concentrations (administered in drinking water):
Controls - Potassium chloride (36 mmol/L = 2584 mg/L)
Low dose - Potassium nitrite (3.6 mmol/L = 306 mg/L)
supplemented with potassium chloride (32.4 mmol/L)
Medium dose - Potassium nitrite (12 mmol/L = 1021 mg/L)
supplemented with potassium chloride (24 mmol/L)
High dose - Potassium nitrite (36 mmol/L = 3064 mg/L)

During the recovery period the drinking water contained potassium chloride. The daily supply of potassium was the same in all groups throughout the experimental period.

Type and Frequency of Observation:

Cage-site observations to check the general condition and behaviour of all rats or to detect signs of ill health and reaction to treatment were conducted once daily.

The individual body weights of all rats were recorded at the start of the experiments and then weekly. In addition, the rats were weighed on the day of their scheduled sacrifice in order to calculate the relative adrenal weight. Water intake was recorded daily and food intake was measured over weekly periods throughout the study. Liquid and food intake per kg body weight were calculated over weekly periods using the average weight of the 2 rats in each cage.

Autopsy:

Starting four weeks before termination the rats were handled individually twice weekly. Sedated rats were sacrificed by decapitation. Following exsanguination macroscopic examination was performed. Adrenals were dissected, weighed and fixed in 4% w/v formaldehyde in 0.067 mol/L phosphate buffer.

Haematology and clinical chemistry:

Blood samples were collected at autopsy in plastic vials containing EDTA. Samples were examined for haemoglobin and methaemoglobin concentrations. Concentrations of nitrate and nitrite in plasma were also measured.

Histopathology:
The fixed adrenal glands were embedded in paraplast and slices stained with haematoxylin-eosin for microscopical examination.

Morphometry:

Morphometry was performed on the stained slices o fthe adrenals to quantitate the size of the zona glomerulosa.

Statistical methods:

data were transofrmed to their natural logarithm to achieve homogeneity of variances and evaluated by analysis of variance using the STATA statistical analysis program. The Bonferroni multicomparison test was used to calculate the significance of the differences. In case the variances were not homogenous after log transformation and for the statistical evaluation of the histopathology score of hypertrophy of the zona

glomerulosa of the adrenals, the non-paramtetric Wilcoxon-Mann-Whitney rank sum test was used to calculate the significance of the differences. p values < 0.05 were considered statistically significant.
Test substance: Chemical name: Potassium nitrite (CAS No. 7758-09-0)
Reliability: (2) valid with restrictions
02-JUN-2005 (19)

Type: Sub-chronic
Species: rat Sex: male/female
Strain: Wistar
Route of administration: drinking water
Exposure period: 90 days
Frequency of treatment: daily
Doses: 0, 100, 300, 1000, 3000 mg/L
Control Group: yes, concurrent no treatment

Year: 1988
GLP: no data
Test substance: other TS

Result: LOEL = 100 mg KNO2/L, equivalent to 5.4 mg NO2/kg bw/day

There were no deaths and the rats appeared to be healthy throughout the study. Ophthalmoscopic examination did not reveal any differences between the test rats and controls that could be ascribed to treatment.

Body weight, food intake and food efficiency were decreased at 3000 mg/L in males. Liquid intake was decreased at 3000 mg/L in males and females and 1000 mg/L in males.

Methaemoglobin was increased at 3000 mg/L. Haemoglobin associated parameters were depressed slightly at 1000 and 3000 mg/L, the changes being slight or very slight. Hypertrophy of the adrenal zona glomerulosa was observed in all test groups in a dose-dependent manner. The incidence was not significally different from controls at 100 mg/L.

Test condition:

Strain and Species: Wistar derived SPF-bred rats

Animal Source: F. Winkelmann (Institute for the Breeding of Laboratory Animals GmbH & Co. KG, Borchen, Germany)

Average Age When Studies Began:6 weeks

Acclimatisation: 13 days

Duration of Exposure: 90 days

Size of Study Groups: 10 rats/sex/dose

Method of Distribution: Animals were distributed randomly into groups

Animals per Cage: 5

Method of Animal Identification: no data

Diet: Grain-based open formula diet (nitrite and nitrate content 2 and 140 mg/kg, respectively) ad lib

Water: Tap water (nitrite and nitrate content <0.01 and 10 mg/L, respectively)

Cages: Stainless steel cages fitted with wire mesh front and floor.

Animal Room Environment:
Temperature: 22+/-2°C
Relative humidity: 40-70%
Photoperiod: 12 hours light/dark cycle
Room air changes: Approximately 10/hour

Type and Frequency of Observation: Rats were weighed weekly and observed daily for condition and behaviour. Ophthalmic onservations were made in all rats of the control and top dose groups prior to the administration of the test substance and during week 13. Food and liquid intake were measured over weekly periods throughout the study.

Method of Sacrifice: Exsanguination from the abdominal aorta whilst under light ether anaesthesia.

Necropsy: Rats were killed in wk 14 and a thorough autopsy was performed. Immediately after evisceration, the adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid and thymus were weighed and the organ to body weight rations calculated.

Clinical Pathology:
Hematology and clinical chemistry: Blood samples were collected from the tip of the tail of all animals in wk 13 and were examined for methaemoglobin concentration, haemoglobin concentration, packed cell volume and erythrocyte, leucocyte and thrombocyte counts and prothrombin times. Whole blood taken from each of the animals after an overnight fast was examined for glucose. At autopsy, heparinised blood samples collected fron the abdominal aorta of all rats were analysed for alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, total bilirubin, urea, creatinine, glutathioneperoxidase, gamma-glutamyltransferase and calcium, inorganic phosphate, chloride, sodium, potassium, nitrite, nitrate and vitamin E.

In week 4,8 and 13 nitrite was determined semiquantitatively in the saliva from all rats of the 1000 and 3000 mg/L groups and from the group receiving KCl instead of KNO2 in the drinking water.

Clinical chemistry:

Histopathology: samples of the organs weighed and of the pituitary, lungs, aorta, parotoid salivary glands, submandibular salivary glands, sublingual salivary glands, oesophagus, forestomach, glandular stomach, small intestine, pancreas, caecum, colon, rectum, urinary bladder,

epididymides, prostate, uterus, mesanteric lymph nodes, axillary lymph nodes and sciatic nerve were fixed in 10% neutral buffered formalin embedded in paraffin wax, stained with haematoxylin and eosin and examined microscopically.

Sperm Motility and Vaginal Cytology:
Test substance: Test Substance: Potassium nitrite (CAS No. 7758-09-0)
Purity: >97%
Supplier: E. Merck, AG Darmstadt, Germany
Reliability: (2) valid with restrictions
02-JUN-2005 (178)

Type: Chronic
Species: rat Sex: male
Strain: Wistar
Route of administration: drinking water
Exposure period: 9 months
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 0.2 % in drinking water
Control Group: yes, concurrent vehicle

Result: The lipoperoxidation in the liver microsome was increased. The free acid phosphatase was increased the total activity, however degraded, which was similarly with that of lysosomal enzyme Cathepsin. The superoxide dismutase activity was elevated in post-mitochondrial expulsion, while it reduced in mitochondria.

The hepatic microsomal lipoperoxidation activity, as measured by malondialdehyde formation, was increased in male Wistar rats given 0.2% sodium nitrite in drinking water. Liver lysosomal enzyme (acid phosphatase and cathepsin) and superoxide dismutase activities were increased compared to the controls. The data suggest that sodium nitrite stimulates generation of superoxide radicals in the liver and causes damage to the cellular and subcellular membranes.
Test condition: In this study the effect of sodium nitrite was investigated on microsomal lipoperoxidation in the liver, for which examines lysosomal enzyme activities in the liver and the cytosomal superoxide dismutase activity in the liver after the treatment in vitro. Six animals per group were used.
Test substance: Chemical name: Sodium ntirite (CAS No. 7632-00-0)
Reliability: (4) not assignable
02-JUN-2005 (45)

Type: Chronic
Species: rat Sex: female
Strain: Long-Evans
Route of administration: oral feed
Exposure period: 21 days
Doses: Dietary conc.= 0, 10, 25 g/kg

Year: 1974

Remark: Sodium nitrite administered in feed at 10 or 25 g/kg to unilaterally ovariectomized Long-Evans rats for 21 days inhibited body weight gain and caused a decrease in the compensatory ovarian hypertrophy that follows

hemicastration. Decreased uterine, liver, and kidney weights and increased spleen weights were also observed.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
02-JUN-2005 (131)
Type: Sub-chronic
Species: rat Sex:
Strain: Wistar
Route of administration: drinking water
Exposure period: 3 months
Frequency of treatment: daily
Doses: 0.3%

Remark: The feeding sodium nitrite to male Wistar rats at 0.3% for 3 months did not affect relative liver weights or the expression of hepatic c-Jun, c-Fos, or c-Myc oncogenes.
Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
02-JUN-2005 (79)

5.5 Genetic Toxicity 'in Vitro'

Year: 2001

Remark:

Sodium nitrite is a direct-acting, base-pair substitution mutagen in organisms ranging from bacteria to mammals. It also has been shown to induce chromosomal effects in mammalian cells in vitro and in vivo.

In an acidic environment, sodium nitrite reacts with amines to form nitrosamines or with amides to form nitrosamides. These compounds are mutagenic in a variety of systems. Nitrosamines require metabolic activation for expression of mutagenic activity, but nitrosamides do not.

Positive results have been reported for sodium nitrite, with and without S9 metabolic activation enzymes, in Salmonella gene mutation studies with strains that revert by base-pair substitution. Genotoxicity of sodium nitrite is not often detected in strains of S. typhimurium that mutate via frameshift mechanisms. Gene reversion and DNA damage were also observed in Escherichia coli WP tester strains after exposure to sodium nitrite in the presence of S9.

Furthermore, sodium nitrite-induced gene mutations were reported in Saccharomyces cerevisiae, Candida utilis , and C. albicans.

In cultured mammalian cells, sodium nitrite was reported to induce gene mutations, chromosomal aberrations and sister chromatid exchanges (SCEs); in none of these experiments was S9 required for the positive response. HeLa cells incubated for 1 to 36 hours had increased levels of unscheduled DNA synthesis (DNA repair) at concentrations above 1 mM sodium nitrite. In vivo, increased frequencies of micronuclei and

8-azaguanine- and ouabain-resistant mutations, but not

chromosomal aberrations, were seen in cells of Syrian golden hamster embryos 24 hours after oral administration of 125, 250, or 500 mg/kg sodium nitrite to the dams. No increases in chromosomal aberrations were noted in lymphocytes of Wistar rats 48 hours after a single gavage treatment of 300 mg/kg sodium nitrite. However, positive results were reported for induction of chromosomal aberrations in bone marrow cells of pregnant female albino rats exposed to 210 mg/kg per day in drinking water for 13 days. In this same study, the liver cells of embryos exposed trans- placentally for the first 13 days of gestation also showed increased numbers of chromosomal aberrations. SCE induction increased with increasing dose in bone marrow cells of Swiss albino mice treated with 2.5 to 200 mg/kg sodium nitrite by intraperitoneal injection. In addition to the demonstrated genotoxicity of sodium nitrite, concerns about the effects of the compound arise from its ability to transform primary and secondary amines into N-nitroso compounds, which are generally mutagenic after S9 activation. Acid conditions facilitate the reaction of nitrite with amines, and this reaction is further catalyzed by alcohols and aldehydes, ultimately producing the actual nitrosating agent, nitrous anhydride.

There are numerous reports of mutagenic activity detected after combined administration of sodium nitrite with amino compounds. For example, sodium nitrite combined with the dye metanil yellow induced dose-related increases in SCEs in the bone marrow of Swiss mice; the increase was significantly greater than the increase in SCEs induced by sodium nitrite alone. Amino acid derivatives in food stuffs (Amadori compounds, <1 micro-M), when reacted with sodium nitrite (>1 mM) under acidic conditions at normal body temperatures (37 degree C), produced mutagenic nitrosated products and induced high levels of unscheduled DNA synthesis in HeLa cells when present in culture medium for 1 to 36 hours.

Transplacental exposure of cells of Syrian golden hamster embryos to a combination of aminopyrine plus sodium nitrite, administered to dams via gavage on days 11 or 12 of gestation, induced a significant increase in 8-azaguanine-resistant mutations compared to either compound administered individually.

In a similar study, transplacental exposure to sodium nitrite plus morpholine, administered simultaneously by gavage to pregnant Syrian golden hamsters on days 11 or 12 of gestation, induced significant increases in 8-azaguanine- and ouabain-resistant mutations, as well as micronuclei, in embryonic fibroblasts.
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Result: Sodium nitrite (100-10,000 ug/plate) was mutagenic in Salmonella typhimurium strain TA100, with and without Aroclor 1254-induced hamster and rat liver S9 enzymes; no

mutagenicity was observed in strain TA98. When sodium nitrite was administered by intraperitoneal injection at 6.25 to 200 mg/kg to male rats three times at 24-hour intervals, no significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in any of the dose groups. The initial trial was judged to be positive, based on the trend test (P=0.001); however, results of a repeat trial, in which 50 mg/kg was the highest nonlethal dose tested, were negative, and the rat bone marrow micronucleus test with sodium nitrite was judged to be negative overall. A similar study in which male mice were administered 7.81 to 250 mg/kg also gave negative results. A third in vivo study, a peripheral blood micronucleus test in male and female mice administered sodium nitrite (375 to 5,000 ppm) for 14 weeks, showed no significant increase in the frequency of micronucleated normochromatic erythrocytes in either males or females. Thus, sodium nitrite demonstrated mutagenic activity in a strain of S. typhimurium that mutates via base-pair substitution, but no indication of chromosomal damage was observed in three micronucleus studies conducted in rats and mice in vivo.

Test condition: The genetic toxicity of sodium nitrite was assessed by testing the ability of the chemical to induce mutations in various strains of Salmonella typhimurium and micronucleated erythrocytes in rat and mouse bone marrow and mouse peripheral blood.

The genetic toxicity studies of sodium nitrite are part of a larger effort by the NTP to develop a comprehensive database that would permit a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). These short-term genetic toxicity tests were originally developed to clarify mechanisms of chemical-induced DNA damage growing out of the earlier electrophilicity/mutagenicity relationship proposed and the somatic mutation theory of cancer. Therefore, the information obtained from these tests applies only to mutagenic carcinogens.

For mutagenic carcinogens, the combination of DNA reactivity and Salmonella mutagenicity is highly correlated with the induction of carcinogenicity in multiple species and genders of rodents and at multiple tissue sites. Data from NTP studies show that a positive response in Salmonella is the most predictive in vitro test for rodent carcinogenicity (89% of the Salmonella mutagens are rodent carcinogens) and that there is no complementarity among the in vitro genetic toxicity tests. That is, no battery of tests that included the Salmonella test improved the predictivity of the Salmonella test alone. Although other in vitro genetic toxicity tests correlate less well with rodent carcinogenicity compared with the Salmonella test, these other tests can provide useful information on the types of DNA and chromosomal effects induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response

in the acute in vivo bone marrow chromosome aberration test or micronucleus test appears to be less than that in the Salmonella test However, clearly positive results in long-term peripheral blood micronucleus tests are associated with high predictivity for rodent carcinogenicity; negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the Salmonella assay and rodent bone marrow cytogenetics tests.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: Sodium nitrite was mutagenic in Salmonella typhimurium strain TA100, with and without Aroclor 1254-induced hamster and rat liver S9 enzymes; no mutagenicity was observed in strain TA98. Results of acute bone marrow micronucleus tests with sodium nitrite in male rats and mice by intraperitoneal injection were negative. In addition, a peripheral blood micro nucleus assay conducted with mice from the 14-week study gave negative results.

Reliability: (2) valid with restrictions

01-JUL-2005 (134)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA1535, TA100, TA98, TA1537
Concentration: 25-2500 µg/plate
Metabolic activation: with and without
Result: positive

Method: other: Ames et al (1975)
Year: 1980

Result: The testing of 25, 250 and 2500 ug/plate with strain TA1535 resulted in definite mutagenic effects at 250 and 2500 ug/plate. A subsequent experiment indicated that a concentration as low 80 ug/plate significantly increased numbers of revertant colonies as compared to the appropriate controls.

A definite mutagenic effect in observed with TA100 but not strong as with TA1535. At lower limit of 250 ug/plate with this strain for minimal detection in indicated. The statistically significant elevation at 25 ug plus 3.125 mg S9 is not substantiated since negative results are observed at 80, 140 and 200 ug/plate. The completely negative effect on strains TA98 and TA1537 is observed.

Test condition: DOSES IN ABSENCE AND PRESENCE OF ACTIVATION:
TA1535: Expt 1 - 0, 25, 250, 2500 µg/plate; Expt 2 - 0, 80, 140, 200 µg/plate

TA100: Expt 1 - 0, 25, 250, 2500 µg/plate; Expt 2 - 0, 80, 140, 200 µg/plate

TA1537 and TA98: 0, 25, 250, 2500 µg/plate

METABOLIC ACTIVATION: S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone

POSITIVE CONTROLS (+/-S9):

Cyclophosphamide (200 µg/plate): TA1535, TA100
Benzo(a)pyrene (10 µg/plate): TA98, TA1537

PLATES/TEST: 3
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Pfalz and Bauer, Inc.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
03-JUN-2005 (101)

Type: Chromosomal aberration test
System of testing: Syrian Hamster Embryo Cell
Concentration: 0, 5, 10, 20, 30, 50 mmol/L
Metabolic activation: without
Result: positive

Year: 1977

Method: Cells were ontained from a near-term whole embryo of a Syrian hamster. In all the experiments the medium used was Eagle's minimal essential medium supplemented with 10% fetal calf serum, Neomycin (50 µg/mL) and Fungizone (1.25 µg/mL). Primary cultures of hamster cells were trypsinized and seeded in TD-40 flasks at a concentration of 1E+06 cells per flask. 24 hours after seeding, the cells were exposed for 24 hours to a medium containing the test substance at the rquired concentrations. the cells were then washed twice with Hank's balanced solution and maintained in a anormal complete medium for 24h. For the last 3h, colcemid (0.3 µg/mL) was added. The chromosomes were prepared by the method of Rothfels and Siminovitch.

Reference:

Rothfels KH and Siminovitch L (1958) an air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technology, 33, 73-77

Result: NaNO2 induced endoreduplications and chromosomal aberrations as well as malignant transformation, in hamster cells in vitro.

Concn NaNO2 (mM)	Abnormal* Metaphase (%)	Aberrations per 100 cells Gaps **	Chromatid breaks	Chromosome breaks	Exchange **	Minutes **
0	3	3	0	0	0	0
5	4	4	0	0	0	0
10	7	6	0	0	1	0
20	14	15	1	0	1	2
30	20	18	7	3	2	6
50	48	43	39	4	32	32
NaCl***	4	3	0	0	0	1

*For control and treated cells, 100 metaphases were counted **

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                  results represent the summation of chromatid type and
                  chromosome type
                  *** Cells were treated with 50 mM NaCl as an osmotic pressure
                  control
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Supplier: Kanto Chemicals, Japan
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
03-JUN-2005                                                              (183)

Type:                    Bacterial reverse mutation assay
System of testing:       Salmonella typhimurium TA92, TA1535, TA100, TA1537,
                         TA94, TA98
Concentration:           up to 10 mg/plate
Metabolic activation:    with and without
Result:                  positive

  Year:           1984

Result:           Positive +/- metabolic activation in TA1535 and TA100

                  2800/plate at 10.0 mg/plate in TA1535 with S9 mix and
                  2069/plate without S9 mix
                  465/plate in TA100 withS9 mix and 314/plate without S9 mix
Test condition:   Solvent; phosphate buffer
Test substance:   Chemcial name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: 97.0%
Reliability:      (2)  valid with restrictions
03-JUN-2005                                                               (93)

Type:                    DNA damage and repair assay
System of testing:       DNA repair test in Escherichia coli WP2, WP67, CM871
Metabolic activation:    with and without
Result:                  positive

  Year:           1984

Method:           Liquid microethod procedure
                  Spot test
Result:           -Liquid micromethod
                  --MIC (ug) without S9 mix
                  WP2; 2500
                  WP67; 2500
                  CM871; 625

                  --MIC (ug) with S9 mix
                  WP2; 3000
                  WP67; 3000
                  CM871; 1250

                  --Potency (Delta MICs/nmol)
                  0.0003

                  -Spot test
                  Positive
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                  Purity: reagent grade
                  Supplier: BDH
Reliability:      (2)  valid with restrictions
06-JUN-2005                                                               (47)
```

Type: Bacterial forward mutation assay
System of testing: Saccharomyces cerevisiae (diploid strain MP1)
Concentration: 0.058-0.43 mM
Result: positive

Year: 1979

Method: Media: Synthetic complete medium. The liquid complete medium YEP is composed of 2% Bacto peptone (Difco No. 0118-01), 1% yeast extract (Difco No. 0127-01) and 2% glucose.

Culturing of yeast cells: About 1000 yeast cells are inoculated in a 300 mL Erlenmeyer flask containing 100 mL YEP, set on a shaker and allowed to grow for 3 days at 25°C into the stationary phase. For the detection of the spontaneous frequency of interallelic recombination, the cultures of strains are stored at 4°C for 3-4 days. During this time their spontaneous frequency is determined by spreading 0.1 mL from the YEP/yeast suspension on solid media selective for interallelic recombinants. Only cultures with a low spontaneous frequency are used for the experiments. The cultures needed for one experiment are mixed together in order to obtain a similar spontaneous frequency of genetic alterations in both experiment and control.

In vitro test: The cultured cells are washed twice with distilled water and the cell titers are adjusted to 5E+08 cells per mL of 0.1 M phosphate buffer of pH 4.5. These cells suspensions are incubated in a test tube on a shaker at 25°C with different concentrations of the test substance. After 210 minutes treatments are stopped and 0.1 mL aliquots of the suspensions (containing 5E+07 cells) are spread on four plates of solid media, selective for interallelic recombinants and mutants, respectively. Similarly, 0.1 mL aliquots of 1E-05 dilutions in distilled water (containing 5E+02 cells) are plated out on ten plates of complete medium to attain the number of survivors (white colonies) and intergenic recombinants (red colonies or red sectors). These cultures are incubated at 25°C and the survivor and recombinant colonies are counted after 4 days. Actidione-resistant colonies are incubated 8 days. The spontaneous frequency of colonies per plate is about 10 to 20 for the mutation system, 20 to 30 for the interallelic recombination system and 1-3 for the intergenic recombination system.
Result: Sodium nitrite shows weak toxic effects and accordingly the dose/effect curves are parallel for all three genetic alterations (slope of about 1) only at the lower dose ranges.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: 99%
Supplier: Schuchardt Munchen
Reliability: (2) valid with restrictions
07-JUN-2005 (58)

Type: Bacterial forward mutation assay
System of testing: Escherichia coli WP2uvrA/pKM101
Concentration: up to 10 mg/plate
Metabolic activation: with
Result: positive

Year: 1982

Result: Dose response effect was seen in the SMr-5 mutagenicity of NaNO2.
Test condition: Mutagenicities of NaNO2 to streptomycin resistance at a concentration of 5 uh/mL (SMr-5) were examined in E. coli WP2 uvrA with plasmid pKM101 derived from S. typhimurium.

Solvent; water
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
06-JUN-2005 (108)

Type: Bacterial reverse mutation assay
System of testing: TA1530, TA1535, TA100, TA102, YG1024, DJ400, DJ460
Concentration: 1, 2.5, 5 mg/plate
Metabolic activation: with and without
Result: positive

Method: Directive 2000/32/EC, B.10
Year: 1994

Result: Nitrite is a direct mutagen in certain S. typhimurium his- strains sensitive to base-pair substitutions (TA1530, TA1535 and TA100), whereas it is inactive in strain TA102 and in frameshift-sensitive strains containing increased acetyltransferase activities (YG1024, DJ400 and DJ460).
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
03-JUN-2005 (13)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA97, TA98, TA100
Concentration: 100 - 1000 µg/plate
Metabolic activation: with
Result: positive

Method: other: Maron and Ames (1984)
Year: 1987

Result: TA97: negative
TA98: negative
TA100: positive (2.1= induced mutagenicity/solvent control)
Test condition: DOSES IN ABSENCE AND PRESENCE OF ACTIVATION:
TA1535: Expt 1 - 0, 25, 250, 2500 µg/plate; Expt 2 - 0, 80, 140, 200 µg/plate

TA100: Expt 1 - 0, 25, 250, 2500 µg/plate; Expt 2 - 0, 80,

140, 200 µg/plate

TA1537 and TA98: 0, 25, 250, 2500 µg/plate

METABOLIC ACTIVATION: S9 from rat liver, induced with Aroclor 1254

POSITIVE CONTROLS:

-S9: 2-NF (TA97, TA98), NaN3 (TA100)
+S9: 2-AA (TA97, TA98, TA100)

PLATES/TEST: 3
REPLICATES: 2
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Merck
Reliability: (2) valid with restrictions
03-JUN-2005 (22)

Type: Unscheduled DNA synthesis
System of testing: HeLa S3 Carcinoma cells
Concentration: 0.0000001-0.006M
Result: positive

Year: 1983

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade
Supplier: JT Baker Chemical Co. (Phillipsburg, NJ)
Reliability: (2) valid with restrictions
15-JUL-2005 (115)

Type: Bacterial reverse mutation assay
System of testing: Salmonell typhimurium TA98, TA100
Concentration: 0, 100, 333, 1000, 1666, 3333, 6666, 10000 ug/plate
Metabolic activation: with and without
Result: positive

Year: 1992

Result: TA 100: Positive +/- S9

TA 98: Negative +/- S9

TA 100

DOSE	NA		30% HLI		30%RLI	
µg/Plate	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	171	5.5	158	12.0	173	6.2
100.000			187	5.6	199	14.7
333.333			182	13.5	233	6.4
1000.000	211	19.9	220	4.7	230	11.8
1666.666	224	10.6				
3333.000	274	21.4	309	11.7	312	10.4
6666.000	376	7.5				
10000.000	443	12.0	451	20.1	353	5.4
POS	878	22.7	894	24.1	555	18.3

TA 98

DOSE	NA		30% HLI		30%RLI	
µg/Plate	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	31	0.09	42	1.7	40	4.8
100.000	27	0.6	40	4.6	33	3.6
333.333	26	4.2	39	3.1	41	3.8
1000.000	30	3.5	47	4.2	29	1.0
1666.666						
3333.000	25	2.6	42	2.1	33	2.8
6666.000						
10000.000	26	2.8	30	5.2	21	5.0

```
                    POS      432 19.0     539     6.1     155    14.3
Test condition:   METABOLIC ACTIVATION: S9 from rat liver, induced with
                  Aroclor 1254; S9 from hamster liver, induced with
                  Aroclor 1254
Test substance:   Chemical name: Sodium ntirite (CAS No. 7632-00-0)
                  Supplier: Pflaltz and Bauer, Inc
Reliability:      (2)  valid with restrictions
03-JUN-2005                                                              (206)

Type:                    Bacterial reverse mutation assay
System of testing:       Salmonella typhimurium TA1530
Concentration:           0, 33.2, 66.5, 133 mmol/L
Cytotoxic Concentration: See RESULT
Metabolic activation:    without
Result:                  positive

  Year:           1980

Result:           -Cytotoxicity
                  Dose (mmol/L)/Survival (%)
                  0/100
                  33.2/100
                  66.5/100
                  133/79.5

                  -Mutation frequency (X10E8)
                  0/2
                  33.2/6
                  66.5/8
                  133/33
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability:      (2)  valid with restrictions
03-JUN-2005                                                               (52)

Type:                    Chromosomal aberration test
System of testing:       CHL (Chinese Hamster Fibroblast cell line)
Concentration:           up to 1.0 mg/mL
Metabolic activation:    without
Result:                  positive

  Year:           1984

Result:           Polyploid (%): 0.0%

                  Structural aberration; 71.0 (%)

                  D20=0.41; TR=52
                  Positive at 24 hr (22%). Positive at 0.25 mg/mL at 24 hr
                  (11.0%). Also positive at 0.5 mg/mL at 24 hr (16.0%) and at
                  48 hr (27.0%)
Test condition:   treatment hour; 24, 48 hours

                  Solvent; saline
Test substance:   Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability:      (2)  valid with restrictions
03-JUN-2005                                                        (91) (93)

Type:                    Chromosomal aberration test
System of testing:       FM3A-Cell (Mammocarcinom-Cell line from C3H Mouse)
```

Concentration: 10E-3 to 10E-1 mol/L
Metabolic activation: without
Result: positive

Year: 1976

Result: The chromosomal preparations demonstrated that severe aberrations were induced in more than 80% of the mitotic plates at 10(-2) M and in nearly 40% at 10(-25) M after 24 and 48 h treatment. According to the results of alkaline sucrose gradient analysis sedimentation profiles of cell DNA treated at as high as 10(-1) M for 24 h scarcely changed from that of control cell DNA. Induction of 8-azaguanine-resistant mutation was demonstrated above 10(-3) M sodium nitrite.
Test condition: Effect of sodium nitrite on cultured FM3A cells, a C3H mouse mammary carcinoma cell line, was examined.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
03-JUN-2005 (106)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Metabolic activation: with and without
Result: positive

Method: other: Maron & Ames (1983)
Year: 1981

Result: -Reverted strains;
TA1535; positive
TA1537; negative
TA1538; negative
TA98; negative
TA100; positive

-Range of activity (nmols/plate)
4-14.5X10E4

-Muatgenic potency (revertants/nmol)
0.005

-Effect of S9 mix (rat liver Aroclor)
slight decrease
Test condition:
METABOLIC ACTIVATION: S9 from rat liver, induced with Aroclor

PLATES/TEST: 3
REPLICATES: 3
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: reagent grade
Supplier: BDH
Reliability: (4) not assignable
Insufficient experimental detail
06-JUN-2005 (46)

Type: other: Chromosomal aberration test, Sister-chromatid exchanges

System of testing: Chinese Hamster V79-H3-Cell
Concentration: 0, 10, 50, 100 mmol/L
Metabolic activation: without
Result: positive

Year: 1981

Result: -Chromosomal aberrations
Concentration (mmol/L)/Abnormal metaphases (%)
0/5
10/6
50/10
100/20

-Sister-chromotid exchanges

Concentration (mmol/L)/SCEs metaphases
0/5.6
10/5.3
50/8.0
100/8.8
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
03-JUN-2005 (184)

Type: other: Chromosomal aberration test, Sister-chromatid exchanges
System of testing: Chainese hamster cell line D-6
Concentration: 1, 3 mmol/L
Result: positive

Year: 1977

Result: -Frequencies of Chromosomal aberration
Dose / Breaks cell
(mL/mL)
1X10E-3 / 0.00
3X10E-3 / >0.24 (the presence of cells with 10 or more breaks, more than 2-fold of control values)

-Frequencies of SCEs
Dose / SCEs cell
(mL/mL)
1X10E-3 / 5.53
3X10E-3 / 11.96 (more than 2-fold of control values)
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
03-JUN-2005 (1)

Type: Sister chromatid exchange assay
System of testing: Human peripheral blood lymphocytes
Concentration: 0, 0.0003, 0.003, 0.01, 0.03 mol/L
Result: positive

Year: 1985

Result: Concentration (mol/L)/No. of SCEs per cell
0/4.40
3X10E-4/4.24
3X10E-3/4.68

```
                1X10E-2/8.94*
                3X10E-2/No mitosis
                *; significantly different from control, p<0.001
Test condition: Treatment time; 72 hours
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                Supplier: Merck
Reliability:    (2)  valid with restrictions
15-JUL-2005                                                          (87)

Type:                   other: Bacterial reverse mutation assay, Chromosomal
                        aberration test
System of testing:      Salmonella typhimurium TA98, TA100, TA1537, Chinese
                        hamster cell (CHL)
Result:                 positive

  Year:         1981

Result:         -Ames test; Positive

                -Chromosomal aberration test; positive
                D20 = 0.4 (mg/mL)
                TR-value = 52 (mg/mL)
Test condition: -Ames test; without S9

                -Chromosomal aberration test; with and without S9
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability:    (4)  not assignable
15-JUL-2005                                                          (92)

Type:                   other
System of testing:      SOS Chromotest Kit;  E. coli PQ37
Concentration:          6.9 ng/mL to 6.9 mg/mL
Metabolic activation:   without
Result:                 negative


Test condition: Solvent; water
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
                Supplier: Merck
Reliability:    (4)  not assignable
                Non-standard test method
06-JUN-2005                                                          (22)
```

5.6 Genetic Toxicity 'in Vivo'

```
Type:           Micronucleus assay
Species:        rat                             Sex: male
Strain:         Fischer 344
Route of admin.: i.p.
Exposure period: 3 days
Doses:          6.25, 12.5, 25, 50, 100 or 200 mg/kg
Result:         negative

Method:         other: NTP method
  Year:         2001
   GLP:         yes

Method:         Male F344/N rats were injected i.p. (three times at 24-hour
                intervals) with sodium nitrite dissolved in phosphate-buffered
```

saline. Solvent control animals were injected with phosphate-buffered saline only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained. 2000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in up to 5 animals per dose group.

The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochrane-Armitage trend test followed by pairwise comparisons between each dosed group and the control group.

Result: 200 mg/kg was found to be the lethal dose. No significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in any of the dose groups. The initial trial was judged to be positive, based on the trend test (P=0.001); however, results of a repeat trial, in which doses of 0, 25 or 50 mg/kg bw were tested, were negative, and the rat bone marrow micronucleus test with sodium nitrite was judged to be negative overall.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions

15-JUL-2005 (134)

Type: Micronucleus assay

Species: mouse Sex: male

Strain: B6C3F1

Route of admin.: i.p.

Exposure period: 3 days

Doses: 7.81, 15.63, 31.25, 62.5, 125, or 250 mg/kg

Result: negative

Method: other: NTP method

Year: 2001

GLP: yes

Method: Male B6C3F1 mice were injected i.p. (three times at 24-hour intervals) with sodium nitrite dissolved in phosphate-buffered saline. Solvent control animals were injected with phosphate-buffered saline only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained. 2000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in up to 5 animals per dose group.

The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochrane-Armitage trend test followed by pairwise comparisons between each dosed group and the control group.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions

15-JUL-2005 (134)

Type: Micronucleus assay

Species: mouse Sex: male/female

Strain: B6C3F1
Route of admin.: drinking water
Exposure period: 14 weeks
Doses: 0, 375, 750, 1500, 3000, or 5000 ppm in drinking water available ad lib.
Result: negative

Method: other: NTP method
Year: 2001
GLP: yes

Method: At the end of the 14 week toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group.
Result: Exposure to 0, 375, 750, 1,500, 3,000 or 5,000 ppm in drinking water was equivalent to approximate daily doses of 0, 90, 190, 345, 750, or 990 mg/kg bw/day in males and 0, 120, 240, 445, 840, or 1,230 mg/kg bw/day in females.

There was no significant increase in the frequency of micronucleated NCEs in either males or females.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
15-JUL-2005 (134)

Type: other: micronucleus formation, chromosomal aberrations, morphological or malignant transformation and drug-resistant mutations in embyonic cells
Species: hamster Sex: female
Route of admin.: gavage
Exposure period: once
Doses: 125, 250, 500 mg/kg
Result: positive

Year: 1979

Result: Marked dose-dependant increases in micronucleus formation, induction of 8-azaguanine- and ouabain-resistant mutations and morphological or neoplastic transformations in the embryo cells were observed. However, there was no marked increase in the frequency of chromosomal aberrations.
Test condition: Animal; Syrian golden hamster, 150 +/- 30g on the 11th or 12th day of pregnancy
Source: Matsumoto Experimental Animal Lab., Chiba, Japan.
Solvent; saline solution
Sample; fetuses excised after dosing of $NaNO_2$, for culture

Transplacental application of $NaNO_2$:

The hamsters were given 0.5-1 mL of physiological saline solution containing 0, 125, 250 or 500 mg $NaNO_2$/kg bw by gavage.The dose of 500 mg/kg bw $NaNO_2$ proved to be above the LD50 dose within 24 h for pregnant hamsters. The hamsters were fed standard laboratory chow (Clea Japan Ltd., Tokyo) and water ad libitum just before and for 24 h after administration of $NaNO_2$. Their fetuses were then excised. The babies from

mothers treated with 500 mg/kg bw NaNO2 and with DMN served as negative and positive controls, respectively.

Primary cultures:

The fetuses were chopped up finely with scissors and digested with 0.25% trypsin at room temperature for 45 min. Primary cultures of trypsinised cells were initiated by seeding 5-10E+05 cells into 10 mL of medium in 75 cm2 plastic flasks. Most cells were grown in Eagle's minimal essential medium supplemented with 10% fetal calf serum but some cells were cultured in Dulbecco's modified Eagle's medium plus 20% FCS at 37°C under 5% CO2 in air.

Examination of chromosomes:

For examination of chromosomes within the first 24 h of primary culture, cells grown in MEM were treated with colcemid at 0.3 µg/mL for 3 h. Mitotic cells in the first cell cycle were then examined. Chromosome preparations were made by the usual air-drying method with a slight modification and stained with Giemsa. The numbers and types of chromosomal abnormalities were assessed by examining 200 well-spead metaphase plates. For examination of micronuclei, samples were made by a modification of the method of Schmid: after culture for 30 h, the cells were collected with 0.1% trypsin, smeared on slide glasses, fixed with methanol and stained with Geimsa. In each experiment, over 5000 resting nuclei were examined for micronuclei.

Induction of morphological transformation:
For determination of transformation, the cells were cultured in DMEM plus 20% FCS for 3-5 days from primary culture and then seeded in inocula of 5000/60 mm dish for studies on colony formation and transformation. The dishes were incubated for 8 days without change of medium then fixed and stained with Giesma solution. The number of colonies was determined. Some transformed colonies were cloned by the cylinder-cup method and cultured in DMEM medium for 7-9 weeks. Then 5-10E+06 cells from the transformed colonies were implanted into the cheek pouches of young golden Syrian hamsters weighing 80-110 g to test for malignant transformation.

Test substance: Name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
01-JUL-2005 (90)

Type: other: chromosomal aberration in bone marrow of rats
Species: rat Sex: male/female
Strain: other: albino
Route of admin.: drinking water
Exposure period: day 0 to 18
Doses: mean 210 mg/kg/day
Result: positive

Year: 1984

Result: Chromosomal aberrations comprising chromatid gaps, breaks (mainly chromatid, including fragments and deletions), centric

fusions, and dicentrics were induced in the bone marrow of adults and the liver of transplacentally exposed embryos. All aberrant cells had one aberration, with the exception of a few cells where two or more aberrations were found.

Pregnant and nonpregnant females treated with nitrite showed a significant increase ($p<0.01$) in chromosomal aberrations of bone marrow cells over the control. However there was no difference between the two groups and their results were pooled.

In the liver of transplacentally exposed embryos, there was also a significant increase ($p<0.001$) in the number of cells with chromosomal aberrations.

The number of cells with hypoploidy was not stastically significant in maternal bone marrow and embryo liver of treated and control groups. Cells with hyperploidy were rare in both bone marrow and embryonic liver.

Adult bone marrow-control/3.31%
Adult bone marrow-treated/9.60%
ratio treated/control=2.90

Embryonic liver-control/1.75%
Embryonic liver-treated/8.62%
ratio treated/control=4.93

Test condition: Animal;15-20 week old

Virgin female rats were mated with males. The day on which sperm were found in the vaginal smears was designated as day 0 of gestation.

Sodium nitrite (1.25 g/L) was given in the drinking water (deionized). The water was changed daily and the volume consumed per rat was recorded. All adults were treated for a period of 13 days, from day 5-day 18 of gestation in the case of pregnant animals. Untreated rats served as controls.

At the termination of treatment the rats were sacrificed and 3-4 living embryos per litter were removed as quickly as possible. The liver of each embryo was transferred to 5 mL MEM supplemented with 10% fetal bovine serum and forced through a stainless steel mesh yielding a cell suspension. The bone marrow cells from the femur of the adult were also collected in 5 mL culture medium. The cell suspensions, to which 0.5 mg colchine was added, were then incubated for 2 h at 37°C in an atmosphere of 5% CO_2 in air. Maternal and embryo chromosomes were prepared in accordance with standard cytogenetic procedures. Slides were prepared and stained with giemsa. At least 50 metaphase spreads wee examined for each adult rat and embryo for chromosomal aberrations.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Matheson, Coleman, Coleman and Bell)

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
01-JUL-2005 (53)

Type: Sister chromatid exchange assay
Species: mouse Sex: male

Strain: Swiss
Route of admin.: i.p.
Exposure period: once
Doses: 2.5, 5, 10, 20, 40, 100, 200 mg/kg
Result: positive

Year: 1986

Result: In vivo sister chromatid exchanges induced by metanil yellow (dye containing secondary amino group), sodium nitrite, and dye combination with nitrite following treatment with acute doses were on mice. The incidence of SCEs was significantly high in both dye- and nitrite-treated series. However, a combination of half the concentrations of dye and nitrite, when used together, gave a frequency of SCE higher than that of either chemical, when given in full dose, indicating the stronger clastogenicity of the nitrosamine formed.
Test condition: Animal: laboratory-bred Swiss albino male mice (Mus musculus), 30g, about 90-100 days old
7 animals/group

Solvent; water
Reliability: (2) valid with restrictions
15-JUL-2005 (65)

Type: Heritable translocation assay
Species: mouse Sex: male
Strain: C3H
Route of admin.: gavage
Exposure period: 14 days
Doses: 60, 120 mg/kg/day

Method: other
Year: 1988
GLP: no data

Result: A lack of heritable translocations, sperm abnormalities, as well as morphological changes, such as changes in eyes, coat colour, testes and body weight, was demonstrated in F1 males originating from treated P males. Significant effects in treated males were found with respect to:
(1) sex-chromosomal univalency in the diakinesis-methaphase I stage after the treatment of stem spermatogonia (the higher dose of sodium nitrite),
(2) sperm-head abnormalities after treatment of differentiating spermatogonia (both doses of sodium nitrite)
(3) fertility after treatment of spermatids (the higher dose of sodium nitrite).
Test condition: In all experiments mature F1 male and female mice (10-12 weeks old) of the hybrid strain C3HX101 were used.

Animals were housed in wire-topped polycarbonate cages under standard laboratory conditions (T=21°C, 60% RH, 12-h day-night rhythm, water and food ad libitum).

Experimental doses were 60 and 120 mg/kg/day for 14 days.

Statistical processing of results was performed using Student's t-test.

Heritable translocation test:

Mice (25 males/group) were dosed by gavage with sodium nitrite solution (0, 60 or 120 mg/kg bw) daily for 14 days. Ten days after the last treatment each male was mated with two virgin females. The interval of mating was seven days after which males were taken out and left for later examination. Numbers of both fertile and sterile females, litter size per fertile female, sex ratio and survival of offspring were noted. Three weeks after birth F1 males were taken out and left until maturation (10-12 weeks). Cytogenetic analyses were performed on spermatocytes (diakinesis-metaphase I) in all F1 males from treated males of the P generation (up to three males from each litter) and in 25 F1 males from controls.

Tests were performed for cytogentic analysis of P males and sperm abnormality tests on P males and F1 males. for all males in the experiment both testes and body weight were determined and changes in eyes and coat colour were noted.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Yugoslav Institute for Meat Technology (Belgrade).
Reliability: (2) valid with restrictions
01-JUL-2005 (5)

Type: other: 8-azaguanine-resistant mutation and neoplastic transformation
Species: hamster Sex:
Route of admin.: gavage
Exposure period: once
Doses: 0, 25, 50, 100 mg/kg
Result: positive

Result: a) 8-azaguanine-resistant mutation
Dose (mg/kg);Induced ratio (induced freq./sponteous freq.)
25; 1.5
50; 1.3
100; 3.7

b)Neoplastic transformation
Dose (mg/kg);Transformation rate (%)
Control; 0.22 +/- 0.23
25; 0.60 +/- 0.41
50; 0.79 +/- 0.26 ($p<0.05$)
100; 0.82 +/- 0.25 ($p<0.05$)
Test condition: On the 11th and 12th days of pregnancy Syrian golden hamsters weighing 150 +/- 40g, from a closed colony.

Solvent; water

Examined tissue; embryonic cell
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
supplier: Wako Chemical Indust., Japan
Reliability: (2) valid with restrictions
01-JUL-2005 (88) (89)

Type: Micronucleus assay
Species: mouse Sex: male
Strain: other: NIH

Route of admin.: gavage
Doses: 0, 10, 15 or 20 mg/kg bw
Result: positive

Year: 2002

Result: The treated animals showed a statistically significant increase (p=0.05) in micronucleated polychromatic erythrocytes at all doses compared with the 0h values (MNPCE/1000 PCE = 1.2+/-0.58 (0h), 4.8+/-0.37 (96h) at 10 mg/kg; 0.4+/-0.24 (0h), 5.0+/-0.31 (96h) at 15 mg/kg; and 1.0+/-0.31 (0h), 5.8+/-0.85 (96h) at 20 mg/kg). No statistical differences were observed in the PE/NE ratio when their results were compared before and after treatment, suggesting that sodium nitrite produced no significant influence on normal bone marrow activity in this study.
Test condition: 0, 10, 15 or 20 mg/kg of sodium nitrite administered orally four times at 24-hour intervals and peripheral blood samples examined after 96 hours.
Test substance: Chemical name: Sodium nitrite (CAS No 7632-00-0)
Supplier: Sigma Chemicals (St Louis MO, USA)
Reliability: (2) valid with restrictions
15-JUL-2005 (48)

Type: other: chromosomal aberration
Species: rat Sex: female
Strain: Wistar
Route of admin.: oral unspecified
Exposure period: once
Doses: 300 mg/kg
Result: negative

Year: 1979

Result: Total aberration (%) = 3
Significant difference was not observed.
Test condition: Test flow

1)administration to rat
2)rat lymphocytes
3)culture
4)harvest
5)score
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
01-JUL-2005 (114)

5.7 Carcinogenicity

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 750, 1,500, or 3,000 ppm in drinking water, available ad libitum
Result: negative
Control Group: yes, concurrent vehicle

Method: other: FDA (21 CFR, Part 58)
Year: 1997
GLP: yes

Result: Survival
Survival of exposed groups was similar to that of the control groups.
Body Weights, Water and Compound Consumption, and Clinical Findings
Mean body weights of males and females exposed to 3,000 ppm were less than those of the control groups throughout the study. Water consumption by males and females exposed to 3,000 ppm was less than that by the controls throughout the study, and that by the other exposed groups was generally less after week 14. Drinking water concentrations of 750, 1,500, or 3,000 ppm sodium nitrite resulted in average daily doses of approximately 35, 70, or 130 mg/kg body weight to males and 40, 80, or 150 mg/kg to females. There were no clinical findings related to exposure to sodium nitrite; the brown discoloration and cyanosis seen in the 14-week studies were not observed.

Determination of Plasma Nitrite and Blood Methemoglobin Concentrations
At 2 weeks and 3 months, no nitrite was detected in the plasma of control male or female rats. Plasma nitrite concentrations tended to increase with increasing exposure concentrations of sodium nitrite. Generally, plasma nitrite concentrations were high at night when the rats were actively feeding and drinking, and were low during the day when the rats were less active. Blood methemoglobin concentrations followed the same pattern.

In 18-month-old male and female rats administered a single dose of 40 mg/kg sodium nitrite by gavage,
plasma nitrite and blood methemoglobin concentrations peaked at 30 minutes.

Pathology and Statistical Analyses
This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the forestomach, mammary gland, liver, kidney, and skin and in the incidences of mononuclear cell leukemia. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group.

Forestomach: The incidences of hyperplasia of the squamous epithelium were significantly increased in males and females exposed to 3,000 ppm. Hyperplasia was generally a minimal change affecting the epithelium of the limiting ridge at the junction of the forestomach and glandular stomach. In a few cases, severe hyperplasia was seen both at and away from the limiting ridge. Hyperplasia was characterized primarily by variable degrees of folding of the squamous epithelium and was usually accompanied by a variable degree of thickening of the overlying keratin layer (hyperkeratosis). No

forestomach neoplasms were observed following exposure to sodium nitrite.

Mammary Gland: The incidence of fibroadenoma was significantly increased in females exposed to 1,500 ppm sodium nitrite and exceeded the historical range for NTP controls given NTP-2000 diet (all routes) or NIH-07 diet (drinking water route) (24%-58%). The incidences in the 750 and 3,000 ppm groups also exceeded the range for the NTP controls given NTP-2000 diet; the incidence in the control group was greater than in these exposed groups and equaled the highest incidence in the NTP-2000 historical control database. Also, when combined with adenomas, there were no significant increases in the incidences of these neoplasms. The incidences of carcinoma were not increased in the exposed groups. The incidences of multiple fibroadenoma were greater in females exposed to 750 ppm and 1,500 ppm than in controls. Fibroadenomas are the most common benign neoplasms that occur in the mammary gland of female F344/N rats. However, unlike benign neoplasms in other tissues that usually progress to malignancy, fibroadenomas are generally considered to represent an end-stage lesion, and progression to carcinoma is rare. Microscopically, fibroadenomas in exposed rats were similar to those in the controls and were characterized by collections of glandular epithelium arranged in acini and ducts and surrounded by fibrous connective tissue. The relative amounts of glandular and fibrous elements varied among neoplasms. Epithelial cells were well differentiated and arranged in a single layer of cuboidal epithelium, which was often vacuolated.

Liver: There were significant increases in the incidences of chronic active inflammation in 1,500 and 3,000 ppm males (0 ppm, 13/50; 750 ppm, 19/50; 1,500 ppm, 25/50, 3,000 ppm, 24/50). This was generally a minor lesion, characterized by a few to several scattered aggregates of mixed mononuclear inflammatory cells, mainly lymphocytes and macrophages. Chronic active inflammation of the liver is a common spontaneous lesion in F344/N rats and may be obscured in rats with mononuclear cell leukemia. Because of the lower incidences of mononuclear cell leukemia, this common background lesion was more easily observed microscopically. The marginally increased incidences of chronic active inflammation in 1,500 and 3,000 ppm males were not considered to be related to sodium nitrite exposure.

Kidney: The incidence of nephropathy was marginally increased in females exposed to 3,000 ppm (14/50, 16/50, 20/50, 23/50). It is unclear whether the slightly increased incidences of nephropathy were directly related to sodium nitrite exposure. Nephropathy is a common spontaneous renal lesion in F344/N rats.

Skin: The incidence of fibroma of the subcutis was significantly increased in males exposed to 1,500 ppm (0/50, 1/50, 6/50, 3/50). The incidence in this group slightly exceeded the historical range for NTP controls (all routes) given NTP-2000 diet. The lack of a dose response for fibroma or a significant increase in the incidences of fibrosarcomas (1/50, 0/50, 0/50, 2/50) and the fact that the combined

incidences of fibroma or fibrosarcoma (1/50, 1/50, 6/50, 5/50) are within the historical range for NTP controls given NTP-2000 diet suggest that these neoplasms were not related to sodium nitrite exposure. Fibromas and fibrosarcomas are the most common neoplasms that occur in the skin of F344/N rats.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia were significantly decreased in males and females exposed to 1,500 or 3,000 ppm and were less than the historical ranges for NTP controls (all routes) given NTP-2000 diet. These findings indicate that sodium nitrite reduced the incidence of mononuclear cell leukemia in F344/N rats, thereby resulting in increased survival.

-Male:
Concentrations in drinking water: 0, 750, 1,500, or 3,000 ppm
Body weights: 3,000 ppm group less than the control group
Survival rates: 29/50, 38/50, 36/50, 36/50
Nonneoplastic effects: Forestomach: epithelial hyperplasia (12/50, 9/50, 10/50, 44/50)
Neoplastic effects: None
Uncertain findings: None
Decreased incidences: Mononuclear cell leukemia: (17/50, 12/50, 7/50, 3/50)
Level of evidence of carcinogenic activity: No evidence

-Female
Concentrations in drinking water: 0, 750, 1,500, or 3,000 ppm
Body weights: 3,000 ppm group less than the control group
Survival rates: 33/50, 31/50, 36/50, 33/50
Nonneoplastic effects: Forestomach: epithelial hyperplasia (8/50, 6/50, 8/50, 40/50)
Neoplastic effects: None
Uncertain findings: None
Decreased incidences: Mononuclear cell leukemia: (15/50, 10/50, 1/50, 1/50)
Level of evidence of carcinogenic activity: No evidence

Test condition: Study Laboratory:
Battelle Columbus Laboratories(Columbus, OH)

Strain and Species:
Rat F344/N

Animal Source:
Taconic Laboratory Animals and Services (Germantown, NY)

Time Held Before Studies:
11 days (males) or 12 days (females)

Average Age When Studies Began:
6 weeks

Duration of Exposure:
105 weeks

Average Age at Necropsy:
110 weeks

Size of Study Groups:
Core study: 50 males and 50 females; special study, 10 males and 10 females; aged sentinel animal study, 15 males and 15 females

Method of Distribution: Animals were distributed randomly into groups of approximately equal initial mean body weights

Animals per Cage:
core study, 2 or 3 (males) or 5 (females); special study, 2 or 3 (males) or 5 (females); aged sentinel animal study, 3

Method of Animal Identification:
Tail tattoo

Diet:
NTP-2000 pelleted diet, irradiated beginning 22 July 1996, changed weekly

Water:
Tap water (Columbus, OH, municipal supply) via amber glass bottles with stainless steel sipper tubes, available ad libitum and changed twice weekly.

Cages:
Solid-bottom polycarbonate

Bedding:
Sani-Chips

Cage Filters:
DuPont 2024 spun-bonded polyester filter (Snow Filtration Co., Cincinnati, OH)

Racks:
Stainless steel

Animal Room Environment:
Temperature: 72 degree +/-3 degree F
Relative humidity: 50% +/- 15%
Room fluorescent light: 12 hours/day
Room air changes: equal or more than 10/hour

Exposure Concentrations:
0, 750, 1,500, or 3,000 ppm in drinking water, available ad libitum

Type and Frequency of Observation:
twice daily. Core study animals were weighed initially and clinical findings and body weights were recorded on day 8, day 36, at 4-week intervals thereafter, and at necropsy. Special study rats were weighed at 2 weeks and 3 months, special study mice were weighed at 12 months, and aged sentinel animal rats and mice were weighed at 18 months. Drinking water consumption by the core study animals was measured over a 1-week period at 4-week intervals, beginning during the first week of the study.

Method of Sacrifice:

CO2 asphyxiation

Necropsy:
Necropsy was performed on all core study animals and five male and five female aged sentinel animals.

Histopathology:
Complete histopathology was performed on all core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, skin, stomach (forestomach and glandular), testis (and epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

Plasma Nitrite and Blood Methemoglobin Concentrations:
Blood was collected from the retroorbital sinus of 10 male and 10 female special study rats at 2 weeks and 3. Blood was collected from two animals per group per time point (0600, 1200, 2100, 2400, and 0300 hours).
Blood was collected from the retroorbital sinus of 15 male and 15 female aged sentinel animals after a single gavage dose of 40 mg/kg at 18 months. Two or three animals were sampled at each time point (2, 5, 10, 30, or 60 minutes after dosing). Plasma nitrite and blood methemoglobin concentrations were determined.

Statistical analyses were conducted about survival rate, neoplasm and non-neoplastic lesion incidences.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: >99%
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Lot No. A42340 and H05714

Conclusion: Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in F344/N rats at approximate doses of up to 130 mg/kg bw/day in males and 150 mg/kg bw/day in females over a two year period.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

18-JUL-2005 (134)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuous
Post exposure period: none
Doses: 0, 750, 1,500, or 3,000 ppm in drinking water, available ad libitum
Result: ambiguous
Control Group: yes, concurrent vehicle

Method: other: FDA (21 CFR, Part 58)
Year: 1997
GLP: yes

Test substance: other TS

Result: Survival
Survival of exposed groups was similar to that of the controls.
Body Weights, Water and Compound Consumption, and Clinical Findings
Mean body weights of exposed groups were generally similar to those of the controls throughout the study, except mean body weights of 3,000 ppm females were consistently less than those of the controls. Water consumption by the exposed groups was generally less than that by the control groups. Drinking water concentrations of 750, 1,500, or 3,000 ppm resulted in average daily doses of approximately 60, 120, or 220 mg/kg for males and 45, 90, or 165 mg/kg for females. There were no clinical findings related to exposure to sodium nitrite.

Determinations of Plasma Nitrite and Blood Methemoglobin Concentrations
At 12 months, no nitrite was detected in the plasma of control or 750 ppm male mice or in any group of female mice. In general, there was an exposure concentration-related increase in plasma nitrite in the 1,500 and 3,000 ppm male mice; peak plasma nitrite concentrations occurred around midnight. Blood methemoglobin concentrations were similar among exposed groups of males and females.

Pathology and Statistical Analyses
This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the forestomach, glandular stomach, lung, and skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group.

Forestomach: The incidences of squamous cell papilloma or carcinoma (combined) in female mice occurred with a positive trend, and the incidence in 3,000 ppm females exceeded the historical range for NTP controls given NTP-2000 diet (all routes) or NIH-07 diet (drinking water route) (range 0%-4%). Because both of these historical databases are rather small, the historical incidences of forestomach squamous cell papilloma or carcinoma (combined) in NTP controls given NIH-07 diet in studies with other routes of chemical administration were evaluated, such as feed controls and inhalation controls.The incidence of squamous cell papilloma or carcinoma (combined) in the 3,000 ppm females exceeded these historical incidences with the exception of corn oil gavage studies, where as many as five papillomas, but no carcinomas, were observed in one control group. Hyperplasia of the squamous epithelium was observed more frequently in 3,000 ppm females than in the controls. Forestomach neoplasms were not observed in male mice exposed to sodium nitrite. Proliferative lesions involving the squamous epithelium represent a continuum, progressing from focal hyperplasia to papilloma to squamous cell carcinoma. Hyperplasia was generally a mild change affecting the

squamous epithelium of the limiting ridge at the junction of the forestomach and glandular stomach. Papillomas consisted of a solitary stalk of lamina propria protruding into the lumen with multiple finger-like projections arising from the stalk. The epithelium covering the projections was hyperplastic. In carcinomas, there was a focal invasion of the squamous epithelium into the lamina propria; however, there was no infiltration of neoplastic cells through the serosa of the forestomach, and there was no metastasis.

Glandular Stomach: The incidence of epithelial hyperplasia was significantly greater in 3,000 ppm males than in the controls. Hyperplasia of the glandular stomach epithelium was focally extensive and was characterized by a distorted and irregular arrangement of the glandular elements of the gastric mucosa. In the hyperplastic areas, the proportion of the gastric glands with mucus-secreting cells was reduced to 25% compared to 50% in the normal area. In addition, the parietal cells in the mid portion of the gastric glands were less distinct in the hyperplastic areas. Instead of large, cuboidal cells with eosinophilic cytoplasm (characteristic of normal parietal cells), the parietal cell area tended to be composed of smaller, elongated, basophilic cells with vesiculated, fusiform nuclei and relatively scant cytoplasm consistent with chief cells. There were no neoplasms of the glandular stomach.

Lung: The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in exposed groups of females were slightly greater than that in the control group. (1/50, 6/50, 5/50, 6/50). However, the incidences of these lesions were within the historical range for controls (all routes) given NTP-2000 diet [17/250 (6.8% +/- 5.6%), range 0%-12%]. The increased incidences were not statistically significant or exposure concentration related and were not accompanied by increased incidences of preneoplastic lesions; therefore, the lung neoplasms in exposed females were not considered to be related bitrite exposure.

Skin: The incidence of fibrosarcoma in 750 ppm females was significantly greater than that in the controls (0 ppm, 0/50; 750 ppm, 5/50; 1,500 ppm, 1/50; 3,000 ppm, 2/50). The incidence exceeded the historical range for NTP controls (all routes) given NTP-2000 diet [3/250 (1.2% +/- 1.8%), range 0%-4%]. The lack of a dose response for fibrosarcomas and the fact that the combined incidence of fibroma or fibrosarcoma (0/50, 5/50, 1/50, 3/50) fell within the historical range for NTP controls given NTP-2000 diet suggest that these neoplasms were not related to sodium nitrite exposure. The most frequent spontaneous mesenchymal neoplasms of the subcutaneous skin in female mice in the order of occurrence were fibrosarcomas, sarcomas, and lipomas.

-Male:
Concentrations in drinking water: 0, 750, 1,500, or 3,000 ppm
Body weights: Exposed groups similar to the control group
Survival rates: 39/50, 45/50, 42/50, 39/50
Nonneoplastic effects: Glandular stomach: epithelial

hyperplasia (0/50, 0/50, 2/50, 10/50)
Neoplastic effects: None
Uncertain findings: None
Decreased incidences: None
Level of evidence of carcinogenic activity: No evidence

-Female
Concentrations in drinking water: 0, 750, 1,500, or 3,000 ppm
Body weights: 3,000 ppm group less than the control group
Survival rates: 40/50, 34/50, 37/50, 41/50
Nonneoplastic effects: None
Neoplastic effects: None
Uncertain findings: Forestomach: squamous cell papilloma or carcinoma (1/50, 0/50, 1/50, 5/50)
Decreased incidences: None
Level of evidence of carcinogenic activity: Equivocal evidence

Test condition: Study Laboratory: Battelle Columbus Laboratories(Columbus, OH)

Strain and Species:Mice B6C3F1

Animal Source:Taconic Laboratory Animals and Services (Germantown, NY)

Time Held Before Studies:13 days (males) or 14 days (females)

Average Age When Studies Began:6 weeks

Duration of Exposure:104 to 105 weeks

Average Age at Necropsy:110 weeks

Size of Study Groups:Core study: 50 males and 50 females; special study, 10 males and 10 females; aged sentinel animal study, 15 males and 15 females

Method of Distribution: Animals were distributed randomly into groups of approximately equal initial mean body weights

Animals per Cage:core and special studies, 1 (male) or 5 (females); aged sentinel animal study, 1 (male) or 3 (females)

Method of Animal Identification:Tail tattoo

Diet:NTP-2000 pelleted diet, irradiated beginning 16 July 1996, changed weekly (males), or twice weekly (females)

Water:Tap water (Columbus, OH, municipal supply) via amber glass bottles with stainless steel sipper tubes, available ad libitum and changed twice weekly (female) or weekly (male)

Cages:Solid-bottom polycarbonate

Bedding:Sani-Chips

Cage Filters:DuPont 2024 spun-bonded polyester filter (Snow Filtration Co., Cincinnati, OH)

Racks:Stainless steel

Animal Room Environment:
Temperature: 72 degree +/- 3 degree F
Relative humidity: 50% +/- 15%
Room fluorescent light: 12 hours/day
Room air changes: equal or more than 10/hour

Exposure Concentrations:0, 750, 1,500, or 3,000 ppm in drinking water, available ad libitum

Type and Frequency of Observation:twice daily. Core study animals were weighed initially and clinical findings and body weights were recorded on day 8, day 36, at 4-week intervals thereafter, and at necropsy. Special study rats were weighed at 2 weeks and 3 months, special study mice were weighed at 12 months, and aged sentinel animal rats and mice were weighed at 18 months. Drinking water consumption by the core study animals was measured over a 1-week period at 4-week intervals, beginning during the first week of the study.

Method of Sacrifice:CO2 asphyxiation

Necropsy: Necropsy was performed on all core study animals and five male and five female aged sentinel animals.

Histopathology:Complete histopathology was performed on all core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, gallbladder, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (female), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, skin, stomach (forestomach and glandular), testis (and epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

Plasma Nitrite and Blood Methemoglobin Concentrations:Blood was collected from the retroorbital sinus of 10 male and 10 female mice at 12 months. Blood was collected from two animals per group per time point (0600, 1200, 2100, 2400, and 0300 hours).
Blood was collected from the retroorbital sinus of 15 male and 15 female aged sentinel animals after a single gavage dose of 62.5 mg/kg at 18 months. Two or three animals were sampled at each time point (2, 5, 10, 30, or 60 minutes after dosing). Plasma nitrite and blood methemoglobin concentrations were determined. Statistical analyses were conducted about survival rate, neoplasm and non-neoplastic lesion incidences.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: >99%
Supplier: J.T. Baker, Inc. (Phillipsburg, NJ)
Lot No. A42340 and H05714

Conclusion: Under the conditions of this study there is no evidence of carcinogenic activity of sodium nitrite in male B6C3F1 mice at doses up to approximately 220 mg/kg bw/day over a two year period. There is equivocal evidence of carcinogenic activity in female mice, based on the positive trend of squamous cell papilloma or carcinoma (combined) in the forestomach.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
18-JUL-2005 (134)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 0.125, 0.25 % in drinking water
Result: negative
Control Group: yes, concurrent vehicle

Year: 1982

Result: Dose (%)
-0.125%; equivalent to 19 mg/rat for male, 15 mg/rat for female
-0.25%; equivalent to 34 mg/rat for male, 25 mg/rat for female

There were no significant differences in the incidence of tumours between control and test groups, apart from a lowere incidence of mononuclear cell leukaemia amongst the test groups compared with controls. This was attributed to slight atrophy of the haematopoietic organs.
Test condition: Animals; 50 animals/sex/group
Age: 8 weeks at start of study.

The carcinogenicity of sodium nitrite was examined in F-344 rats. Sodium nitrite was administered in the drinking-water for 2 yr at levels of 0.125 or 0.25%.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: 98.5%
Supplier: Koso Chemical Co Ltd
Reliability: (2) valid with restrictions
18-JUL-2005 (116)

Species: rat Sex: male
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 weeks
Frequency of treatment: ad libitum
Post exposure period: 24 weeks
Doses: 0.3% in drinking water
Result: negative
Control Group: yes

Year: 1993

Result: NaNO2 itself had no carcinogenic potential.

In a multi-organ model in which rats were initiated with various carcinogens, 0.3% sodium nitrite in drinking water strongly enhanced the development of forestomach lesions but inhibited the development of glandular stomach lesions when these animals were given catechol or 3-methoxycatechol, with or without prior carcinogen exposure.
Test condition: In a multi-organ model in which rats were initiated with various carcinogens (4 weeks)
15 animal/group
Starting 3 days after the completion of these carcinogen treatments, animals were given diet containing test chemicals, or basal diet either alone or in combination with 0.3% $NaNO_2$ for about 24 weeks, when complete autopsy was performed.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Reliability: (4) not assignable
15-JUL-2005 (77)

Species: other: rat and hamster Sex: male/female
Strain: other: Sprague-Dawley and Syrian golden
Route of administration: oral feed
Exposure period: rat; F1+F2 generations (F1 + F2 at 125 weeks), hamster; 110 weeks
Frequency of treatment: continuous
Post exposure period: non
Doses: 1000 ppm
Result: positive
Control Group: yes

Year: 1976
GLP: no

Result: Most combinations of the two chemicals induced a high incidence of hepatocellular carcinoma in rats and lower incidence in hamster. The highest level of nitrite and morphpline (1000 ppm of each) had a stronger potential for carcinogenesis in both rats and hamsters than did a dietary level of 50 ppm of preformed N-nitrosomorpholine. Nitrite and morpholine also induced angiosarcoma in both species, most frequently in the liver, with the lung as the next most common site. The nitrite concentration in the diet seemed to have a greater effect on the incidence of hepatocellular carcinoma and angiosarcoma in the rat than did the concentration of morpholine. Dietary concentrations of 5 ppm each of nitrite and morpholine induced hepatocellular carcinoma and angiosacroma in some rats.

High concentrations of sodium nitrite alone associated with a relatively high incidence (27%) of lymphoreticular tumor, control (6%).
Test condition: Concentration;
various dietary concentrations of nitrite and morphine (up to 1000 ppm of each) or N-nitro-somorpholine (5 or 50 ppm).
Test substance: Chemical name: sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
15-JUL-2005 (159)

Species: rat Sex: male
Strain: Fischer 344

Route of administration: drinking water
Exposure period: Expeiment-1; 4 weeks, Experiment-2; 52 weeks
Post exposure period: no
Doses: 0.3% in drinking water
Control Group: yes

Year: 1994
GLP: no data

Result: Experiment-1;
It was noteworthy that the heights of the mucosa in animals treated with NaNO2 alone were more than in those receiving basal diet alone, and these were further increased by the additional with sodium ascorbate without phenolic anti-oxidants.

Experiment-2;
In MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) treated animals, incidences of forestomach papilomas and carcinomas were significantly enhanced in the NaNO2 alone group (84 and 47%, respectively) as compared with the basal diet group (30 and 10%), with further significant increase in carcinomas occurring with additional sodium ascorbate (79%, $p<0.05$) and ascorbic acid (85%, $p<0.05$) treatment. In animals without MNNG, all animals in the NaNO2 group demonstrated mild hyperplasia, additional administration of sodium ascorbate or ascorbic acid remarkably enhancing the grade of hyperplasia, and resulting in 53% and 20% incidences, respectively, of papillomas. Thus NaNO2 was demonstrated to exert promoter action for forestomach carcinogenesis, with sodium ascorbate and ascorbic acid acting as co-promoters. The results strongly indicate that combined treatment with sodium ascorbate or ascorbic acid and NaNO2 may induce forestomach carcinomas in the long term.
Test condition: Experiment-1;
120 rats were divided into 24 groups, each one of five phenolic compounds in the diet, basal as a control, or additional treatment 1% sodium ascorbate in diet and/or 0.3% NaNO2 in drinking water for 4 weeks.

Experiment-1;
120 rats in groups were given 150 mg/kg bw of MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) in vehicle (DMS:water = 1:1) by stomach tube after 16h starvation. Starting 1 week later, animals were divided into 6 groups. And another 6 groups of 15 animals each were treated as pretreated groups. Sodium ascorbate and ascorbic acid were mixed in powdered basal diet at a dose of 1%, and NaNO2 was dissolved in tap water at dose of 0.3% for the end of test period (52 week).
Test substance: Chemcial name: Sodium nitrite (CAS No. 7632-00-0)
Purity: food grade
Reliability: (4) not assignable

15-JUL-2005 (205)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: other: Exp-1; oral feed, Exp-2; drinking water
Exposure period: 106 weeks
Frequency of treatment: ad libitum

Post exposure period: until death
Doses: 0.2% in feed, drinking water (2000 ppm)
Result: positive
Control Group: yes, concurrent no treatment

Method: other
Year: 1984
GLP: no data

Result: 2000 ppm;
drinking water
equivalent to 27 mg/animal/day (male and female)

oral feed
equivalent to 57 mg/animal/day (male)
equivalent to 38 mg/animal/day (female)

There was little or no life-shortening effect in any treatment group. None of the four amines administered alone induced an increase in the incidence of any tumour in comparison with the untreated control groups. In the male rats given diphenhydramine, chlorpheniramine or N,N-dimethyldodecylamine-N-oxide concurrently with nitrite there was a significant increase in the incidence of liver neoplasms (hepatocellular carcinomas and neoplastic nodules). In the groups given untreated feed or drinking-water there were, respectively, five and three male rats that had liver tumours. In contrast the number of male rats with liver tumours was ten in the group given dimethyldodecylamine-N-oxide plus nitrite, 11 in that given diphenhydramine plus nitrite and 14 (eight with carcinomas) in the group given chlorpheniramine plus nitrite.

These results suggest that the ingestion of dimethyldodecylamine-N-oxide, diphenhydramine hydrochloride or chlorpheniramine under conditions when they could be nitrosated with nitrite in the stomach might present an increased carcinogenic risk.

Test condition: Feed containing 0.2% allantoin or diphenhydramine (as the hydrochloride) or 0.1% chlorpheniramine (as the maleate), with or without 0.2% sodium nitrite, was given ad lib. to groups of 20 or 24 male and 20 or 24 female F344 rats for 106 wk. Groups of 24 male and 24 female F344 rats were given drinking-water that contained N,N-dimethyldodecylamine-N-oxide at a concentration of 0.1%, with or without 0.2% sodium nitrite, for 93 wk. Control rats were given untreated feed or drinking-water and nitrite-treated controls were given sodium nitrite at a concentration of 0.2% in feed or drinking-water. At the end of the treatment period the rats were given untreated feed and water and observed until death.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Fisher Scientific Co., Pittsburgh, PA, US.

Conclusion: In female rats given nitrite alone the incidence of liver neoplasmas (carcinomas plus neoplastic nodules) was significantly increased (P=0.015; nitrite in feed) and P=0.060; nitrite in drinking water vs untreated control), but in male.

Reliability: (4) not assignable
15-JUL-2005 (111)

Species: mouse Sex: male/female
Strain: ICR
Route of administration: drinking water
Exposure period: 109 weeks
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 0.125, 0.25, 0.5 % in drinking water
Result: negative
Control Group: yes, concurrent no treatment

Year: 1979

Result: Dose (%)
-0.125%; equivalent to 702 mg/kg/day for male, 471 mg/kg/day female
-0.25%; equivalent to 429 mg/kg/day for male, 244 mg/kg/day female
-0.5%; equivalent to 241 mg/kg/day for male, 173 mg/kg/day female

As a result, development of various tumors, including thymic lymphoma, nonthymic lymphoid leukemia, pulmonary adenoma and carcinoma, and benign and malignant tumors in soft tissue, was seen in these mice. However, as to the incidence of tumors as well as the developmental time of each histologically classified tumor, no apparent difference was detected between those in the experimental groups and the control group.
Test condition: Sodium nitrite has been widely used as one of the most effective food additives to tinge color on cured meat. However, it has been elucidated that this chemical is not merely a precursor of N-nitroso compounds, many of which are strongly carcinogenic, but also a mutagenic substance in biological tests. In order to ascertain the possible tumorigenicity of sodium nitrite itself, chronic toxicity of the agent in mice, by means of daily oral administration as drinking water for more than 18 months, in the concentration of 0.5 (maximum tolerated dose), 0.25, and 0.125%, was tested.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (4) not assignable
18-JUL-2005 (86)

Species: rat Sex: male
Strain: Fischer 344
Route of administration: oral feed
Exposure period: Continued on diets for 115 weeks, or until sacrifice moribund.
Frequency of treatment: continuous
Doses: 0.2 or 0.5% (w/w)
Result: negative
Control Group: yes, concurrent vehicle

Year: 1989

Result: Increased frequency of focal or diffuse interstitial cell hypoplasia. Appeared to increase with increasing dose, but not statistically significant.

Significant reductions in RBCs, resolved by 52 weeks.

Reduced MCV, Hct, and Hb in highconcentration group.

Concentration-related decrease in body weight gain, statistically significant at the high concentration.

Evidence of impaired feed utilization.

Better survival rate for treated than control animals, but not statistically significant.

Significantly lower incidences of lymphomas, leukemias, and testicular interstitial cell tumors.

Sodium nitrite exposure was associated with RBC (red blood corpuscle) count reduction over the first eight weeks of treatment. RBC's remained low until at least week 28 of the study, and then gradually rose to reach normal levels by approximately week 52. The reductions in RBC's seen in the high-concentration group were associated with reductions in the MCV (mean red cell volume), Hct (hematocrit), and Hb (hemoglobin concentration).

Body weight gain in treated rats showed a dose-related reduction, which was statistically significant at the higher sodium nitrite concentration. The body weight reductions relative to the pattern of feed intake, indicated that feed utilization was impaired by sodium nitrite exposure. Mortality data, on the other hand, showed a more favorable survival rate for treated animals, as compared to controls. This difference, however, was not statistically significant.

Compared to controls, treated animals suffered significantly lower incidences of lymphomas, leukemias, and testicular interstitial cell tumors. The authors suggested that the reduced weight gain of treated animals might have reduced susceptibility to tumor formation, and exerted a positive influence on life span.

On the other hand, findings of focal or diffuse testicular interstitial cell hypoplasia were more frequent among treated animals. While these changes appeared to increase with increasing dose, statistical significance was not reported. The study authors postulate that the hyperplasia might have been a function of hormonal imbalance related to geriatric changes in older animals.

Test condition: 5-6 week old male rats fed low-protein diets containing sodium nitrite at 0, 0.2, or 0.5% (w/w).

50 animals/treatment group. 20 controls.

Continued on diets for 115 weeks, or until sacrifice moribund.

Groups of 5-6-week old, male, F344 rats were fed reduced-protein diets contain sodium nitrate at concentrations of 0.2 or 0.5% (w/w). Fifty rats were

assigned to each treatment group, and 20 control rats were given the reduced-protein diet without added sodium nitrite. Animals were continued on their diets until they were sacrificed as moribund, or after 115 weeks of treatment.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: BDH Ltd, Dagenham Essex
Conclusion: There was no evidence of carcinogenicity in a detailed examination of a wide range of tissues from 50 male rats given up to 5000 ppm sodium nitrite (about 260 mg/kg/day) in a low protein diet, for up to 115 weeks.
Reliability: (4) not assignable
18-JUL-2005 (68)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 646 days
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 800, 1600 ppm
Control Group: yes, concurrent vehicle

Year: 1989

Result: The first tumor was found on day 441 in the liver of a rat given a diet containing 800 ppm sodium nitrite. On day 646, liver tumors were found in 1 of 22 rats (4.5%) on an 800-ppm sodium nitrite diet and in 5 of 19 rats (26.3%) on a 1,600-ppm sodium nitrite diet. The incidence of liver tumors in the rats fed 1,600 ppm sodium nitrite was significantly different from that in controls as judged by the t-test ($P < 0.05$).
A hepatocellular carcinoma and a hemangioendothelial sarcoma of the liver were found on day 646 in 2 rats fed 1,600 ppm sodium nitrite. One mammary tumor but no liver tumors were found in the 19 control rats. The concentration of sodium nitrite decreased after preparation of the pellet diet, but it was still at least 70% of the initial amount when the pellets were given to the rats.
Test condition: Sodium nitrite was given to male noninbred Wistar rats at levels of 800 ppm and 1,600 ppm in a pellet diet for 646 experimental days.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade
Supplier: Wako Pure Chemical Company, Japan
Conclusion: Liver tumors (incidences 5/19 or 26%) were induced in Wistar rats given 1,600 ppm in pelleted feed.
Reliability: (4) not assignable
18-JUL-2005 (10)

Species: mouse Sex: male/female
Strain: Swiss
Route of administration: drinking water
Exposure period: 40 weeks
Frequency of treatment: continuous
Post exposure period: no
Doses: 1.0 g/liter (concentration)
Control Group: yes

Year: 1971

Result: Compound/Control/NaNO2
Dose/None/1.0g per liter
Initial No. of mice (females;males)/80;80/40;40
Effective No. of mice (females/males)/71;73/38;36
Total adenoma-bearing mice (%)/14/19
Adenoma per mouse/0.18/0.2
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Analytical grade
Supplier: J.T. Baker Chemical Company, Phillipsburg, New Jersey
Conclusion: Treatment with sodium nitrite alone produced no effect. This study suggested that the induction of lung adenomas in animals treated with secondary amins (piperazine, morphine and so on) and nitrite is due to in vivo nitrosation (presumably in the stomach) with the formation of carcinogenic nitrosamines.
Reliability: (4) not assignable
18-JUL-2005 (71)

Species: mouse Sex: male
Strain: Strain A
Route of administration: drinking water
Exposure period: 20-25 weeks
Frequency of treatment: continuous
Doses: 1.0 g/liter (concentration)
Control Group: yes

Year: 1973

Result: Compound	Control	NaNO2
Dose (g/L)	0	1.0
Initial No. of mice	40	40
Effective No. of mice	37	37
Total adenoma-bearing mice (%)	32	30
Adenoma per mouse	1.4	1.2

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Practical grade
Supplier: J.T. Baker Chemical Company, Phillipsburg, New Jersey
Conclusion: Negative result was obtained with NaNO2 alone.
Reliability: (4) not assignable
18-JUL-2005 (70)

Species: mouse Sex: male/female
Strain: Swiss
Route of administration: drinking water
Exposure period: 26 weeks
Frequency of treatment: continuous
Post exposure period: 12 weeks
Doses: 0.65 g/mouse
Result: negative
Control Group: yes

Year: 1972

Result: Compound	Control	NaNO2
Dose (g/mouse)	0	0.65
Initial No. of mice (F/M)	30;30	30;30

	Autopsied No. mice (F/M)	27;26	30;30
	Total adenoma-bearing mice (%)	13.2	8.3
	Adenoma per mouse	0.15	0.08

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (69)

Species: other: rat and mouse Sex: male/female
Strain: other: F344 and C57BL/6
Route of administration: oral feed
Exposure period: 365 days
Frequency of treatment: continuous
Post exposure period: 120 days
Doses: 0.5%
Control Group: yes, concurrent vehicle

Year: 1979

Result: Days in study and incidence of neoplasms

-Rat
Group/Effective No. of animals/Days in study/Animals with neoplasms
Control (stock diet);
Male / 50 / 472 / 30 (60 %)
Female / 44 / 473 / 20 (45 %)

0.58% butylurea;
Male / 16 / 396 / 7 (44 %)
Female / 16 / 437 / 5 (31 %)

0.50% sodium nitrite;
Male / 16 / 432 / 8 (50 %)
Female / 16 / 447 / 6 (37 %)

0.50% sodium nitrite and 0.58% butylurea;
Male / 46 / 262 / 43 (93 %)
Female / 45 / 233 / 44 (98 %)
-Mouse
Group/Effective No. of animals/Days in study/Animals with neoplasms
Control (stock diet);
Male / 95 / 475 / 8 (8 %)
Female / 92 / 457 / 17 (18 %)

0.58% butylurea;
Male / 26 / 480 / 7 (27 %)
Female / 24 / 418 / 4 (17 %)

0.50% sodium nitrite;
Male / 11 / 430 / 2 (18 %)
Female / 12 / 418 / 2 (17 %)

0.50% sodium nitrite and 0.58% butylurea;
Male / 39 / 325 / 35 (90 %)
Female / 40 / 293 / 27 (67 %)

Test condition: Number of Animals used

-Rat
Control (stock diet); male/female = 50/55

0.58% butylurea; male/female = 50/50
0.50% sodium nitrite; male/female = 20/20
0.50% sodium nitrite and
0.58% butylurea; male/female = 50/50

-Mouse
Control (stock diet); male/female = 100/100
0.58% butylurea; male/female = 50/50
0.50% sodium nitrite; male/female = 20/20
0.50% sodium nitrite and
0.58% butylurea; male/female = 50/70

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (110)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: drinking water
Exposure period: 104 weeks
Frequency of treatment: continuous
Post exposure period: until death
Doses: 0.2%
Control Group: no

Year: 1975

Result: Most of the animals given heptamethyleneimine hydrochloride or sodium nitrite alone survived 2 years or more after the beginning of the treatment, and no tumors attributable to the treatment were seen at death; tumors appearing were those of endocrine origin found commonly in untreated controls. In the group receiving the combined treatment, most females were dead at 50 weeks and most males were dead at 80 weeks, 27 of 30 having tumors not seen in either control group. A total of 16 had squamous carcinomas in the lung; 25 had tumors of the oropharynx, tongue, esophagus, and forestomach; and there were a few animals with tumors in the nasal cavity and trachea. The expericment showed that squamous tumors of the lung could be induced by ingestion an amine and sodium nitrite.

NaN02, 0.2% - Nonendcrine tumor-bearing rats/total no. of rats
Male - 0/27
Female - 0/26 (4 animals were still alive at 140 wks.)

Test condition: Groups of 15 males and 15 females Sprague-Dawley rats were given 20 ml of drinking water solution containing either 0.2% heptamethyleneimine hydrochloride or this salt together with 0.2% sodium nitrite, 5 days a week for 28 weeks. Another group of 17 male and 30 female rats was given 0.2% sodium nitrite solution for 104 weeks.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (175)

Species: rat Sex: male
Strain: Wistar
Route of administration: drinking water

Exposure period: 94 weeks
Frequency of treatment: continuous
Post exposure period: no
Doses: 0, 0.1, 0.3%
Control Group: yes, concurrent no treatment

Year: 1989

Remark: The carcinogenic activity of endogenously synthesized N-nitrosobis(2-hydroxypropyl)amine (BHP) was investigated in male Wistar rats administered bis(2-hydroxypropyl)amine (BHPA) mixed in powder diet at a concentration of 1%, and sodium nitrite (SN) dissolved in distilled water at concentrations of 0.15 and 0.3%, for 94 weeks. Urinary excretion of BHP was detected in rats given 1% BHPA and 0.3% SN but not in the groups receiving either of these precursors alone. Nasal cavity, lung, esophagus, liver and urinary bladder tumors were found in animals treated with combinations of 1% BHPA and 0.15 or 0.3% SN, suggesting that the target organs of the endogenously synthesized BHP are similar to those affected when the carcinogen is administered exogenously. The incidences of nasal cavity and lung tumors reached 74 and 58% in rats given 1% BHPA and 0.3% SN, respectively. Tumors at sites other than target organs were only found at levels similar to those previously reported for spontaneous tumors in male Wistars. The present results clearly indicated the tumor inducibility of a nitrosatable amine, BHA, through an endogenous nitrosation by feeding to rats in conjunction with nitrite, and provide further suggestive evidence that endogenous nitrosations of environmental nitrosatable amines can be a potential risk factor in human cancer development.

Result: (Treatment)/(No. of rats-initial;effective)/(Body weight-initial;final)/(Daily intake per rats as sodium nitrite (mg))
Non-treated / 20; 19 / 189.5; 404.7/ 0.0
0.15% NaNO2 / 20; 18 / 189.5; 401.3/ 29.6
0.30% NaNO2 / 20; 16 / 185.6; 358.9/ 51.3

Incidence of tumors (%)	Non-treated	0.15% NaNO2	0.30% NaNO2
Nasal cavity	/ 0	/ 0	/ 0
Lung	/ 0	/ 0	/ 0
Esophagus	/ 0	/ 0	/ 0
Liver	/ 0	/ 0	/ 0
Urinary bladder	/ 0	/ 0	/ 0
Thyroid	/ 15	/ 6	/ 13
Kidney	/ 0	/ 0	/ 0
Stomach	/ 0	/ 6	/ 0
Pancreas	/ 0	/ 11	/ 0
Adrenal gland	/ 21	/ 6	/ 31
Testis	/ 47	/ 56	/ 63
Pituitary gland	/ 5	/ 6	/ 0
Mammary gland	/ 5	/ 6	/ 0
Others	/ 0	/ 0	/ 0

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Wako Pure Chemical Industry Ltd., Japan

Reliability: (4) not assignable

18-JUL-2005 (203)

Species: rat Sex: male/female

Strain: ICR
Route of administration: gavage
Exposure period: 10 weeks
Frequency of treatment: once a week
Post exposure period: until 18 months after the first dose
Doses: 0, 70 mg/kg/week
Control Group: yes, concurrent vehicle

Year: 1993

Remark: Carcinogenic potential of ethylenethiourea (ETU) in combination with sodium nitrite was investigated in ICR mice of both sexes. Groups of 30 males and 30 females each were given 10 weekly oral administrations of ETU and sodium nitrite with the following combinations of dosing (ETU vs sodium nitrite, mg/kg/wk): 0 vs 0, 100 vs 0, 0 vs 70, 25 vs 17.5, 50 vs 35, and 100 vs 70. Thereafter, the animals were allowed to live without treatment up to 18 mo after the first administration.

Result: Cumulative incidence of tumors at 18 months

-Male

(Incidence of tumors)	/ (Control)	/ ($NaNO_2$-treated)
Malignant lymphoma	/ (3/30)	/ (4/30)
Lung adenoma, adenocarcinoma	/ (9/30)	/ (11/30)
Forestomach squamous cell papilloma, carcinoma	/ (0/30)	/ (0/30)
Harderian gland adenoma	/ (3/30)	/ (1/30)

-Female

(Incidence of tumors)	/ (Control)	/ ($NaNO_2$-treated)
Malignant lymphoma	/ (6/30)	/ (12/30)
Lung adenoma, adenocarcinoma	/ (3/30)	/ (5/30)
Forestomach squamous cell papilloma, carcinoma	/ (0/30)	/ (0/30)
Harderian gland adenoma	/ (0/30)	/ (4/30)
Uterine adenocarcinoma	/ (0/30)	/ (0/30)

Concurrent administration of ETU and sodium nitrite caused earlier development of tumors and/or dose-dependent increases in the incidences of tumors in the lymphatic tissue, lung, forestomach, Harderian gland, and uterus, whereas treatment with either ETU or sodium nitrite
failed to show carcinogenic activity. In addition, carcinomas in the forestomach and uterine horn were limited to mice receiving concurrent administrations of ETU and sodium nitrite.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Wako Pure Chemical Industry Ltd., Japan
Conclusion: These results indicate that ETU is most probably converted in vivo into N-nitroso ETU and that the N-nitroso ETU has a greater carcinogenic potential in mice than ETU alone.
Reliability: (4) not assignable
18-JUL-2005 (204)

Species: mouse Sex:
Strain: Swiss
Route of administration: drinking water

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Exposure period:         6 months
Doses:                   1 g/liter (concentration)
Control Group:           yes

  Year:           1972

Result:           Compound            /Control/NaNO2
                  Dose                /None   /1.0g per liter
                  Initial No. of mice /144    /74
                  Adenoma per mouse   /0.2    /0.2
Test substance:   Chemical name: Sodium ntirite (CAS No. 7632-00-0)
Reliability:      (4)  not assignable
18-JUL-2005                                                          (125)

Species:                 rat                              Sex:
Strain:                  Sprague-Dawley
Route of administration: other: various
Exposure period:         26 months or until death
Frequency of treatment:  continuous
Doses:                   0, 250, 500, 1000, 2000 ppm
Control Group:           yes, concurrent vehicle

  Year:           1979

Remark:           Rats were exposed to sodium nitrite in food or water at
                  concentrations of 0, 250, 1000, and 2000 ppm.
Result:           -Treatment/Dose(ppm)/Proportion (%) Malignant
                  lymphomas;Immunoblastic cell proliferation

                  Semipurified diet (agar gel)/   0/ 3.7 ;7.3
                  Semipurified diet (agar gel)/ 250/ 7.3 ;6.6
                  Semipurified diet (agar gel)/ 500/ 8.1 ;16.9
                  Semipurified diet (agar gel)/1000/ 8.1 ;10.3
                  Semipurified diet (agar gel)/2000/ 10.3;13.4

                  In water (agar diet)        /1000/ 11.8 ;12.5
                  In water (agar diet)        /2000/ 10.3 ;13.4

                  Commercial lab chow (purina)/   0/  6.8 ;3.8
                  Commercial lab chow (purina)/1000/ 10.4 ;8.9
                  Commercial lab chow (purina)/2000/  9.0 ;8.3

                  Casein diet                 /   0/  7.4 ;7.4
                  Casein diet                 /1000/ 13.2 ;8.0

                  Lymphoma was increased in all groups fed nitrite; the
                  overall combined incidence was 5.4 percent in 573 control
                  rats and 10.2 percent in 1383 treated rats. The mechanism of
                  cancer induction did not appear to be through the formation of
                  nitrosamines but through a more direct effect of nitrite on
                  the lymphocyte.
Test substance:   Chemical name: Sodium ntirite (CAS No. 7632-00-0)
Reliability:      (4)  not assignable
18-JUL-2005                                                          (130)

Species:                 mouse                            Sex: male
Doses:                   50 micro-gram/gm
Control Group:           yes, concurrent no treatment

  Year:           1982
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Result: Histopathlogical examination of liver revealed that mice given DEA-HCl and NaNO2 had significantly more liver tumors compared to mice given only NaNO2 or DEA-HCl. This study suggested the formation of a potent carcinogen in vivo by the interaction of DEA-HCl and NaNO2.

(Treatment)	(Dose)	(No of mice with tumors-No of examined)
Distilled water	-	2 - 17
NaNO2	50	3 - 11
DEA-HCl	50	5 - 15
DEA-HCl + NaNO2	50+50	17 - 23

Test condition: To investigate the in vivo interaction of DEA-HCl and NaNO2 infant C57BL X C3H F1 male mice were given these substances (50 micro-gram/gm BW) intragastrically either singly or in combination. There were from 30 to 35 mice per group. Animals were sacrificed periodically up to 110 weeks.
Test substance: Chemical Name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (145)

Year: 1980

Remark: Incidences of malignant lymphoma were significantly increased in all exposed groups compared to the controls, and immunoblastic cell proliferation was observed in some animals in each exposed group. However, these results were not confirmed by a committee specially formed to review the data.
Reliability: (4) not assignable
18-JUL-2005 (59)

Species: mouse Sex:

Year: 1997

Remark: A study of the effect of sodium nitrite (SN) on leukemia development in mice induced by Rauscher Leukemia virus (RLV) (Balb/c mice), Mazurenko leukemia virus (MaLV) (CC57Br mice), and Gross leukemia virus (GLV) (AKR/J mice) was performed. SN was administered in water (at concentrations of 5.0, 50.0, 500.0, and 2000.0 mg/l, by NaNO2). A moderate, yet statistically significant acceleration of leukemia development was observed in some groups of SN-treated mice. Our findings and the literature provide evidence that SN has the capacity to enhance the carcinogenic effect of leukemia viruses in vivo.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (84)

Species: mouse Sex: male/female
Strain: Balb/c

Year: 1975

Remark: The spontaneous incidence of pulmonary tumors in intact virgin BALB/c mice of both sexes was 9 % in males and 11 % in females.
Ethambutol and sodium nitrite administered singly did not

tumors. However, when administered together they increased the adenoma and carcinoma incidence to 67 % in males and to 76 % in females and that of lymphomas (lymphocytic, histiocytic and mixed) to 22 % in males and to 14 % in females.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (17)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: oral feed
Exposure period: 104 weeks
Frequency of treatment: continuous
Post exposure period: sacrified at 130-week
Doses: 0, 2000 ppm
Control Group: yes, concurrent vehicle

Year: 1984

Result: Simultaneous feeding to rats of thiram with sodium nitrite was carried out to assess the possibility of formation of carcinogenic N-nitroso derivatives in vivo. Following the administration of feed containing 500 ppm thiram plus 2000 ppm sodium nitrite for 104 w, a high incidence of tumors of the nasal cavity was found in both sexes, 18 of 24 males and 15 of 24 females. No nasal-cavity tumors were seen in untreated rats, or those given 500 ppm of thiram or 2000 ppm of sodium nitrite alone. A 20% incidence of papillomas of the forestomach was also seen in the rats of both sexes given the combined treatment. The other significant difference in incidence of tumors between the rats given thiram with or without nitrite was a decreased number of animals with monocytic leukemia, which is a common neoplasm in untreated F344 rats.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Conclusion: No nasal-cavity tumors were seen in untreated rats, or 2000 ppm of sodium nitrite alone.
Reliability: (4) not assignable
18-JUL-2005 (112)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: oral feed
Exposure period: 78 weeks
Frequency of treatment: continuous
Post exposure period: until death
Doses: 0.2%
Control Group: yes

Year: 1980

Remark: A mixture of 0.1% disulfiram together with 0.2% sodium nitrite in powdered rat diet was fed to 20 male and 20 female Fischer 344 rats for 78 wk, after which the animals were observed until death. Ten of the males and 12 of the females died with tumours of the oesophagus, tongue, squamous stomach and nasal cavity. None of these tumours was observed in rats fed either disulfiram or sodium nitrite alone at similar doses. The tumours were attributed to the

reaction of disulfiram and nitrite in the stomach, with the formation of nitrosodiethylamine, which has given rise to these tumours in Fischer rats from the same colony.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (113)

Species: rat Sex: male/female
Strain: Wistar

Year: 1980

Remark: Large doses of dimethylnitramine (DMNM), N-nitroso-L-proline (NPRO), and sodium nitrite were administered in the drinking water to MRC Wistar rats for at least 1 year, and the rats were maintained for life. DMNM (total dose, 20 g/kg) produced liver tumors in 25 (69%) of the 36 rats and nasal cavity tumors in 9 (25%) of the rats. NPRO (total dose, 36 g/kg) induced no tumors in 37 treated rats. In the group receiving NaNO2 (3.0 g/liter drinking water; total dose, 63 g/kg), 8 (18%) of 45 rats had forestomach squamous papillomas. The tumor incidence in the NaNO2-treated group was significantly greater than that of 2% in a control group started 11 months earlier, which suggested that the NaNO2 was tumorigenic in this experiment.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
18-JUL-2005 (124)

Species: rat Sex:

Year: 1983

Remark: Administration to rats of ascorbate with morpholine and nitrite was previously shown to inhibit the liver tumor production and to enhance the induction of forestomach tumors, as compared to treatment with morpholine and nitrite. In a repetition of this experiment, 10 g morpholine/kg in the diet and 2 g sodium nitrite/liter in the drinking water were administered for life to male MRC-Wistar rats without (group 1) or with (group 2) 22.7 g sodium ascorbate/kg in the diet. Group 3 was untreated. Group 2 showed a lower liver tumor incidence with a longer latency than group 1, indicating a 78% inhibition by ascorbate of in vivo N-nitrosomorpholine (NMOR) formation. The incidence of forestomach papillomas was 3% in group 1, 38% in group 2, and 8% in group 3. The difference between groups 1 and 2 was not significant due to the shorter life-span of group 1. Group 1 and especially group 2 had more forestomach hyperplasia and hyperkeratosis than group 3. Ascorbate might have enhanced induction of these lesions because of an action synergistic with that of NMOR. However, it is most likely that the lowered NMOR dose and concomitantly increased survival produced by the ascorbate were solely responsible for the increased incidence of forestomach papillomas and other lesions in group 2.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (4) not assignable

18-JUL-2005 (126)

Year: 1990

Remark: Presented are the literature data as well as the results of our own investigations on the genotoxic and carcinogenic effects of sodium nitrite (SN). The carcinogenicity of SN detected in animal experiments appears to be related to the formation of nitroso compounds from endogenous nitrosable precursors. Sodium nitrite possesses transforming and promoting effects in cell cultures, as well as mutagenic effects in the bacterial systems, where the predominant effect of SN was compared to that of N-nitrosodimethylamine (NDMA). Prolonged pretreatment with SN amplifies the liver DNA damage in rats in case of NDMA endogenous synthesis.
Reliability: (4) not assignable
18-JUL-2005 (151)

Species: rat Sex: male
Strain: Wistar
Route of administration: drinking water

Year: 1995

Remark: Cancers and precancerous lesions of the esophagus were efficiently induced in rats by the simulation of a clinico-epidemiological setting; that is, the administration of precursors of nitrosamine. Six week old non-inbred male Wistar rats were given 2g/kg bodyweight of sarcosine ethyl ester hydrochloride (SEEH) and concurrently 0.3g/kg bodyweight of sodium nitrite (NaNO2), precursors of N-nitrososarcosine ethyl ester (NSEE), in 2% sucrose as drinking water. Group 1 received the precursors twice a week for 6 weeks followed by 8 weeks observation, and group 2, once every 3 days for 7 weeks followed by 26 weeks observation. At the end of treatment, no tumor had developed in the esophagus of rats in group 1, but the [3H]-thymidine labeling indices in both basal and superficial layer cells were higher than in the control group. On subsequent observation, papillomas appeared in group 1 (33.3%), and carcinomas in group 2 (33.3%), within 4 weeks. The tumors induced in group 1 were mostly papillomas and rarely carcinomas. When the observation was prolonged in group 2, 100% of the animals had cancer in week 20. The pathological changes of the lesions paralleled the sequential development of human squamous cell carcinoma of the esophagus. Our system has the advantages in that papillomas and cancers can be induced in rats in a short time and the agents used are less toxic than preformed nitrosamines administered previously by gastric intubation. It would serve as a useful experimental tool to study premalignant lesions and cancers of the esophagus.

Reliability: (2) valid with restrictions
18-JUL-2005 (201)

5.8.1 Toxicity to Fertility

Remark: See section 5.8.2 and 5.8.3
19-JUL-2005

5.8.2 Developmental Toxicity/Teratogenicity

Remark:

Data available in the published literature included studies of the effects of pre- and/or postnatal exposure to sodium nitrite on mice, guinea pigs, and rats. Additional relevant information on the developmental toxicity of compounds related to sodium nitrite is also discussed below.

Studies conducted in mice have not provided clear and consistent evidence for adverse effects of in utero exposure to sodium nitrite on measures of fetal viability, weight, sex ratio, or the frequencies of external or skeletal malformations. Nor did the study provide clear, dose-dependent evidence for adverse effects of prenatal exposure on parameters of postnatal growth or viability. Treatment protocols used in all three of these studies covered at least a major portion of the organogenesis phase of development, and oral doses of sodium nitrite ranged from 20 mg/kg/day to 243 mg/kg/day.

The study was aimed primarily at detecting sodium nitrite-induced changes in fetal erythropoiesis in CD-1 mice. From the commencement of gestation until sacrifice, according to a time course schedule, pregnant animals were given a daily dose of 20 mg sodium nitrite/kg bw by gavage. Treatment was associated with evidence for alterations in the proportions of hepatic erythroblasts at distinct stages of maturation. The authors interpreted the observed changes as indicative of a treatment-dependent increase in embryonic production of erythroid cells. No increase in peripheral red blood cell counts could be demonstrated, however, hence the functional significance of these findings remains unclear.

In pregnant guinea pigs, administration of 45 mg sodium nitrite/kg bw by s.c. injection during the last week of gestation resulted in spontaneous abortion of litters, but only in ascorbic acid-deficient females. Neither ascorbic acid deficiency alone, nor sodium nitrate in the presence of sufficient ascorbic acid, was associated with excess abortions. No gross abnormalities were noted in any living or aborted fetuses. In another study using guinea pigs, all pregnant sows given a dose of 70 mg sodium nitrite/kg bw by s.c. injection died within 60 minutes of treatment. All animals given a lower dose of 60 mg/kg bw aborted their litters. Co-administration of methylene blue, a MetHb antagonist, exerted a protective effect on fetuses.

Guinea pigs were also adversely affected by prenatal exposure to potassium nitrite in maternal drinking water. The percentage of fetal loss was higher in all treated litters than in controls, and appeared to generally increase with increasing dose (ranging from approximately 110 to 3520 mg potassium nitrite/kg bw/day), but no statistical analysis

was provided. At the two highest doses, all fetuses and one dam died. Placental degeneration, and inflammation of the reproductive tract were noted in females having resorbed or mummified fetuses.

There were no studies available from the published literature which used rats in a standard developmental-toxicity protocol involving prenatal-only administration of sodium nitrite. There are, however, a series of studies which exposed pregnant rats to sodium nitrite during the latter half of gestation, and evaluated postnatal effects on behavior and neurological development. In other studies, sodium nitrite was administered to pregnant and lactating rats; in some cases, the weaned offspring were then exposed directly. Reported effects of such treatment have included increased postnatal mortality and depressed postnatal growth, as well as decreases in open-field locomotor activity, and effects on pup organ weights and iron status.

Simple learning in response to either a reward or an aversive stimulus was not affected in two month old male offspring of female rats exposed to sodium nitrite in the latter half of gestation. Discriminatory learning of both visual and auditory cues, however, was impaired in the nitrite-exposed animals, as was long-term retention of a conditioned passive-avoidance response. Effects of prenatal nitrite exposure did not diminish as the animals aged; at 24 months, nitrite-exposed animals were unable to discriminate between light and dark areas of the test cage in response to avoidance training. Open-field activity levels, social behavior, and corticosterone levels in response to stress were also all affected in adult rats prenatally exposed to nitrite. Upon necropsy at 28 months of age, absolute and relative adrenal weights were found to be significantly higher in treated animals than in controls.

A study of cholinergic and serotonergic nerve fiber outgrowth in the hippocampus and neocortex of neonatal rats found significant delays associated with prenatal exposure

date: 04-JAN-2006

to sodium nitrite. The authors attributed their findings to nitriteinduced prenatal hypoxia, leading to retarded development of certain neurotransmitter pathways, in turn causing long-lasting dysfunctions in the developing rat brain. In the histopathological study, as in the behavioral studies, co-adminsitration of the Ca2+ channel-blocking drug, nimodipine, prevented the adverse effects associated with nitrite exposure alone. The protective effects of nimodipine were attributed to its antihypoxic activity, as it would be expected to block increases in intracellular Ca2+ concentrations associated with perinatal brain damage of hypoxic/ischemic origin.

Pre- and postnatal exposure of rats to sodium nitrite has been reported to have adverse effects on hematological parameters, including dose-dependent decreases in Hb content, RBC counts, and MVC values. A later study by the same group investigated the possible role of iron deficiency in producing the adverse postnatal effects associated with

sodium nitrite exposure. In the absence of supplemental iron, sodium nitrite-treated pups developed severe microcytic anemia by the second week of postnatal life. The anemia was associated with significant depressions in hematological parameters, as well as with depressed growth and increased pup mortality. Iron supplementation mitigated or eliminated all of these adverse effects.

No adverse effects on pup weights or mortality were observed in a rat multigeneration study, in which animals were fed on a sodium nitrite-containing, cured-meat diet providing daily sodium nitrite doses of approximately 5, 20, or 100 mg/kg bw.

Two of the studies in which rats were exposed to sodium nitrite both pre- and post-natally presented data on parameters evaluated at birth. A paper described the results of a cross-fostering experiment, in which offspring were exposed during either, both, or neither the pre- and post-natal periods. Although data were not presented, the authors stated that prenatal-only exposure led to no statistically significant differences in body weights from control pups, but there was an apparent effect of prenatal exposure evident on postnatal days one through eight. Additionally, offspring exposed only prenatally to sodium nitrite showed a significant reduction in MCV on postnatal day 21. In a later study by the same group, pup sex ratio and litter size at birth were found to be unaffected by prenatal exposure to sodium nitrite, but birth weights were significantly lower (by an ANOVA test of sex and treatment as sources of variation) in treated than in control animals.

The U.S. FDA summarized the results of submitted data from teratogenicity studies on sodium and potassium nitrate, which were performed in rats, mice, hamsters, and rabbits.

The Agency concluded that none of these studies demonstrated adverse effects of either compound on development. Conversely, the same N-nitroso compounds which can induce transplacental carcinogenicity in experimental animals when given late in pregnancy, may also induce a teratogenic response when given early in pregnancy
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Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: Studies conducted in mice have not provided clear and consistent evidence for adverse effects of in utero exposure to sodium nitrite on standard parameters of developmental toxicity. Administration of sodium nitrite to pregnant guinea pigs, particularly ascorbic acid deficient guinea pigs, has resulted in abortion of litters. Behavioral and neurodevelopmental studies conducted in rats have indicated life-long consequences of prenatal exposure to sodium nitrite. These effects have been attributed to nitrite-induced prenatal hypoxia. Fetal, as well as

maternal, methemoglobinemia has been reported in guinea pigs and dairy cows, following administration of sodium nitrite to the maternal animal. In dairy cows, mean fetal PO2 levels were found to be decreased following maternal infusion with sodium nitrite.

Reliability: (2) valid with restrictions
Reliable review of exsisting literature data.

18-JUL-2005 (29)

Species: guinea pig Sex: female
Route of administration: s.c.
Exposure period: During last week of gestation
Control Group: other

Year: 1970

Remark: Three experiments were performed in an attempt to clarify the relationship between ascorbic acid deficiency and sensitivity to sodium nitrite toxicity. Guinea pigs were fed on a diet lacking in ascorbic acid, until their plasma ascorbic acid decreased to a level considered to be "subnormal." In the experiment A, administration of 50 mg/kg sodium nitrite by subcutaneous (s.c.) injection produced significantly higher MetHb levels in 12 ascorbic acid deficient guinea pigs than in 6 controls. Higher MetHb levels were found at all time points tested: 60, 90, and 120 min post injection. No control animals died, while mortality was 83.3% in treated/deficient animals.

The experiment B and C; See TC and RS

The authors attributed the more severe effects of sodium nitrite on ascorbic-acid deficient guinea pigs to: "(1) a relative decrease in ascorbic acid available as a reducing substance, and (2) decreased hemoglobin and PCV values, which increased the ratio of nitrite per erythrocyte."

Result: Experiment B;
-Fetal Effects
No gross abnormalities.
All litters aborted in sodium nitrite treated, ascorbic acid-deficient animals.
No abortions in non-deficient, treated animals, or in deficient animals not given sodium nitrite.

-Maternal Effects
No deaths in non-deficient group; 83.3% mortality in deficient group.
Higher MetHb levels in deficient than in non-deficient dams.

Experiment C;
-Fetal Effects
Fetal MetHb levels did not differ between deficient and non-deficient groups.

-Maternal Effects
Maternal Hb, PCV, and plasma ascorbic acid lower in ascorbic acid-deficient dams than in their offspring.
MetHb levels significantly higher in deficient than in non-deficient dams.

Test condition: Experiment B;

11 pregnant sows given 45 mg sodium nitrite/kg bw, s.c.
Ascorbic acid status of animals varied.
Experiment C;
8 pregnant sows, 5 having low ascorbic acid status, were given 40 mg sodium nitrite/kg bw, s.c.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: These data indicated that the fetal deaths were related to higher levels of methemoglobin in maternal blood of ascorbic acid-deficient guinea pig.

Reliability: (2) valid with restrictions

18-JUL-2005 (105)

Species: guinea pig Sex: female
Strain: other: not specified
Route of administration: s.c.
Exposure period: Last 15 days of pregnancy
Control Group: yes

Year: 1971

Result: Experiment-1:
All guinea pigs given 50 mg/kg $NaNO_2$ as well as the controls gave birth to normal littles.

All 3 guinea pigs given 60 mg/kg $NaNO_2$ aborted 1-4 days after treatment.

The four given 70 mg/kg died within 60 minutes.

Experiment-2:
There was 96% mortality of fetuses from nitrite-treated dams examined at 3 or more hours after nitrite administration. All fetuses of control animals were alive.

Methemoglobin was present in maternal and fetal blood up to 6 hr after administration, with maternal concentrations being much higher. Plasma NO_2-N was detected at low concentrations at 1/4 and 11/2 hr in both maternal and fetal blood but was not detected at 3 hr or later. Heinz bodies were not seen.

Experiment-3:

Methemoglobin concentrations increased significantly during the experiment in both dams and fetuses. Fetal methemoglobin levels were significantly lower than corresponding maternal values. fetuses from dams were viable at 20 and 40 min, but fetuses in 4/6 litters were dead by 60 minutes.

Experiment-4:
fetuses were apparently protected by administration of methylene blue to the dams at the time of nitrite administration. Thoses gives nitrite alone aborted 3-4 days after treatment.

Experiment-5:
In guinea pigs given $NaNO_2$ (60 mg/kg) there was a corresponding reduction in maternal and fetal PO_2 values with the increases in methemoglobin values.

Test condition: Experiment-1:

Four pregnant guinea pigs were each given an s.c. injection of sodium nitrite (2% solution) at a dose of 50 mg/kg, three were given 60 mg/kg and 4 were administered 70 mg/kg. Two controls were given 2% NaCl, 50 mg/kg and one 60 mg/kg. The number of litters born alive, the number aborted and the number of dams killed was recorded.

Experiment-2:

A group of nine guinea pigs was injected s.c. with sodium nitrite solution (2%) at a dose of 60 mg/kg. A control group of nine guinea pigs was injected with sodium chloride (2%) s.c. at a dose of 60 mg/kg. One nitrite-treated and one control guinea pig eac hwere killed at the following intervals following administration of the sodium nitrite or sodium chloride: 0.25, 1.5, 3, 6, 18, 24, 24.5, 48 and 56 hours. Hysterotomies were performed and the fetuses removed and examined. Maternal blood samples were collected as were blood samples from live fetuses. Hemoglobin, methemoglobin and plasma nitrite levels were determined on all blood samples. Blood samples were stained with 1% methylene blue and examined for the presence of Heinz bodies. At necropsy samples of adrenal, brain, heart, intestine, kidney, liver, lung, pancreas, placenta, spleen and uterus were examined.

Experiment-3:

To determine maternal and fetal values of methemoglobin and plasma nitrite following sodium nitrite administration, 18 pregnant guinea pigs were each given sodium nitrite (60 mg/kg) and a hysterotomy was performed on 6 each at 20, 40 and 60 minutes. Maternal and fetal blood samples were obtained and analysed. The viability of fetuses was ascertained and recorded.

Experiment-4:

Methylene blue (10 mg/kg) was administered i.p. to 6 pregnant guinea pigs during the last 15 days of pregnancy. Three were simultaneously given $NaNO_2$ (60 mg/kg, s.c.). One nitrite-treated animal and 1 given only methylene blue were anesthetised and hysterotomies were performed 3, 24 and 100 hr after treatment. Six pregnant guinea pigs wre selected. Two were given methylene blue (10 mg/kg) and $NaNO_2$ (60 mg/kg). Two were given only methylene blue (10 mg/kg) and the remaining 2 guinea pigs were given $NaNO_2$ (60 mg/kg). Toxicity for the fetuses was evaluated as in prior experiments.

Experiment-5:

Six pregnant guinea pigs at approximately day 60 of gestation were used. Three were given a s.c. injection of $NaNO_2$ (60 mg/kg). Blood samples wre then collected 30, 60 and 90 min after the injection. Maternal blood was drawn from uterine blood vessels and the fetal blood from umbilical blood vessels. Maternal and fetal blood samples were also collected from the 3 untreated guinea pigs. A Radiometer was used to determine PO_2.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (2) valid with restrictions
18-JUL-2005 (164)

Species:	mouse Sex: female
Strain:	CD-1
Route of administration:	gavage
Exposure period:	from 0 to 14, 16, or 18 days of gestation
Frequency of treatment:	daily
Doses:	20mg/kg bw/day (ca 0.5 mg/mouse/day)
Control Group:	yes, concurrent vehicle

Year: 1978

Remark: Although the dose level is only one, this study can be used as a key study because the protocol is similar to guideline-study.

CAL/EPA (California Environmental Protection Agency) (2000). Evidence on Developmental and Reproductive Toxicity of Sodium Nitrite, Reproductive and Cancer Hazard Assessment Section (RCHAS), Office of Environmental Health Assessment (OEHHA) evaluated this study as follows:

A study of erythropoiesis in fetal CD-1 mice following maternal exposure to sodium nitrite was conducted. The study was intended to test a working hypothesis that sodium nitrite-induced fetal methemoglobinemia would be expected to result in increased fetal erythropoietic activity.

Result: No differences were found between treated and control animals, at any of the time points evaluated, for litter size, mean litter weight, mean embryo weight, mean number of resorption sites, or percentages of dead implants. There were no statistically significant differences between treated (n=42) and control (n=37) animals in the frequencies of skeletal abnormalities or variations, but the authors noted a tendency toward talipomanus and talipes in the nitrite-treated group. No data were provided to support this tendency.

Hepatic hematopoeisis was evaluated as percentages of total hepatic erythroblasts which were at various distinct stages of maturation. The overall frequency of red blood cells was significantly increased in treated animals at 14 and 16 days of gestation, but not at 18 days. The percentage of mature erythrocytes in hepatic tissue was reduced in treated animals on gestation days 14 and 16, but not on gestation day 18. The percentages of pro- and basophilic erythroblasts were significantly decreased in treated animals of 14 and 16 days gestation age, but not on gestation day 18. Polychromatophilic erythroblasts were significantly more frequent in treated than control animals on gestation day 14, and significantly less frequent on gestation day 16, with no difference between treated and control animals on gestation day 18. Orthochromatophilic erythroblasts were also found at a higher percentage in treated than control animals on gestation day 14, and to be unchanged from controls on gestation day 18. This cell type, however, was found to be more frequent in treated than in control animals on gestation day 16.

The overall frequency of white blood cells was slightly, but not significantly, reduced at all three time points. The proportion of neutrophil granulocytes was significantly increased in treated animals at day 18, but was unchanged from control values at the other time points.

The authors interpreted their results as indicating a treatment-dependent increase in embryonic hepatic production of erythroid cells. The lack of a sustained increase in mature red blood cells in the livers of day 18 fetuses was considered to be due to a normal developmental shift in the main site of erythropoesis from the liver to the bone marrow and spleen. The authors acknowledge, however, that no increase in red blood cell counts could be demonstrated in the peripheral circulation. Hence the functional significance of their findings remains unclear.

Test condition: CD-1 mice were time-mated, and the day of plug detection was considered to be day one of gestation. Treated animals were given 0.5 mg sodium nitrite per mouse, by intubation, daily throughout gestation, until sacrifice on gestation day 14, 16, or 18. For an approximate average body weight of 25 mg, this provided a dose of 20 mg/kg/day. Control animals were given distilled water, also by gavage. Twenty-three control and 23 treated litters were sacrificed for examination on gestation day 14; 12 control and 9 treated litters were examined on day 16; and four control and four treated litters were evaluated on gestation day 18.

Litter size, the number of resorption sites and dead implants, and embryo/fetal weights were recorded at the time of sacrifice. Embryos/fetuses were examined for gross anatomical defects, and, following removal of the livers for analysis, their carcasses were prepared for skeletal examinations. Parameters of erythropoiesis were determined for hepatic tissues, as the liver is the main site of erythrocyte production between gestation days 12 and 19 in the mouse.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Supplier: Matheson, Coleman and Bell

Conclusion: -Fetal Effects
No significant differences between groups for: litter size, weight, resorptions, fetal death, or skeletal abnormalities or variations.
Significant changes in hepatic erythropoiesis.
No sustained increase in hepatic mature red blood cells on day 18.

Reliability: (2) valid with restrictions
19-JUL-2005 (66)

Species: mouse Sex: male/female
Strain: CD-1
Route of administration: gavage
Exposure period: gestation days 6-15, dosing started after implantation.
Frequency of treatment: daily
Doses: 0, 20, 40, 80, or 120 mg sodium nitrite given to groups of 25 pregnant mice on gestation days 6 - 15.
Control Group: yes

Year: 1987

Result: One maternal death occurred in the 40 mg/kg/day group; no other dams died. No clinical symptoms of toxicity were reported during the course of the study. Maternal body weights on gestation day 17, and maternal body weight gains over the gestation period, were significantly reduced at the two highest doses of 80 and 120 mg/kg/day. Kidney and liver weights showed significant decrements from controls at some doses, but there was no evidence for a dose-response relationship. Furthermore, when considered relative to body weights, organ weights were found not to differ between groups. There were no differences between groups in hemoglobin or hematocrit values.

There were no differences between groups in the frequency of pregnancy among mated females, nor were there any incidents of total litter loss or premature delivery. The number of implantations per litter was significantly reduced in the high-dose group, as was mean litter size. As it is likely that implantation preceded or coincided with the beginning of treatment, the observed changes in implantation frequency are unlikely to have resulted from treatment. In turn, the change in litter size on gestation day 17 may have been an artifact of non-treatment related variation in implantation frequency.

The percentage of living fetuses at each dose was significantly reduced at both 20 and 120 mg sodium nitrite/kg bw, but not at the intervening doses of 40 and 80 mg/kg. The total number of dead fetuses, and the number of early fetal deaths was significantly increased at the high dose. At the low dose, the total number of dead fetuses was significantly decreased. Fetal weights were significantly increased over controls at all doses of sodium nitrite, with no apparent relationship to administered dose.

Isolated incidences of cleft palate were observed in all groups (treated and control), with no evidence for a treatment-related effect. No other external abnormalities were observed. There were some significant differences between treated and control fetuses in the pattern of ossification of skeletal elements, but there was no consistent treatment related pattern of skeletal retardation or advancement. As compared to controls, the frequency of a 14th rib was significantly lower in the fetuses of animals given 20 or 80 mg sodium nitrite, but significantly higher in fetuses of the 40 or 120 mg/kg/day groups. No differences were observed between groups in the frequency of litters having at least one fetus with 14 ribs.

For the five females in each dose group who delivered their fetuses normally, only the high-dose group showed significant reductions in body weight, relative to controls, on gestation day 17. There were no significant differences between groups in the rate of body weight gain during gestation. When these dams were necropsied subsequent to weaning of their offspring, uterine weights were significantly reduced in the 20 and 120 mg/kg groups, but not in the two intervening dose groups. Hemoglobin was

significantly increased in the 40 mg/kg dose group, and hematocrit was significantly decreased in the 120 mg/kg dose group.

Litter size at postnatal day 70 was significantly reduced from control levels in the 40 and 80 mg/kg groups. No offspring deaths were reported for the first 14 days following birth. During the interval between postnatal day 14 and postnatal day 70, one pup died in each of the control and low-dose groups, and two pups died in the high-dose group.

Pup body weights on postnatal day 14 were significantly reduced in the 20 and 120 mg/kg dose groups, but not in the 40 or 80 mg/kg/day dose groups. Hence, the dose-response pattern for body weight reductions mirrors that observed for litter size. In other words, the data suggest that pups in the smaller litters of the 40 and 80 mg/kg dose groups had less competition for milk, and thus were able to maintain a normal body weight.

By postnatal day 70, there were no differences between dose groups in the mean weights of male or female pups. Some significant differences in mean organ weights were found between dosed groups and the control group, but these differences were not apparent when organ weights were considered relative to body weights.
Compared to controls, hematocrits were significantly reduced in all groups of sodium nitrite-exposed female pups. Hemoglobin content was also reduced in treated animals, reaching statistical significance for males in the 20 and 80 mg/kg groups, and for females in the 40 mg/kg group.

Test condition: Groups of 25 time-mated female Crj:CD-1 mice (10 wk-old) were exposed to sodium nitrite by gavage, at doses of 0, 20, 40, 80, or 120 mg/kg/day, on gestation days 6-15. On gestation day 17, 20 pregnant mice from each group were sacrificed for examination of their uterine contents. The five remaining mice in each group were allowed to deliver their offspring normally for postnatal observations.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Reagent grade

Conclusion: -Fetal Effects
Significant decreases in: implantation frequency and mean litter size at 120 mg/kg/day; living fetuses at 20 and 120 (not 40) mg/kg/day; dead fetuses at 20 mg/kg/day.
Significant increases in: dead fetuses and early fetal deaths at 120 mg/kg/day; increased fetal weights at all doses, with no dose response.

-Maternal Effects
1 maternal death at 40 mg/kg
No clinical symptoms of toxicity.
Body weights on day 17, and gestational weight gain significantly reduced at 80 and 120 mg/kg/day.
Some decreases in absolute, but not relative, liver and kidney weights.

Reliability: (2) valid with restrictions
19-JUL-2005 (161)

Species: mouse Sex: female

Strain: ICR
Route of administration: drinking water
Exposure period: gestation day 7-18
Frequency of treatment: continuous
Doses: 0, 100, 1000 mg/L = appl. 0.27, 243 mg/kg bw/day
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: > 1000 mg/l
NOAEL Teratogenicity: > 1000 mg/l

Year: 1989

Result: There were no statistically significant changes in water consumption or gestational weight gain of treated dams. Nor were there any significant changes in the numbers of corpora lutea, live fetuses per litter, or resorbed or dead fetuses per litter. No significant differences were reported between groups for sex ratio, the frequency of runting, fetal body weights, or the frequencies of external or skeletal malformations. No significant changes were noted in the frequency of chromosomal gaps or breaks in maternal bone marrow cells, or in fetal liver cells.

Although the numbers of corpora lutea had been predetermined before dosing, the result is consistent

-Fetal Effects
No significant changes in: live fetuses per litter, fetal weights, sex ratio; or in frequencies of resorptions, dead fetuses, runting, or external or internal malformations.
No significant changes in the frequency of chromosomal gaps or breaks in liver cells.

-Maternal Effects
No significant changes in: water consumption, gestational weight gain, or frequency of chromosomal gaps or breaks in bone marrow cells.
Test condition: Animal; sexually mature virgin female ICR mice (25-33g)

Females were sacrificed, and their uterine contents evaluated on gestation day 18.
Test substance: Chemical name: Sodium ntirite (CAS No. 7632-00-0)
Conclusion: Teratogenic and mutagenic effects of NaNO2 were absent in mice at the dose of 100 and 1000 mg/L.
Reliability: (2) valid with restrictions
19-JUL-2005 (160)

Species: rat Sex: female
Strain: Wistar
Route of administration: drinking water
Exposure period: from gestation day 13 to parturation
Duration of test: Two months postnatal
Doses: 2g/L
Control Group: yes, concurrent no treatment

Year: 1990
GLP: no data

Method: Behavioral testing of male offspring at 2 months postnatal age.
Male offspring were divided into test groups, and subjected

to either discrimination or passive avoidance testing. Auditory discrimination for a drinking water reward (conducted following 23 hours of water deprivation) was tested, as well as visual discrimination in a (footshock) active avoidance situation. The passive avoidance test system consisted of avoiding footshock by learning not to enter a darkened compartment. Extinction and retention of these behaviors were also evaluated. Five to nine animals were subjected to each testing series.

Number animals/group not stated.

Result: Not standard test, single dose level, number of the dam not reported, but noteworthy.

Acquisition of the initial phase of all three tasks showed no significant differences between treatment groups. For all animals, performance of the auditory conditioned response was "almost errorless" within four training sessions. Acquisition of the visually cued, one-way avoidance response was also comparable between groups ($F_{2,13} = 0.1$; $P = 0.9$). More than 50% of the animals from all groups showed maximal passive avoidance following the first footshock experience.

Animals exposed prenatally to nitrite, however, were found to be impaired both in discrimination learning behavior (both auditory and visual), and in long-term retention of the passive-avoidance response. In the discrimination phase of the auditory test, the nitrite-treated rats made more errors than did either the controls or the nitrite plus nimodipine -treated group in response to the non-reinforced stimulus ($F_{1,12} = 21.26$; $P<0.001$ and $F_{1,16} = 10.68$; $P<0.01$, respectively). A similar pattern was found for the error rate in response to the reinforced stimulus ($F_{1,16} = 5.89$; $P<0.05$ and $F_{1,16} = 6.32$; $P<0.05$, for differences from the control and nimodipine plus nitrite groups, respectively).

Impairment of long-term retention of the passive avoidance response (intertrial intervals of 3 or 4 days) was shown for nitrite-exposed animals compared to the control group ($U = 33$; $P = 0.02$). The effect of nitrite was at least partially reversed by nimodipine, as no difference was found between the controls and the nitrite plus nimodipine group ($U = 75$; $P = 0.6$). However, there was no significant difference between the nitrite only group, and the nitrite plus nimodipine group ($U = 43$; $P = 0.09$). In the first two sessions of the retention test, significantly more individuals of the nitrite plus nimodipine group showed the avoidance response as compared to the nitrite treated group ($X2 = 4.53$; $P<0.05$; and $X2 = 4.11$; $P<0.05$, respectively). Differences between the nitrite-only group and the control group were also significant ($X2$; $P<0.05$). By the third trial, the extinction process was too far advanced in all groups to reveal any between-group differences.

-Offspring Effects
No changes in initial acquisition of learned responses.
Nitrite-exposed animals significantly impaired for discrimination learning behavior, and for long-term retention of passive-avoidance.
Co-administration of nimodipine prevented these effects.

Test condition: Pregnant Wistar rats were given 2 g/L sodium nitrite in drinking water, from gestation day 13 until parturition. Apart from untreated controls, other experimental groups were given either the calcium antagonist nimodipine, at a dose of 10 mg/kg bw, by gavage, from gestation day 11 through parturition, or both nimodipine and sodium nitrite. The number of animals assigned to each treatment group was not stated in the paper.

At two months postnatal age, male offspring were divided into test groups, and subjected to either discrimination or passive avoidance testing. Auditory discrimination for a drinking water reward (conducted following 23 hours of water deprivation) was tested, as well as visual discrimination in a (footshock) active avoidance situation. The passive avoidance test system consisted of avoiding footshock by learning not to enter a darkened compartment. Extinction and retention of these behaviors were also evaluated. Five to nine animals were subjected to each testing series.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: The results support the hypothesis that Ca++ homeostasis of neurons is important factor for normal development of brain behavior.

Reliability: (2) valid with restrictions

19-JUL-2005 (137)

Species:	rat	Sex:	
Strain:	Wistar		
Route of administration:	drinking water		
Exposure period:	from gestation day 13 to parturation		
Frequency of treatment:	ad libitum		
Duration of test:	10 days post natal		
Doses:	2 g/L		
Control Group:	yes, concurrent no treatment		
Result:	-Offspring Effects: Significant effects of sodium nitrite on ingrowth of cholinergic and serotonergic nerve fibers into the hippocampal dentate gyrus and the parietal neocortex.		

Method: other: brain histopathology on days 1, 3, 5, 7, 10 postnatal

Year: 1994

Result: Detail are as follows:

Brains of male pups were processed for histopathological staining of acetylcholinesterase (AchE) and serotonin (5-hydroxytryptamine: 5-HT)-positive fibers. Brains were sampled on each of post-natal days 1, 3, 5, 7, 10, and 20. From each litter group, only a single pup was assigned to one of the age-groups, to avoid possible litter-based confounding effects.

Prenatal nitrite exposure was shown to modulate the development of AchE and 5-HT positive fiber ingrowth into the hippocampal dentate gyrus (DG) and parietal neocortex during the first week of postnatal life. Fiber densities were evaluated from serial sections. Effects on both fiber types were region-specific in the hippocampus, and restricted to the DG. Based on an ANOVA test of treatment effects, nitrite was found to significantly influence

cholinergic fiber density in the DG ($F_{1,39} = 3.92$, $P < 0.05$). The number of fiber crossings in the DG region was also decreased in nitrite-treated postnatal day 5 and 7 animals. Co-administration of nimodipine prevented these effects.

In the parietal cortex, the delay in cholinergic fiber ingrowth was more pronounced in deep than in superficial layers, while the serotonergic innervation was influenced more evenly. By postnatal day 10, the differences between nitrite-treated and control rats were no longer apparent. ANOVAs carried out for postnatal days 3-10, revealed a treatment effect on cholinergic fibers restricted to the deeper layers ($F_{2,66} = 3.71$, $P < 0.05$), with a specific effect of nitrite (without nimodipine) on lowering fiber density ($F_{1,39} = 5.67$, $P < 0.02$). On postnatal day 7, 5-HT fiber density was significantly lower in nitriteexposed animals than in controls, in both layers of the parietal cortex. Again, the effect of nitrite was reversed in the presence of nimodipine.

The authors concluded that the impact of prenatal nitrite exposure was most evident during the early postnatal period, when rapid fiber proliferation normally takes place.

They attributed the effects of nitrite to prenatal hypoxia, and the mitigating effects of nimopidine to its neuroprotective or antihypoxic action. Nimopidine would be expected to block increases in intracellular Ca2+ concentrations associated with perinatal brain damage of hypoxic/ischemic origin. They postulated that the transitory effects of prenatal hypoxia on fiber ingrowth could, in turn, result in long-lasting functional deficits in the rat brain.

Test condition: 2 g sodium nitrite/liter drinking water from gestation day 13 through birth. 1000 ppm nimodipine in food pellets to some animals (with or without sodium nitrite), from gestation day 11 through birth.

Number animals/group not stated. Brain histology of male pups on post-natal days 1, 3, 7, 10, and 20.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: The results indicated that prenatal hypoxia evokes a temporary delay in the cholinergic and serotonergic fiber outgrowth in cortical target areas in a region-sepecific manner. The hypoxia-induced growth inhibition is prevented by the calcium antagonist nimodipine, which supports the importance of the intracellular Ca++ homeostasis of cells and growth cones in regulating axonal proliferation.

Reliability: (2) valid with restrictions
Not standard test, single dose level, number of the dam not reported, but noteworthy.

19-JUL-2005 (135)

Species: rat Sex:
Strain: Wistar
Route of administration: drinking water
Exposure period: from gestation day 13 to parturation
Frequency of treatment: daily
Duration of test: 28 months postnatal
Doses: 2g/L

Control Group: yes, concurrent no treatment

Method: other: Behavioral testing of male offspring at 26 months postnatal age.
Year: 1994

Result: -Offspring Effects
Details are as follows: Pregnant Wistar rats were given 2 g/L sodium nitrite in drinking water, from gestation day 13 until parturition. Apart from untreated controls, other experimental groups were given either the calcium antagonist nimodipine, at a dose of 10 mg/kg bw, by gavage, from gestation day 11 through parturition, or both nimodipine and sodium nitrite. All treatments ceased at parturition. There were eight to ten dams in each treatment group, and no significant differences in litter size or pup birth weight were noted.

Behavioral testing was performed on adult male offspring at the ages of 5 months, 19 months, and 23-26 months. Spontaneous behaviors, such as open-field activity and social behavior, were measured repeatedly. Learning tests were applied only during old age, in order to avoid repeated use of footshock. Plasma corticosterone responses were measured at 28 months of age.

By 2-way ANOVA, both age and treatment-dependent effects were apparent on measures of open-field activity (latency to start locomotion and number of crossings of lines marking the test arena). A statistically significant interaction between these two factors ($F_{2,128} = 2.44$; $P<0.05$) indicated that the effect of prenatal nitrite exposure on start latency was more profound at advanced ages. Line-crossing activity also decreased with age ($F_{2,128} = 46.75$; $P<0.001$). Nitrite-only exposed rats were less active than controls at both 5 and 19 months of age ($P<0.05$), but not at 23 months.

Total duration of social interactions, as measured by recording the duration of activities such as inspection, mounting, grooming, and play fighting, showed a marked effect of treatment ($F_{2,43} = 76.31$; $P<0.001$). The number of social interactions occurring during the five minute observation periods also varied between treatment groups ($F_{2,43} = 3.42$; $P<0.05$); this was attributed primarily to the high activity levels of nitrite plus nimodipine-exposed animals at 23 months, as compared to the nitrite-only group.

At 24 months of age, rats exposed prenatally to nitrite showed no evidence of ability to discriminate between light and dark areas of the test cage, following avoidance training by administration of footshocks in the dark area. The vehicle controls, and nitrite plus nimodipine-exposed animals showed dark-avoidance behavior in initial trials, as well as extinction of this response in later trials, in response to withdrawal of the aversive stimulus. Nearly 60% of nitrite-exposed animals showed an extreme response to the footshock stimulus, and vocalized, jumped, and/or bit the grid floor. This was a significant increase over the frequency of such reactions in vehicle controls, or in animals prenatally exposed to nitrite plus nimodipine

(P<0.02, by X2).

At the age of 28 months, plasma corticosterone levels were measure 15, 30, and 90 minutes after test animals were subjected to a novel stress. Compared to basal corticosertone levels, determined from samples taken at one minute prior to application of the novel stressor, an increased corticosterone response was induced in all three groups ($F_{3,42} = 9.53$; $P<0.001$). The shape of the stress-response curve depended on the nature of prenatal treatment ($F_{3,42} = 2.71$; $P<0.02$). For vehicle control and nitrite plus nimodipine-exposed rats, corticosterone levels had returned to baseline levels by 90 minutes following the stress incident. In the nitrite-only exposed animals, plasma corticosterone was still significantly elevated above baseline levels at 90 minutes poststress ($P<0.05$). Upon necropsy, adrenal weights, and adrenal weights relative to body weights were found to be significantly ($P<0.05$) higher in rats prenatally exposed to nitrite alone, as compared to control animals. Animals exposed to nitrite plus nimodipine had higher adrenal weights, but also higher body weights than controls, hence relative adrenal weights were comparable between the latter two groups.

Test condition: 2 g sodium nitrite/liter drinking water from gestation day 13 through birth.
10 mg nimodipine/kg bw by gavage to some animals (with or without sodium nitrite), from gestation day 11 through birth.
8-10 dams/group.
Behavioral testing of male offspring at 5, 19, and 23-26 months.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: -Offspring Effects
Age and treatment had significant effects on measures of open field activity.
Impaired discrimination learning at 24 months of age (not tested at earlier time points). Hyperreactivity to footshock.
Significantly prolonged

Reliability: (2) valid with restrictions

19-JUL-2005 (136)

Species: rat Sex: female
Route of administration: drinking water
Exposure period: From pregnancy to lactation
Frequency of treatment: continuous
Doses: 2000, 3000 mg/L
Control Group: yes, concurrent vehicle

Year: 1972
GLP: no

Result: Reported effects for pups included: increased mortality, decreased weight gain, and poor appearance of the fur. Mean litter size (presumably at birth) was 10 for controls, 9.5 at the low dose, and 8.5 at the high dose. No statistical analysis was provided. Mortality was reported as 6% for controls, 30% for the low-dose group, and 53% for the high-dose group. It is not stated, however, during what period that mortality occurred. Birthweights were similar

for treated and control animals, but growth rates were substantially slower for treated animals. At weaning, the mean weight of control pups was 51.5 g, while the mean weights of low and high-dose group pups were 29.5 and 18.5 g, respectively. Subsequent to weaning, growth rates of the dosed groups improved. No evidence was found of abnormally high MetHb in pups, but mean Hb levels were approximately 20% lower in treated than control animals.

-Offspring Effects
Mean litter sizes lower in treated than control animals; no statistical analysis.
Birthweights similar between groups.

Test condition: 0, 2000, or 3000 mg sodium nitrite/L drinking water to groups of 7-12 rats during gestation and lactation.

Groups of 12 pregnant rats were given sodium nitrite in drinking water at concentrations of either 2000 or 3000 mg/L. A group of seven controls was given plain tap water. While the experimental details are not provided, it appears that litters were delivered normally and remained with their dams until weaning. Treatment of the dams' drinking water continued during the lactation period. At weaning, the pups were put on plain drinking water.

Conclusion: Rats born of dam chronically exposed to nitrites in drinking water bduring gestation period showed high mortality rates and poor growth and development as compared to controls.

Reliability: (2) valid with restrictions
22-JUL-2005 (163)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: for 14 days prior to mating, throughout the breeding period (1-14 days), and throughout gestation and lactation. Weaned offspring were continued on the same diets for the duration of the study
Frequency of treatment: continuous
Duration of test: same as exposure period to day 90 post-natal
Doses: 0, 0.0125, 0.025, or 0.05% (w/w) equivalent to 0, 10.75, 21.5 or 43 mg/kg bw/day
Control Group: yes, concurrent vehicle

Method: other: reproductive performance and behavioral test on offsprings
Year: 1984
GLP: no

Remark: Although offsprings were fed with test substance containing feed for 90 days post-natal, and manifested effects are indiscriminative from pre- or pat-natal effect, study is comprehensive and well described.
Result: NOAEL (Reproduction) = 43 mg/kg bw/day
NOAEL (Development) = 10.75 mg/kg bw/day

Parental observations:

There were no significant reductions in food consumption or body weight in treated animals before breeding or in treated

animals before breeding or in treated females during gestation or lactation. Sodium nitrite had no significant effect on reproductive performance (Table 1) and no malformations were seen.

Table 1: Reproductive Performance of rats fed 0-43 mg/kg bw/day sodium ntirite in the diet

	Values of reproductive performance parameters			
Parameter	Negative Control	Positive Control	Sodium nitrite groups (mg/kg bw/day)	
			10.75	21.5	43
No. females w/ sperm	35	32	22	24	28
Females delivering (%)	77.1	78.1	68.2	79.2	64.3
No. litters < 8 live pups	2	15	0	5	3
Gestation length (days)	22.2+/-0.2	22.3+/-0.2	21.9+/-0.2	22.4+/-0.2	21.9+/-0.2
No. born/litter	11.0+/-0.4	8.5+/-0.7	10.9+/-0.4	11.0+/-0.9	10.9+/-0.7
Total No. offspring delivered	297	212	163	209	196
Mean male/female ratio	1.25	0.71	1.08	0.91	1.21
No. litters tested after birth	25	10	15	14	14

Preweaning observations:
Mortality: Sodium nitrite produced significant increases in offspring mortality in the 43 mg/kg bw/day group during the preweaning period but not at birth or after day 24, and in the 21.5 mg/kg bw/day group at birth but not thereafter. The 10.75 mg/kg bw/day group exhibited no significant increase in mortality.
Body weight: Sodium nitrite decreased preweaning body weights in the 21.5 and 43 mg/kg bw/day groups compared with negative controls.

Behaviour:
The preweaning appearances of surface righting, pivoting, negative geotaxis, and auditory startle behavior were all unaffected by exposure to sodium nitrite. Control of swimming direction and control of head height while swimming, however, were delayed in sodium nitrite exposed preweaning pups. These effects were more pronounced at the

two higher sodium nitrite concentrations. Open-field activity was decreased in preweaning females of the middle concentration group (P<0.05). The effect is considered the basis of NOEL of offsprings.

Open field activity on postnatal days 40-45 was significantly reduced in both males and females of the high and low-sodium-nitrite concentration groups, but not in the mid-concentration group. As compared to controls, the low-concentration group also showed significantly longer response latencies on the first day of post-weaning testing.

Body and organ weights:

At necropsy on postnatal day 90, there were no significant reductions in body or brain weights with sodium nitrite treatment. Females of the 10.75 and 21.5 mg/kg bw/day groups showed a 4-5% reduction in eye weights (P<0.05).

Toxicological meaning of the finding is unknown.

Due to the lack of a clear dose-response relationship, the authors considered that interpretation of responses at the low and mid-concentrations of sodium nitrite was problematical. They concluded, however, that the effects observed in the high-concentration group gave evidence that sodium nitrite is capable of inducing moderate decreases in open-field locomotor activity.

Test condition: Animals: Sprague-Dawley rats
Sex: male/female
Weight: 200-220 g

Fed 0 (two control groups), 0.0125, 0.025 or 0.05 w/w sodium nitrite in diet (equivalent to 0, 10.75, 21.5 or 43 mg/kg bw/day, respectively).

Negative control dams received no treatment. Positive control dams were given two i.p. injections of 2 mg 5-azacytidine/kg on day 16 of gestation.

Dietary treatments were given continuously to both males and females for 14 days before mating and for 1-14 days during breeding, and to females only during gestation (22 days) and lactation (21 days).

After weaning, offspring were given dietary sodium nitrite at the level their parents had received throughout the remainder of the experiment (up to 90 days of age for most animals and longer for those in avoidance testing).

Litters with fewer than eight live offspring were not kept beyond day 1 after birth. Litters of more than 12 were reduced to 12 by a random selection procedure that balanced the sex distribution as much as possible.

Parental bodyweights were measured weekly except during breeding and food consumption was measured on selected rats during all phases of the experiment.

Date of birth and length of gestation recorded for all

litters. On the day following birth all litters were examined and data collected on litter size, sex distribution, weight and number of dead and/or malformed offspring
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Purity: Food grade
Supplier: EI du Pont de Nemours & Co.
Reliability: (2) valid with restrictions
19-JUL-2005 (189)

Species: rat Sex: female
Route of administration: gavage

Year: 1982

Method: Virgin female rats (170-200 g) were paired overnight with proven breeders. The orning that a positive vaginal smear was observed was noted as day 1 of pregnancy.

Postnatal study (exp 1): Five to 12 mated females were randomnly assigned to each experimental group. On day 15 of gestation two groups of rats were given an orally intubated dose of 40 or 60 mg/kg ETU/kg followed immediately by a dose of 80 mg NaNO2/kg or two successive doses of distilled water. The dams were allowed to deliver normally and progeny were examined at birth and frequently thereafter, the animals being maintained for 140 days to assess survival and development. pups that died while on test were autopsied to determine cause of death.

Prenatal studies (expt 2): All test chemicals were administered to dams (12-17 dams/group) orally on day 13 of gestation. All the dams were autopsied on the last day of pregnancy. Uteri were searched for resorptions and for dead and live fetuses. All live fetuses were weighed and examined for external malformations and after appropriate processing 2/3 of them wee studied for gross visceral changes and the remainder for skeletal anomalies using standard procedures.

In expt 2 the effects of NaNa2 on the ETU-induced teratogenicty were assessed. An oral dose of 60 mg ETU/kg was folllowed immediately by an oral dose of 80, 100 or 120 mg NaNO2/kg. Control groups received either 60 mg ETU/kg or 100 or 120 mg NANO2/kg or distilled water alone.
Result: Expt 1: No treatment related adverse effects were observed on pregnancy or parturition in any test or control group.

Expt 2: Two of the 17 dams in the group given distilled water plus 120 mg/kg NaNO2 died. Periodic weight measurements during pregnancy failed to show any significant treatment related effects. No foetal effects were observed in rats dosed with 100 or 120 mg NaNO2/kg.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (2) valid with restrictions
22-JUL-2005 (103)

Species: rat Sex:
Strain: Long-Evans
Route of administration: drinking water
Exposure period: Day 0 gestation to day 20 of lactation

Frequency of treatment: daily
Doses: 0, 2.0, 3.0 g NaNO2/l
Control Group: yes, concurrent vehicle

Method: other: Effect of ion to the manifestation of nitrite toxicity.
Year: 1988
GLP: no

Remark: A series of three experiments was performed to evaluate the possible role of iron deficiency in the etiology of sodium nitrite-mediated developmental toxicity.

Result: Results of experiment I included the finding that fluid consumption was significantly reduced in treated animals over the period of gestation, but maternal gestational weight gain was not significantly affected. At birth, pup sex ratio and litter size were unaffected by treatment, but pup weights were significantly lower in treated than control litters. Birth weights were analyzed by 2-way ANOVA, examining the contributions of sex and treatment.

By the second week postpartum, pups not supplemented with iron developed severe microcytic anemia. These pups showed reduced postnatal weight-gain, and significantly increased mortality before day 20 postpartum. Hemoglobin, RBC counts, and MCV values were all significantly lower in treated than in control pups; iron supplementation ameliorated or eliminated all of these effects.

As in experiment I, treated experiment II females (both pregnant and unmated) consumed significantly less water than their respective controls. At the same time, weight gains over 22 days (the gestation period in the pregnant animals) were unaffected by treatment. Mean lactational weight gain in experiment II was significantly reduced in treated dams, relative to controls. As evaluated on postnatal day 15, treated dams showed significant reductions in MCV, hemoglobin, and plasma iron levels. RBCs were unchanged from control values. In unmated females, nitrite treatment did not affect hematological parameters or plasma iron levels.

Litter size and sex ratio were unaffected in this experiment. Birth weights were reduced in treated male and female pups, relative to controls, but these differences were not statistically significant. Postnatal pup growth was significantly depressed. By postnatal day 15, pups of treated dams were demonstrably anemic, and had significant reductions in MCV, RBC, and hemoglobin levels.

In experiment III, the pattern of results for maternal fluid consumption and weight gain during gestation and lactation was similar to that seen in the other two experiments. Litter size was significantly increased in sodium nitrite-exposed pups, and pup weights were significantly decreased. While the decrease in birth weight may have been an artifact of the larger mean litter size, the ANOVA used to evaluate these data is stated to have examined only sex and treatment as sources of variation in birth weight. Litter size was evidently not incorporated into the analysis.

As in the previous experiments, postnatal pup growth was adversely affected by sodium nitrite exposure, as were hematological variables. On gestation day 15, heart weights relative to body weights were found to be significantly increased in treated pups. Relative spleen weights of these same pups were significantly decreased. Liver iron content was significantly decreased, and liver copper content significantly increased following sodium nitrite exposure. Milk from sodium nitrite-treated dams was found to have a significantly lower iron content than the milk of control animals. The authors postulate that sodium nitrite exposed-dams suffered from iron deficiency, and hence produced iron-deficient milk, which in turn was responsible for the adverse effects observed on their offspring.

Test condition: In experiment I;
Pregnant Long-Evans rats were assigned to either control or treated conditions, with six animals in each group. Control animals were given plain tap water, while treated animals were given drinking water containing 3 g sodium nitrite/liter. Treatment began on gestation day 0, and continued throughout lactation. Litters were delivered normally, and culled to ten pups. Half the pups in each litter were given supplemental iron by i.p. injection, on postnatal days 0, 7, and 14. On each of postnatal days 7, 14, and 21, selected pups were sacrificed for hematological and histopathological analysis.

In experiment II;
Four groups of animals were delineated: ten pregnant controls, five virgin controls, 13 pregnant treated animals, and four virgin treated animals. Treated animals were given 2.0 g sodium nitrite/liter drinking water. All animals were sacrificed on postnatal day 15 for analysis of hematological parameters.

In experiment III;
Pregnant females were maintained throughout gestation and lactation on drinking water containing 0 (n=7) or 2.0 (n=8) g sodium nitrite/liter drinking water. All pups and dams were sacrificed for evaluation on postnatal day 15.

Test substance: Chemical name:Sodium nitrite (CAS No. 7632-00-0)

Conclusion: It appears that nitrite-consuming dams have a reduced capacity to transfer iron to their pups. The nitrite-associated toxicities in the pups are actually a result of an iron deficiency.

Reliability: (4) not assignable

19-JUL-2005 (148)

Species: rat Sex: female
Strain: Long-Evans
Route of administration: drinking water
Exposure period: Day 0 gestation to day 20 of lactation
Frequency of treatment: daily
Doses: 0, 0.5, 1.0, 2.0, 3.0 g NaNO2/l
Control Group: yes, concurrent vehicle

Method: other: Three studies in three methods are reported.
Year: 1987
GLP: no data

Remark: Postnatal endpoints were investigated in Long-Evans rats following exposure to sodium nitrite in drinking water during pregnancy and lactation.
Three related experiments were reported in a single paper: I - pilot dose-response; II - dose-response; and III - crossfostering.

Result: No effects on maternal gestational weight gain were found in experiments I or II; data on maternal weight gain were not presented for experiment III. In some instances, maternal fluid consumption was significantly reduced in treated animals as compared to controls: at both doses in experiment I, and in the high concentration group in experiment II. Treatment had no effect on litter size, sex ratio, or mean pup weights in any of the three experiments.

Postnatally, adverse effects became manifest for both dams and pups. In experiment I, maternal water consumption and weight gain in the treated groups were significantly depressed during the lactation period. A dose-response trend for reduced maternal body weight was also apparent in experiment II, although the differences from controls did not reach statistical significance. Fluid consumption in experiment II was not significantly decreased by treatment.

In experiment I, pup growth was significantly depressed, and pup mortality was increased, both in a dose-dependent fashion. Affected pups were described as "pale, weak, and in generally poor condition with distended bellies." In experiment II, only the high-concentration group pups showed significant growth impairment. Pups from the lowest concentration group were significantly heavier than controls on postnatal days 3 and 6. There was no postnatal pup mortality in experiment II

In experiment III, the strongest effects on postnatal weight were seen for pups exposed to sodium nitrite during both gestation and lactation. Pups exposed during lactation alone were also affected, although to a lesser degree. Pups exposed only during the prenatal period showed no significant differences from controls in postnatal body weights, although there was an apparent influence of prenatal exposure on pup growth between postnatal days 1 and 8.

Hematological parameters were significantly affected in treated animals in all three experiments. In experiment I, hemoglobin content and red blood cell counts (RBCs) were significantly decreased in a dose-dependent manner on postpartum days 9 and 16. Maximum corpuscular volume (MCV) was significantly reduced at both concentrations of sodium nitrite, when measured on postnatal day 16. MVCs were also reduced in treated, relative to control, animals on postnatal day 9, but the differences were not statistically significant. Methemoglobin levels were not significantly different from controls at either sodium nitrite concentration, or on either day.

Methemoglobin levels were not determined in experiment II, but hemoglobin levels for the high concentration group (2.0 g $NaNO_2$/liter) were significantly lower than controls

starting from postnatal day 7. RBCs for this group were significantly reduced relative to controls by postnatal day 9. Hematological effects were also seen in the lower concentration groups, but only at later timepoints. The most sensitive endpoint was MCV, which was significantly reduced, in a concentration-dependent fashion, at all three concentrations on postnatal day 16.

The cross-fostering experiment (experiment III) demonstrated that the observed anemia was predominantly postnatal in origin. The prenatal-exposure-only group, however, did show a significant reduction in MCV on postnatal day 21. Methemoglobin was not measured in this experiment.

Test condition: In experiment I, three groups of 8-10 pregnant females were given 0, 2.0, or 3.0 g sodium nitrite/liter drinking water. According to the study authors, these concentrations resulted in total sodium nitrite consumption, over 18 days of gestation, of 3.94 and 5.40 mg/g bw (219 and 300 mg/kg bw/day, respectively). Lactating dams given the same drinking water solutions had higher sodium nitrite intakes of 420 and 514 mg/kg/day. Litters were delivered normally, and culled to eight pups on postnatal day 1. Hematological parameters were determined on postnatal days 9 and 16.

In experiment II, four groups of 5-8 pregnant animals were given 0, 0.5, 1.0, or 2.0 g sodium nitrite/liter drinking water. These concentrations were reported to result in total sodium nitrite consumption, over 18 days of gestation, of 1.3, 2.14, and 3.6 mg/g bw (72.2, 119, 200 mg/kg bw/day, respectively). Litters were culled to ten pups on postnatal day 2, and blood samples were taken on postnatal days 7, 9, 13, 16, and 20.

In experiment III, pregnant females were given either plain tap water, or tap water containing sodium nitrite at a concentration of 2.0 g/liter. All pups were fostered at birth, such that four groups were created: control (prenatal)/control (postnatal); control (prenatal)/treated (postnatal); treated (prenatal)/control (postnatal); treated (prenatal)/treated (postnatal). Each litter consisted of ten pups, and there were 5-6 litters per group. Pups were sacrificed for hematological and histological assessment on postnatal days 7, 14, or 21.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Conclusion: Administration of 1g $NaNO_2$/Liter resulted in hematological effects but did not affect growth or mortality. $NaNO_2$ (0.5g/liter) was at or near the no observed effect level.

Reliability: (4) not assignable

22-JUL-2005 (149)

Species: rat Sex: male/female

Route of administration: oral feed

Exposure period: Treated diets were provided to F0 animals from ten weeks prior to mating, and continued until animals were terminated on the study. Subsequent to weaning, groups of 60-100 F1 animals were continued on the respective F0 diet.

Frequency of treatment: continuous

Doses: fed cured meat to which 0, 200, 1000, or 4000 mg/kg expressed as sodium nitrite had been added. After

processing the food, contents may have changed. See "remarks".
Control Group: other: One control group received casein as a protein source; another control group was given
NOAEL Maternal Toxity: >= 100 mg/kg bw
NOAEL Teratogenicity: >= 100 mg/kg bw
Result: see "remarks"

Method: other: see "remarks"
Year: 1984
GLP: no

Remark: Study protocol is somewhat similar to one generation feeding study. The spiked diet were processed supposedly to cause potential nitrosoamine formation in food.

A cohort of 70 male and 140 female rats was divided into groups and fed cured meat to which 0, 200, 1000, or 4000 mg sodium nitrite/kg had been added. One control group received casein as a protein source; another control group was given fresh chopped pork. Treated diets were provided to F0 animals from ten weeks prior to mating, and continued until animals were terminated on the study. Subsequent to weaning, groups of 60-100 F1 animals were continued on the respective F0 diet.

Following canning, autoclaving, and storage prior to experimental use, the treated diets were determined to have nitrite contents of 6, 47, or 580 mg/kg. Data on feed consumption were not provided in the paper, but the doses of sodium nitrite consumed in the form of treated diets have been estimated at 5, 25, or 100 mg/kg bw. No differences were noted between treated and control F0 animals in appearance or behavior, nor were effects noted on reproductive parameters such as pregnancy rate, litter size, mean pup weight, or pup survival. No morphological
abnormalities were observed in F1 offspring. There were no significant differences in the rate of postnatal mortality between treated and control pups.
Test substance: Chemical name: Potassium nitrite (CAS No. 7758-09-0)
Reliability: (4) not assignable
19-JUL-2005 (138)

Year: 1989

Remark: Another information of N-nitroso compounds

Recent investigations have suggested that drugs that are amines can undergo endogenous or exogenous nitrosation reactions to form N-nitroso compounds. These compounds have been extensively characterized in animal models as carcinogens, mutagens and teratogens. In order to examine the possible effects of exposure to nitrosatable drugs during gestation on pregnancy outcome, data were utilized from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke. Pregnancy outcomes for 6061 pregnancies in which the mother ingested a drug known to undergo nitrosation were compared with 6921 randomly sampled pregnancies without such

exposure. The major outcome factors of interest were birth defects, fetal, neonatal and infant death and birthweight. Our findings suggest that no significant increases in risk of fetal, neonatal and infant death or low birthweight were associated with nitrosatable drug exposure during pregnancy. However, the risk of a tumour in the offspring of exposed mothers was increased (relative risk, RR = 2.29; 95% Cl 0.99-5.26). Increases in relative risk of major malformations was also observed and this increase was greater when exposure during the first four months of pregnancy was examined separately (RR = 1.33; 1.11-1.58). There were specific individual malformations that were observed to have increased relative risks (for example: eye malformations, hydrocephaly, craniosynostosis and meningomyelocoele/meningocoele) but interpretation was difficult due to multiple comparisons and some of these observations were associated with wide confidence intervals. These types of adverse pregnancy outcomes were consistent with animal study outcomes.

Reliability: (4) not assignable
19-JUL-2005 (139)

Species: guinea pig Sex: female
Route of administration: drinking water

Year: 1968

Remark: Concentrations of 0, 300, 1000, 2000, 3000, 4000, 5000 ro 10000 mg/L (ppm) estimated to provide doses of 0, 110, 270, 940, 1110, 1190, 1490 or3520 mg/kg bw/day.

Result: Reproduction in the female was impaired by 30,000 ppm of KNO3 with reproductive performance only 8% of that of the controls. Fetal losses were 100% in females given 5000 and 10,000 ppm of KNO2. Reproduction was maintained at lower levels of treatment. Male fertility was apparently not impaired since conception took place at all levels of treatment. Food and water consumption and weight gains were normal except for a diminished rate of gain at 10,000 ppm of KNO2. Uterine and cervical inflammatory lesions and degenerative placental lesions were present in females in which the fetuses had been aborted, mummified, or absorbed.

Test condition: Guinea pigs were given KNO3 in doses ranging from 300 to 30,000 ppm in the water and KNO2 in amounts from 300 to 10,000 ppm for periods ranging from 100 to 240 days.

Test substance: Test Substance; Potassium nitrite (CAS No. 7758-09-0)

Reliability: (4) not assignable
19-JUL-2005 (165)

Species: mouse Sex:
Strain: C57BL
Route of administration: drinking water
Exposure period: Parents, after mating to 21 days post-natal. Offsprings from partulation to 21 days p-n.t
Frequency of treatment: continuous
Doses: 1 g/L
Control Group: yes, concurrent vehicle
Result: Aggression score increased

Method: other: Aggression test
Year: 1974

GLP: no

Remark: Five mated pairs of C57Bl6J mice were given sodium nitrite in their drinking water at a concentration of 1 g/L. Five additional pairs served as controls. When young were born, the adult males were removed. Litters were left with their dams for 21 days; treatment of the sodium nitrite exposed females was continued during this time. At weaning (21 days postnatal), 12 male offspring were chosen at random from each group and removed to isolation cages. Males from the sodium nitrite-treated group were continued on the 1 g sodium nitrite/L drinking water solution. Aggression testing was commenced following an eight week isolation period. Each animal was placed in a "fighting cage" with another animal for a period of ten minutes, once each week, for six weeks. Four of the sessions were with other males from the same group (treated or control), and two sessions were with males from the other group. The level of aggression was scored according to a predetermined scale having a maximum score of 20. Following this first round of aggression testing, the treated animals were switched to plain tap water, and after a break of two weeks, aggression testing was repeated.

For the first round of testing, aggression scores were significantly elevated for the treated animals. Aggression scores were higher for treated males paired with control males (mean=12.95), than for treated males paired with other treated males (mean=10.88). These differences disappeared following a two-week recovery period after withdrawal of sodium nitrite.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (3) invalid
Method is not popular. Reporting is poor and down graded.

19-JUL-2005 (72)

5.8.3 Toxicity to Reproduction, Other Studies

Remark:

Integrative Evaluation
There were no available data on the potential of sodium nitrite to cause reproductive toxicity in humans. Among the available animal studies, none were conducted under a standard multi-generation reproductive toxicity study protocol, and only a limited number included the treatment of both sexes during the mating period. None of the pair-based studies provided evidence for an effect of sodium nitrite on fertility, or on other reproductive parameters evaluated. A study of the effects of potassium nitrite in guinea pigs also reported no effect of treatment on fertility.

Two studies have provided some evidence of testicular changes at the histopathological level in male rats, but the observed effects could not be confidently attributed to sodium nitrite exposure. No evidence of testicular pathology was identified in animals subjected to a 3-day regimen of sodium nitrite injections.

No evidence for adverse effects of sodium nitrite on female reproduction was obtained from the studies reviewed. In addition to fertility, relevant endpoints addressed by at least one of these studies included: mean live litter size, pup birthweight and viability, post-delivery estrous cycle parameters, gross and histopathological evaluation of the ovaries and uterus, and timing of vaginal opening in exposed female offspring. There is some suggestion that sodium nitrite might affect milk production, as postnatal weight gain was reduced in the offspring of dams given approximately 245 mg sodium nitrite/kg/day during pregnancy and lactation. Alternatively, this might have been the indirect result of poor palatability of sodium nitrite-treated water leading to reduced water consumption (and hence to reduced milk production), or due to a direct effect on the pups of sodium nitrite secreted in the dams' milk.

A study on the effects of potassium nitrite in pregnant guinea pigs reported that fertility was retained, although fetal loss appeared to increase with increasing dose of potassium nitrite. It is not known whether the difference in results from the studies using sodium nitrite is due to the use of the potassium salt, or to the generally higher doses given, or to a greater sensitivity of the guinea pig as compared to the rat and mouse
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Van Logten, M., Tonkelaar, R., Kroes, R., Berkvens, J., Esch, G. (1972). Long-term experiment with canned meat treated with sodium nitrite and glucono-gamma-lactone in rats. Fd. Cosmet. Toxicol. 10, 475-488

NTP (National Toxicology Program) (1990). Final Report on the Reproductive Toxicity of Sodium Nitrite (CAS No. 7632-00-0). Research Triangle Park, NC: National Toxicology Program. Report No.: NTP 90-266

Olsen, P., Gry, J., Knudsen, I., Meyer, O., Poulsen, E. (1984). Animal feeding study with nitrite treated meat. IARC Scientific Publication 57, 667-675

Vorhees, C. V., Butcher, R. E., Brunner, R. L., Wootten, V. (1984). Developmental toxicity and psychotoxicity of sodium nitrite in rats. Food Chem. Toxicol. 22, 1-6

Conclusion:

-Male Reproductive Toxicity
There were no available data on the potential of sodium nitrite to cause male reproductive toxicity in humans. Among the available animal studies, none were conducted under a standard multi-generation reproductive toxicity study protocol, and only a limited number included the treatment of both sexes during the mating period. None of the pair-based studies provided evidence for an effect of sodium nitrite on fertility, or on other reproductive parameters evaluated. Two studies provided some evidence of testicular changes at the histopathological level in male rats, but the observed effects could not be confidently attributed to sodium nitrite exposure. No evidence of testicular pathology was identified in animals subjected to a 3-day regimen of sodium nitrite injections.

-Female Reproductive Toxicity
There were no available data on the potential of sodium nitrite to cause female reproductive toxicity in humans. With regard to the available animal toxicity studies, no evidence for adverse effects of sodium nitrite on female reproduction was obtained. In addition to fertility, relevant endpoints addressed by at least one study included: mean live litter size, pup birthweight and viability, post-delivery estrous cycle parameters, gross and histopathological evaluation of the ovaries and uterus, and timing of vaginal opening in exposed female offspring. There is some suggestion that sodium nitrite might affect milk production, as postnatal weight gain was reduced in the offspring of dams given approximately 245 mg sodium nitrite/kg/day during pregnancy and lactation. Alternatively, this might have been the indirect result of poor palatability of sodium nitrite-treated water leading to reduced water consumption (and hence to reduced milk production), or due to a direct effect on the pups of sodium nitrite secreted in the dams' milk.

A study on the effects of potassium nitrite in pregnant guinea pigs reported that fertility was retained, although fetal loss appeared to increase with increasing dose of potassium nitrite. It is not known whether the difference from the studies using sodium nitrite is due to the use of the potassium salt, to the generally higher doses given, or to a greater sensitivity of the guinea pig as compared to the rat and mous

Reliability: (2) valid with restrictions
22-JUL-2005 (29)

Remark: There is evidence for placental transfer of sodium nitrite to rat and mouse fetuses. Sodium nitrite administered by oral intubation to pregnant mice (0.5 mg/mouse per day) did not cause fetal mortality or resorption or changes in embryo weight or incidence of skeletal malformation. The treatment did stimulate fetal hepatic erythropoiesis, probably related to fetal methemoglobinemia.

Nitrite and methemoglobin were detected in fetal blood after pregnant rats were given a single dose of 2.5 to 5.0 mg sodium nitrite/kg body weight.
References cited:

Anderson, L. M., Giner-Sorolla, A., Haller, I. M., Budinger, J. M. (1985). Effects of cimetidine, nitrite, cimetidine plus nitrite, and nitrosocimetidine on tumors in mice following transplacental plus chronic lifetime exposure. Cancer Res. 45, 3561-3566

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Vorhees, C. V., Butcher, R. E., Brunner, R. L., Wootten, V. (1984). Developmental toxicity and psychotoxicity of sodium nitrite in rats. Food Chem. Toxicol. 22, 1-6

Reliability: (2) valid with restrictions
Reliable evaluation

22-JUL-2005 (134)

Type:	other: reproductive
In Vitro/in vivo:	In vivo
Species:	mouse
Strain:	CD-1 Sex: male/female
Route of administration:	drinking water
Doses:	0, 0.06, 0.12, 0.24% w/v. (approximate doses of 125, 260, 425 mg/kg/day)
Control Group:	yes, concurrent vehicle

Method: other: RACB protocol
Year: 1997

Remark: Swiss CD-1 mice were subjected to the National Toxicology Program's (NTP) Reproductive Assessment by Continuous Breeding (RACB) protocol. Forty mating pairs served as controls, and 20 mating pairs were assigned to each dose group. Sodium nitrite was given in the drinking water at concentrations of 0, 0.06, 0.12, and 0.24% w/v. As calculated from water consumption and body weight data, these concentrations resulted in approximate doses of 125, 260, and 425 mg/kg/day.

Nine animals died during the 14-week breeding period: 3, 4, 0, and 1, in the control through high dose groups, respectively. These deaths were not considered to be treatment related. The experiment revealed no treatment-related effects on the mean number of litters per pair, cumulative days to deliver each litter, mean litter size, or pup birth-weight or viability.

The males were removed after 14 weeks of continuous breeding, and the females allowed to deliver and rear their last litters. Exposure of dams was continued through the lactation period. Postnatal mortality was not affected by treatment, but weights of the high-dose group pups were reduced by 12-17% from postnatal day 7 to postnatal day 21. The authors were unsure whether this was a direct effect of sodium nitrite exposure, or an indirect result of reduced maternal water consumption, and hence reduced milk production.

Only the high-dose and control group pups were carried through to breed a second generation. Following weaning, these (F1) animals were exposed to sodium nitrite in their drinking water, at the same dose their parents had received. Non-siblings were mated within their treatment group at approximately 74 days of age, and the females allowed to carry and deliver their first litters. No effects of treatment on fertility or reproductive success were noted. Post-delivery estrous cycle and sperm parameters were not altered by treatment. The number, weight and viability of F2 young were also unaffected, as were F1 terminal body and organ weights.

Result: No treatment-related effects on: mean number of litters/pair, cumulative days to deliver each litter, mean litter size, birth weight, or pup viability.

No effect on postnatal mortality of pups exposed during lactation.

Reduced body weights of nursing pups.

No effects on reproduction of high-dose F1 animals, or on viability and weight of F2 offspring.

No effects on post-delivery estrous cycle or sperm parameters.

9 deaths during the study period were not considered to be treatment related.

No effect on F1 terminal body and organ weights.

Test condition: Continuous breeding protocol.

Swiss CD-1 mice.
20 mated pairs/dose group; 40 mated pairs as controls.

Sodium nitrite in drinking water to doses of 0, 125, 260, and 425 mg/kg/day.

High dose and control pups were continued on to breed a 2d

generation.
Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Conclusion: Affected sex: Unclear
Study confounder: None
NOAEL reproductive toxicity: 0.24% (425 mg/kg/day)
NOAEL general toxicity: 0.12% (260 mg/kg/day)
F1 more sensitive than F0?: No
Postnatal toxicity: Unclear
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
25-JUL-2005 (35) (133)

Year: 1996

Remark: A cohort of 70 male and 140 female rats was divided into groups and fed cured meat to which 0, 200, 1000, or 4000 mg sodium nitrite/kg had been added. One control group received casein as a protein source; another control group was given fresh chopped pork. Treated diets were provided to F0 animals from ten weeks prior to mating until animals were terminated on the study. Subsequent to weaning, groups of 60 - 100 F1 animals were continued on the respective F0 diet.

Following canning, autoclaving, and storage prior to experimental use, the treated diets were determined to have nitrite contents of 6, 47, 580 mg/kg. Data on feed consumption were not provided in the paper, but the doses of sodium nitrite consumed in the form of treated diets have been estimated at 5, 25, or 100 mg/kg bw. No differences were noted between treated and control F0 animals in appearance or behavior. Nor were effects noted on reproductive parameters such as pregnancy rate, litter size, mean pup weight, or pup survival. No morphological abnormalities were observed in F1 offspring. There were no significant differences in the rate of postnatal mortality between treated and control pups.
Result: No effects of treatment noted on pregnancy rate, litter size, mean pup weight, pup survival, or malformation frequency.

No differences noted in appearance of behavior of treated F0 animals.
Test condition: 70 male and 140 female rats (total) fed on meat containing sodium nitrite to doses of 0, 5, 25, or 100 mg/kg bw.

Diets given from 10 weeks prior to mating, throughout gestation and lactation, and to weaned F1 offspring.
Reliability: (4) not assignable
22-JUL-2005 (138) (197)

Year: 1963

Remark: In a multigeneration study of teratogenesis and transplacental carcinogenesis, groups of 30 to 37 rats were given sodium nitrite in drinking water to achieve doses of 0 or 100 mg/kg bw. The study was continued for three generations, with F1 and F2 animals being fed 500 mg diethyamine/kg bw, as well as sodium nitrite. Mean lifespan

was lower for treated than control animals, for all three generations (730 days for controls; and 630, 625, and 610 days for nitrate exposed animals of successive generations), but the authors concluded that nitrite alone, or in combination with diethylamine, at the doses used, had no carcinogenic or teratogenic effects. Reproductive parameters were only noted in passing, but it is noted that 15 matings of the P0 generation resulted in 94 offspring (an average of 6.3 pups/litter), and that 22 matings of the F1 generation resulted in 149 offspring (an average of 6.7 pups/litter).

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)

Reliability: (4) not assignable

19-JUL-2005 (50)

Type:	other: a study of transplacental and chronic carcinogencity
In Vitro/in vivo:	In vivo
Species:	mouse
Strain:	other: see TC Sex:
Route of administration:	drinking water
Exposure period:	until natural death
Frequency of treatment:	ad libitum
Duration of test:	until natural death
Doses:	0, 0.184, or 1.84 g/L (equivalent to to 0, 30.7 or 310 mg/kg bw/day)
Control Group:	yes, concurrent vehicle

Year: 1985

Remark: The authors state that these doses were chosen as equivalent to common human exposure levels (the low dose), and to a level ten times higher than that amount (the high dose). Other treatments investigated included: cimetidine (CM), nitrocimetidine (NCM), and CM plus nitrite.

The authors state that data were collected on: the number of females becoming pregnant, the average time from introduction of the male until birth of a litter, average gestational weight gain, number of stillborn litters, mean litter sizes at birth and at weaning, and the numbers of male and female offspring. While the data are not provided in the paper, it is stated there were no significant differences between groups for these parameters. Twenty litters were born to the control group, and 14 and 15 litters to the low and high-dose sodium nitrite-treated groups, respectively. Sodium nitrite treatment did not affect survival times or body weights in this experiment. Common non-neoplastic lesions included cystic seminal vesicles and preputial glands in males, and cystic uteri, ovaries, and mammary glands in females. Incidences of these lesions were not correlated with chemical treatment.

Result: No significant changes in measures of fertility, or offspring viability or sex ratio.

No effect on survival times, body weights, or gestational weight gain.

Incidences of non-neoplastic lesions were not correlated with chemical treatment.

Test condition: In a study of transplacental and chronic carcinogencity,

treatment was initiated in 7 to 8-week old C57BL/6 female, and BALB/c male mice. Sodium nitrite was provided in drinking water, at concentrations of 0, 0.184, or 1.84 g/L.

Mice were bred after two weeks of treatment, producing a generation of B6CF1 progeny.

These progeny were weaned at four weeks postnatal age, and continued on treated drinking water until their natural deaths. (Animals were bred after 2 weeks; P0 dams and F1 progeny continued on treatment.) Treatment of the dams was also continued.

The concentrations of sodium nitrite used were determined to have provided doses of 0, 30.7 mg/kg, or 0.31 g/kg, respectively.

Test substance: Chemical name: Sodium Nitrite (CAS No. 7632-00-0)
Conclusion: None of NaNO2 had large effects on reproductive parameters, survival, or incidence of non-neoplastic lesions.
Reliability: (2) valid with restrictions
22-JUL-2005 (9)

Type: other: reproductive and carconogenicity
In Vitro/in vivo: In vivo
Species: rat
Strain: Wistar Sex: male/female
Route of administration: other: diet containing 40% meat
Exposure period: 29 months (max.)
Frequency of treatment: diet
Duration of test: 29 months (max.)
Doses: 0, 0.02, 0.5% in diet
Control Group: yes
Result: No effect to reproductive toxicity and carcinogenicity

Year: 1972

Remark: -Female Reproductive Toxicity

The authors fed groups of 30 male and 30 female rats on a cured meat diet containing 0, 0.02 or 0.5% sodium nitrite. The 40% meat diet, in the absence of sodium nitrite, resulted in mean body weights which were higher than those of standard-diet controls. Meat with added sodium nitrite resulted in lower body weights. These changes appear to have been dose-related, and were statistically significant in many cases. Mean feed consumption, however, was not reported to be different between any of the meat-fed groups. There were no differences between groups in mortality. Organs evaluated at the gross and histopathological levels included the ovaries and uterus. No treatment-related changes were found.

-Male Reproductive Toxicity

The authors fed groups of 30 male and 30 female rats on a cured meat diet containing 0, 0.02 or 0.5% sodium nitrite. The study was terminated after 29 months. The 40% meat diet, in the absence of sodium nitrite, resulted in mean body weights which were higher than those of standard-diet

controls. Meat with added sodium nitrite resulted in lower body weights.
These changes appear to have been dose-related, and were statistically significant in many cases. Mean feed consumption, however, was not reported to be different between any of the meat-fed groups. There were no differences between groups in mortality. Organs evaluated at the gross and histopathological levels included the testes and prostate. No treatment-related changes were found.

Result: No treatment-related changes in gross or histological appearance of testes and prostate, or ovaries and uterus.

Reductions in mean body weights appeared to be dose-related.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
22-JUL-2005 (187)

In Vitro/in vivo: In vivo
Species: rat
Strain: Fischer 344 Sex: male
Route of administration: i.p.
Exposure period: 3 days
Frequency of treatment: every 3 hours for 3 days.
Doses: 15 mg/kg

Year: 1981

Result: At sacrifice 1 hr following final injection, no evidence of histopathological damage to testes.

Treatment supported a steady MetHb level of 2-40%.

At sacrifice 1 hr following final injection, no evidence of histopathological damage to brain or liver.

This regimen supported a steady MetHb level of 2-40%.
Sodium nitrite-treated animals were sacrificed one hour following the final injection, and histopathological examinations were made of the brain, liver, and testes. No evidence of pathology was found.

Test condition: male rats given 15 mg sodium nitrite/kg bw, i.p., every 3 hours for 3 days.

In a study of the effects of nitrobenzene (NB) exposure on male Fischer-344 rats, sodium nitrite was used as a positive control for induction of methemoglobinemia. Animals exposed to a single dose of NB developed hepatic and testicular lesions, as well as elevated levels of methemoglobin. In order to distinguish the effects of methemoglobinemia from other potential mechanisms of NB toxicity, five additional male rats were given sodium nitrite by i.p. injection, at a dose of 15 mg/kg, every three hours, for three days.

Test substance: Chemical name: Sodium nitrite (CAS No. 7632-00-0)
Reliability: (4) not assignable
22-JUL-2005 (20)

5.9 Specific Investigations

Endpoint: other: CANCER

Remark: No adequate epidemiology studies of sodium nitrite and human cancer were found in the literature. A study was reported no associations between nitrite intake and risk of laryngeal or oral cancer. In epidemiological studies, it was reported a direct association between nitrite intake and stomach cancer risk. As comparing subjects with low methionine (<1.5 mg/day) and low nitrite (<2.7 mg/day) intake with subjects with high methionine (>1.9 mg/day) and high nitrite (>2.7 mg/day) intake, it was reported an association between nitrite intake and stomach cancer risk. Another study reported a significantly elevated risk for stomach cancer for users of well water compared with those who used central water supplies in Germany. The study did not report measurements of nitrate in the water but assumed that well water contained considerable amounts of nitrate. An increased death rate from gastric cancer among the residents of the English town of Worsop was thought to be related to the high concentration of nitrate (90 mg/L) in the public water supply. In Columbia, where gastric cancer is common, the high intake of nitrate is reflected in the high urinary excretion rate of nitrate. Patients with precancerous gastric lesions had high nitrite concentrations in their gastric juice.

A study demonstrated that nitrate intake resulted in a significant rise in mean salivary nitrate and nitrite concentrations and that N-nitrosodimethylamine and N-nitrosopiperidine were detected in the urine samples.

Reliability: (2) valid with restrictions
Reliable evaluation

22-JUL-2005 (134)

Endpoint: other: DEVELOPMENTAL TOXICITY

Remark: Auxiliary Information: THE INTEGRATED EVALUATION BY CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY (2000)
Two epidemiological studies conducted in Australia examined the possible relationship between birth defects and high-nitrate concentrations in drinking water. Statistically significant results from these studies indicated that elevated risk for congenital malformations, particularly malformations of the CNS, was associated with maternal consumption of drinking water containing sodium nitrate at concentrations in excess of 5 ppm. Findings from a case-control study conducted in Canada were far less striking than the Australian data. Nitrate content of the water was generally associated with source (spring, tap, or well), with well water tending to have the highest nitrate content (26.03 ppm at the 87.5 percentile). For women who drank spring or tap water during pregnancy, the nitrate level, per se, was not shown to be associated with increased risk for birth defects. A moderate, non-statistically significant increase in risk for CNS malformations was associated with maternal consumption of well water having nitrate levels of 26 ppm or more.

N-nitroso compounds, which are known to be teratogenic and/or carcinogenic in experimental animals, have also been implicated as potentially responsible for the increased frequency of major malformations observed in offspring of women given nitrosatable drugs during pregnancy. Nitrite is a precursor of N-nitroso compounds, but it has not been directly established that the necessary reactions occur in toxicologically significant quantities in vivo, under normal food intake conditions. However, the association between frequent consumption of hot dogs and/or other cured meat products during pregnancy, and an increased risk for childhood brain tumors in offspring suggests that such reactions might occur.

Conclusion: Epidemiological studies support an association between maternal consumption of nitrite containing cured meats during pregnancy and childhood cancers, particularly brain tumors. While these data cannot implicate sodium nitrite in isolation from other dietary components, they are consistent with the results of transplacental carcinogenicity studies in which sodium nitrite, in combination with amine or amide precursors of N-nitroso compounds, were given to experimental animals.

Reliability: (2) valid with restrictions
Reliable evaluation

22-JUL-2005 (29)

Endpoint: other: ADI (2003)

Remark: ADI = 0.07 mg/NO2/kg/day (JECFA) (2003)

The Committee concluded that the pivotal observed toxic effects of nitrate are consequent on its conversion to nitrite in vivo. The Committee at its present meeting established an ADI of 0-0.07 mg/kg bw for nitrite. As the new data on nitrite would not provide a basis for a significant change in the previous ADI for nitrate, the Committee retained the ADI of 0-5 mg/kg bw expressed as sodium nitrate, or 0-3.7 mg/kg bw, expressed as nitrate ion, established at its forty-fourth meeting.

-RATIONALE
The few new studies on the toxicokinetics and metabolism of nitrate in animals that have become available since the forty-fourth meeting of the Committee confirm that the rat is not a good surrogate species for humans in this respect, as it does not show salivary transport of nitrate and therefore has limited conversion of nitrate to nitrite.

In a study of the conversion of nitrate to nitrite in humans, in which sodium nitrate was administered in drinking-water at a single dose of 7.3 mg/kg bw, expressed as nitrate ion, neither blood pressure nor methaemoglobin concentration was affected. The nitrite concentration of the gastric juice was approximately six times higher after administration of nitrate in combination with pretreatment with omeprazole at 40 mg/day (which increased the gastric pH) than after nitrate alone. Nitrate was absorbed rapidly, the concentration in plasma increasing within 10 min, and

the half-life of nitrate in plasma was about 6.5 h; about 70% of the dose was excreted in urine within 10 h of dosing. The plasma concentration of nitrite did not change after nitrate administration. About 8% of the total nitrate administered was converted to nitrite in saliva, as found in other studies. The Committee at its forty-fourth meeting concluded that the range of nitrate conversion is 5-7% for normal individuals and 20% for individuals with a high rate of conversion.

The results of studies in humans on the potential of a high nitrate intake to cause methaemoglobinaemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinaemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinaemia. No increase in methaemoglobin concentration was seen in volunteers after a single administration of sodium nitrate in drinking-water providing a dose of 7.3 mg/kg bw, expressed as nitrate ion.

A study in humans showed that nitrate in vegetable matrices and from other sources, such as drinking-water, is almost totally bioavailable.

As nitrate shares a common transport mechanism with iodide, studies were conducted to determine whether nitrate affects thyroid function. A 28-day study with volunteers given sodium nitrate in drinking-water at a concentration equivalent to 15 mg/kg bw per day (11 mg/kg bw per day expressed as nitrate ion) showed no effects on thyroid function and no increase in the per cent of methaemoglobinaemia. A 90-day study of toxicity in rats showed that sodium nitrate at a dose of 50 mg/kg bw per day did not affect the thyroid or the zona glomerulosa of the adrenals.

In studies in humans, consumption of drinking-water containing sodium nitrate at a concentration of 2800 mg/l concomitantly with volatile N-nitrosatable amines in the diet (in cod, salmon or shrimp) led to a two- to threefold increase in urinary excretion of N-nitrosodimethylamine and N-nitrosopiperidine.

Several studies were reviewed on the effect of administration of nitrate on the release of nitric oxide at the junction of the oesophagus and the stomach in humans, which, it was speculated, might be associated with an increased incidence of cancer at this site. However, no such association has been observed in epidemiological studies.

A number of epidemiological studies have been published since the forty-fourth meeting of the Committee on the relationship between nitrate intake and cancer risk. At its present meeting, the Committee ranked the study designs according to their capacity to provide evidence of a relationship. In the descriptions below, relative risk estimates are given for those studies in which levels of intake of nitrate were provided.

Six ecological (correlation) studies were reported on nitrate in drinking-water and mortality from or incidence of cancer. Elevated risks were found for prostate cancer and for brain tumours (each in one study), but the results of six studies on gastric cancer were conflicting. The results of ecological studies (in which populations are the units of measurement) cannot be extrapolated to the individual level. Furthermore, most of the ecological studies were based on limited data on nitrate concentrations and on cancer mortality rates (rather than incidence rates), and none took an induction period for cancer into account.

Three of the studies were cross-sectional, involving measurement of, e.g., salivary nitrate in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore these studies cannot contribute to a causal interpretation of the results of studies of nitrate intake and cancer risk.

Seven case-control studies on nitrate in drinking-water and/or food and cancers at various sites were reviewed. In the studies on nitrate in drinking-water, conflicting results were reported with regard to an association with non-Hodgkin lymphoma, and no association was found with brain tumours. In the studies on dietary nitrate, no association was found with oral, oesophageal, gastric or testicular cancer. No other cancer sites have been studied.

Three prospective cohort studies have been conducted on nitrate intake and cancer risk. A cohort study in the Netherlands, with 6 years of follow-up, found no significant association between the incidence of gastric cancer and intake of nitrate from food or drinking-water, with relative risks for increasing quintiles of total nitrate intake of 1.0 (reference quintile), 1.2, 0.7, 0.9 and 0.9 for mean intakes of 60, 85, 100, 120 and 180 mg/day, respectively. Neither the relative risks nor the trend across relative risks was significant. A further analysis of the effect of nitrate within tertiles of vitamin C intake also did not reveal a positive association between nitrate intake and gastric cancer. A Finnish cohort study on dietary nitrate, with 24 years of follow-up, reported no association with the risks for tumours of the stomach, colorectum or head and neck. The average nitrate intake in this cohort was reported to be 77 mg/day. A cohort study in Iowa, USA, with 11 years of follow-up, revealed no consistent association between intake of nitrate from drinking-water and the risks for cancers at many sites, and an inverse association was reported with cancers of the uterus and rectum. Positive associations with nitrate intake were observed only for cancers of the ovary and urinary bladder, although it was not possible to determine whether other factors in drinking-water were responsible for these associations. In addition, no evidence of a dose-response relationship was found for any of the cancer sites addressed in the study in Iowa. The cohort studies included control for various potential confounders, such as intake of vegetables, age and

smoking.

Overall, the epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. These data, combined with the results of the epidemiological studies considered by the Committee at its forty-fourth meeting, do not provide evidence that nitrate is carcinogenic to humans.

A number of studies were performed to determine whether there are associations between nitrate intake in drinking-water and insulin-dependent diabetes mellitus, neural tube defects or sudden infant death syndrome. In none of these studies was a hypothesis proposed for the mechanism of an association. Two studies were conducted on the incidence of insulin-dependent diabetes mellitus and nitrate intake via drinking-water. One study in Yorkshire, United Kingdom, suggested a positive association, but the authors considered that the finding required confirmation. A study in the Netherlands with a larger number of subjects did not show a positive association. The two studies on nitrate intake and neural tube defects also showed no association. In a recent ecological study in Sweden, a correlation was reported between the nitrate concentration in drinking-water and the occurrence of sudden infant death syndrome; however, no confounding factors were taken into account. The Committee considered that it would be premature to include these observations in its safety assessment.

Reliability: (2) valid with restrictions
Reliable evaluation. Based on cumulative information and the newest.

22-JUL-2005 (97)

Endpoint: other: Special studies, Effects on adrenal and thyroid glands
Species: rat
Strain: Wistar Sex: male
Result: See "remark".

Year: 1996

Remark: -Effects on adrenal and thyroid glands

Hypertrophy of the zona glomerulosa of the adrenals of rats was reported after administration of low doses of nitrite for 90 days; the effect was considered to be due to its conversion to nitrate. A study was therefore conducted to compare the effects of nitrate and nitrite on the zona glomerulosa. Three groups of 10 male Wistar rats were given drinking-water containing potassium chloride (control), potassium nitrite or potassium nitrate at a concentration of 36 mmol/l for 90 days. The body-weight gain of rats given nitrite or nitrate was slightly slower than that of controls, but no differences in food intake per kilogram body weight were observed between the three groups. The water intake of the group given nitrite was statistically significantly lower than that of the other two groups. The rats receiving nitrite appeared cyanotic during the first month of treatment but not thereafter, perhaps because the water intake was greater during the first month. At the end

of the observation period, the concentrations of methaemoglobin and nitrite in blood were significantly increased; the nitrate concentration in plasma in the groups given nitrite or nitrate groups were similar but were higher than those of controls. Treatment with nitrite or nitrate had no consistent effect on the concentrations of thyroxin, free thyroxin, thyroid stimulating hormones, adrenocorticotrophic hormone, corticosterone or aldosterone in blood. Microscopic examination revealed slight hypertrophy of the adrenal zona glomerulosa in all rats given nitrite and minimal hypertrophy in 2/10 rats given nitrate. The results of morphometric analyses of the adrenals were in line with those of microscopic examination. In the rats given nitrite, the fraction of the surface area of the zona glomerulosa in median sections was significantly greater than that in the controls or rats given nitrate. The minimal hypertrophy of the adrenal zona glomerulosa observed occasionally in rats given nitrate was barely detectable by morphometric analysis. It was concluded that nitrate ion does not play a role in the etiology of hypertrophy of the zona glomerulosa of the adrenal glands in rats.
Auxiliary information: THE INTEGRATED EVALUATION BY JECFA (2003)
JECFA (FAO/WHO Joint Expert Committee on Food Additives) (2003). Food Additives Series 50 has determined that nitrate ion does not play a role in the etiology of hypertrophy of the zona glomerulosa of the adrenal glands in rats (Boink et al., 1996). Based upon the determination, ADI was revised. This paper confirmed the determination.

This conclusion implies that safety evaluation should not be derived from the NOEL for minimal hypertrophy of the adrenal zona glomerulosa, used by the committee at its forty-forth meeting, but on NOELs for other end-points.

Reliability: (2) valid with restrictions
Based on a comprehensive study. Lately revised.

22-JUL-2005 (97)

Endpoint: other: ADI (1995)

Year: 2003

Remark: ADI = 0.06 mg/NO2/kg/day (JECFA) (1995)

Nitrite (and potential endogenous formation of N-nitroso compounds)
Nitrite was reviewed at the sixth, eighth, seventeenth and twentieth meetings of the Committee. At its sixth meeting, the Committee allocated an ADI of 0-0.4 mg per kg of body weight to this substance, expressed as sodium nitrite. This ADI was based on a marginal reduction in body-weight gain at a dose level of 100 mg per kg of body weight per day in a long-term study in rats. At its seventeenth meeting, the Committee lowered the ADI to 0-0.2 mg sodium nitrite per kg of body weight and made it temporary. At that time, the Committee used a safety factor higher than normal (500) because a marginal effect level was considered and there was a possibility of the endogenous formation of N-nitroso compounds from the nitrite and N-nitrosatable compounds

present together in food and the gastrointestinal tract. At its twentieth meeting, the Committee considered the reports of a WHO task group and of a working group of the International Agency for Research on Cancer on N-nitroso compounds, but concluded that they did not provide sufficient evidence to revise the temporary status of the ADI. Since the previous evaluation of nitrite, numerous toxicological and epidemiological data have become available.

The toxic effects of nitrite are of the following three types: the formation of methaemoglobin; hypertrophy of the adrenal zona glomerulosa in rats; and genotoxicity.

Methaemoglobinaemia is seen particularly after acute and subacute exposure. However, it is not the sole determinant of the NOEL. In a 2-year oral toxicity study in rats, the NOEL was 6.7 mg nitrite per kg of body weight per day (67 mg/l of drinking-water per day), expressed as nitrite ion. At the next higher dose level of 67 mg nitrite per kg of body weight, methaemoglobin accounted for 5 % of total haemoglobin; in addition, dilatation of coronary arteries and of the bronchi with infiltration of lymphocytes and alveolar hyperinflation were also seen. Methaemoglobin is particularly important where it exceeds 10 % of total haemoglobin, leading to toxic effects such as cyanosis. Young infants (below the age of 3 months) seem especially vulnerable to methaemoglobin. There is also evidence that fetal haemoglobin is more readily oxidized to methaemoglobin. and that in the neonate methaemoglobin reductase is less effective in the reduction of methaemoglobin to normal haemoglobin.

In a 90-day toxicity study in Wistar rats, the incidence and degree of hypertrophy of the adrenal zona glomerulosa observed at a dose level of 5.4 mg per kg of body weight per day, expressed as nitrite ion, were not significantly different from those among controls, whereas at higher dose levels the hypertrophy was both significant and dose-related.

In another 90-day toxicity study carried out by other investigators with a different Wistar substrain, slight hypertrophy of the adrenal zona glomerulosa was seen from 28 days onwards, but only at dose levels three times as high. The NOEL for hypertrophy in these studies was 5.4 mg per kg of body weight per day, expressed as nitrite ion.

Nitrite both with and without nitrosatable precursors was found to be genotoxic in several in vitro and in vivo test systems. However, DNA repair was not affected by nitrite.

Carcinogenicity studies with nitrite were negative, with the exception of those in which extremely high doses of both nitrite and nitrosatable precursors were administered. In addition, there was no evidence for an association between nitrite and nitrate exposure in humans and the risk of cancer. The Committee noted that few epidemiological studies were available in which cancers other than gastric cancer were investigated.

Although it has been shown in several controlled laboratory studies that, when both nitrite and N-nitrosatable compounds are present together at high levels, N-nitroso compounds are formed endogenously, there are quantitative data only on those N-nitroso compounds which are readily formed endogenously, such as N-nitrosoproline, which is not carcinogenic. As there was no quantitative evidence of the endogenous formation of carcinogenic N-nitroso compounds at intake levels of nitrite and nitrosatable precursors achievable in the diet, a quantitative risk assessment of nitrite on the basis of endogenously formed N-nitroso compounds was not considered to be appropriate. The safety evaluationwas therefore based on the toxicity studies on nitrite.

As previously mentioned, the NOEL was 5.4 mg per kg of body weight per day (expressed as nitrite ion) in 90-day toxicity studies in rats in which hypertrophy of the adrenal zona glomerulosa was observed and 6.7 mg per kg of body weight per day (expressed as nitrite ion) in a 2-year toxicity study in rats in which toxic effects in the heart and lungs were observed. On the basis of these results and a safety factor of 100, the Committee allocated an ADI of 0-0.06 mg per kg of body weight to nitrite, expressed as nitrite ion. This ADI applies to all sources of intake. Nitrite should not be used as an additive in food for infants below the age

of 3 months. The ADI does not apply to such infants.

A toxicological monograph summarizing both relevant information from the previous monograph and the information that has become available since the previous evaluation was prepared. The existing tentative specifications for potassium nitrite and sodium nitrite were revised, and the "tentative" designation was deleted.

Reliability: (4) not assignable
22-JUL-2005 (96)

5.10 Exposure Experience

Type of experience: other: NTP assessment

Remark: Auxiliary information: THE INTEGRATED EVALUATION BY NTP (2001)
Humans
In humans, sodium nitrite causes smooth muscle relaxation, methemoglobinemia, and cyanosis. Fatal poisonings of infants
resulting from ingestion of nitrates in water or spinach have been recorded. Longterm ingestion of water containing high levels of nitrate may increase the risk of gastric cancer. However, prospective cohort study did not support an
association between the intake of nitrate and nitrite and gastric cancer risk. The LD50 value for sodium nitrite has been estimated to be about 1 g in adults; a 17-year-old woman died after taking a single 1-g tablet. Fatal methemoglobinemia was reported after ingestion of a

laxative
solution contaminated with 15 g/L sodium nitrite.
Reliability: (2) valid with restrictions
19-OCT-2004 (134)

Type of experience: other: National Institute of Public Health and Environmental Hygiene, Netherlands.

Remark: Auxiliary Information: National Institute of Public Health and Environmental Hygiene, Netherlands (1986)
The lowest acute oral lethal dose of nitrite reported for man varied from 27-255 mg/kg b.w., in which the lowest figures applied for children and elderly people. Nitrite is also more toxic to young infants (3 months) than adults giving rise to relatively higher methaemoglobin levels in the blood. The lowest toxic dose reported was 1 mg NO2/kg b.w., whereas in another study 0.5-5 mg NO2/kg b.w. did not cause any toxic effect.

No information on short-term or long-term toxicity in man is available except for the epidemiological studies on the relation between nitrate or nitrite intake and tumour incidences.

No firm relationship was found between the estimated nitrate uptake and gastric cancer in normal healthy individuals, although in several studies an association was indicated. In occupational situations no increased gastric cancer incidences were found. The populations studied were,
however, small.

Individuals with stomach lesions or disorders, especially those with atrophic gastitis, PA-patients and persons on cimetidine and other antacid medication present special risk
groups in which a correlation between nitrate or nitrite intake and incidence of gastric cancer cannot be excluded.

Both for healthy individuals and those of the special risk groups, the possible correlation between the nitrate or nitrite intake and gastric cancer is based on the formation of carcinogenic N-nitroso compounds. Combining the facts that for N-nitroso formation also amine or amide have to be present in relevant concentrations and that both nitrate and nitrite were not carcinogenic in animal studies under well-defined conditions, it can be concluded that nitrate and nitrite itself have no carcinogenic properties.
Reliability: (2) valid with restrictions
22-JUL-2005 (129)

Type of experience: Direct observation, poisoning incidents

Result: A four year old boy was treated with two liniment solutions containing sodium nitrite at 30 g/L (Liniment A) and 140 g/L (Liniment B). Liniment A was applied all over the boy's body, causing listlessness and vomiting. Liniment B was applied all over the boy's body a few days later. The boy went into shock and suffered severe cyanosis. He was hospitalised immediately, but died after two hours in

intensive care. The boy's blood methemoglobin level was found to be 76%.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JUL-2005 (155) (156)

Type of experience: other: recurrent urticaria: clinical investigation of 330 patients

Remark: -Provocation tests

The patients were recommended a diet free from salicylates and dyes for at least 4-5 days prior to admission. No antihistamines were given. For safety reasons and to allow close examination, all the provocations were performed in the wards. A challenge test battery with several controls, was used from 1974-1978. Changes in the schedule were often made. The tests could only be done in patients with no or slight symptoms. The chemicals were given in titanium dioxide-whitened gelatin capsules with lactose added to complete the fillings and the patients were unaware of the type of compound tested. If the patients reacted to lactose,
wheat starch was used instead as filling material. The first
dose was given at 8 o'clock in the morning and additional doses at1-h intervals. Only one type of additive was given per day. If the test was positive, it was necessary to wait with the next provocation until the reaction had subsided. Provocation tests are judged as positive when the patients develop clear signs of urdcaria or angio-oedema within 24 h.
About half of the patients reacted within 6 h. Judging whether a reaction is positive or negative is not always easy. When in doubt,the doctor should therefore can the reaction uncertain and repeat the test later. One or more positive reactions were found in 31%. of the patients tested
and totally negative tests in 36%. In 33% one or several tests were uncertain. They are those reported by the doctors
on the questionnaire for provocation tests. Some patients reacted to more than one drug. Patients reacting to lactose were tested again With starch¥ In five patients the repeated
test with lactose was positive but the test with starch was negative. The tests with other chemicals were then done with
starch as filling. In four other lactose-positive patients,the repeat tests with lactose were negative. Several patients with ongoing urticaria had questionable tests to both lactose and starch. Further tests then had to be postponed until the patient was in a more stable phase. Questionable tests to other chemicals were often repeated.
□@Patients with positive or questionable tests were always recommended a diet free from the additive:they had reacted to. In patients with positive reactions to additives which have not before been known to provoke urticaria, repeated tests with two placebo capsules were done within a year. The

additive tested and the number of patients reacting out of the number provoked were as follows: nitrateand nitrite 1 of
2. The patients had often found flare-ups of their weals when they had ingested by mistake the additive they should have avoided.

Result: Provocation test in patients with recurrent urticaria. Test period 1974-1978

Result

No. of patients tested: 60
Positive (%); 6
Uncertain (%); 12
Negative (%); 82

Test substance: a mixture of 100 mg sodium nitrite and 100 mg sodium nitrate.

Reliability: (3) invalid
Down graded because test material is a mixture.

24-NOV-2004 (98)

Type of experience: other: food antigens and additives

Remark: Oral challenge with an unstated amount of sodium nitrite cited un specified allergic response in two of 15 subjects suffering from an underfined food allergy.

Challenges were performed the additive and placebo.

Result: Oral challenges with the food additive in 15 patients with allergy to various foods.

No. of patients; 15
Negative; 13
positive; 2

Reliability: (4) not assignable
Down graded because of too small number of subjects.

24-NOV-2004 (127)

Type of experience: Human - Exposure through Food

Remark: A risk assessment was made on nitrate, nitrite and N-nitroso compounds encountered in the human diet. Mean estimates of nitrate intake range from 31 - 185 mg/day in various European countries, with vegetables supplying 80-85%. The intake of nitrite is much lower in various European countries and averages 0.7 - 8.7 mg/day, with both vegetables and cured meats being the major sources.

Reliability: (2) valid with restrictions

22-JUL-2005 (63)

Type of experience: Human - Exposure through Food

Remark: Nitrite occurs in plants at low concentrations, normally between 1-2 mg/kg fresh weight and rarely over 10 mg/kg, although potatoes have been reported to contain 2-60 mg/kg, with a mean concentration of 19 mg/kg

Reliability: (2) valid with restrictions

22-JUL-2005 (121)

Type of experience: Human - Exposure through Food

Remark: Diet constitutes an important source of exposure to both nitrite and nitrate. The major dietary source of nitrate is vegetables. Lettuce, spinach, celery and beetroot commonly contain more than 1g nitrate/kg fresh weight and may reach 3-4 g/kg
Reliability: (2) valid with restrictions
22-JUL-2005 (190)

Type of experience: Direct observation, poisoning incidents

Remark: Report of the fatal case of a nurse who probably ingested a 1g tablet of sodium nitrite (670 mg NO2-). Death occurred two hours after admission to hospital. Post mortem methaemoglobin level was 35%, implying a much higher level on admission. Serum nitrite level was 13 mg/L.
Reliability: (2) valid with restrictions
22-JUL-2005 (67)

Type of experience: Direct observation, poisoning incidents

Remark: Report of a case of methaemoglobinaemia associated with three previously healthy children (two four year old boys and a two year old girl). One of the children had mistaken a bag of sodium nitrite crystals for sugar and added it to cups of tea at concentrations of 5100, 5000 and 4900 mg/L. Methaemoglobin levels of 77% and 38% were measured for two of the children.
Reliability: (2) valid with restrictions
22-JUL-2005 (60)

Type of experience: Direct observation, poisoning incidents

Remark: Report of two cases of methemoglobinaemia attributable to nitrite contamination of potable water through boiler fluid additives. In the first of these, 49 schoolchildren were affected after eating soup which had been diluted with hot water from the tap. The soup was found to contain 459 ppm nitrite. Methaemoglobinaemia was diagnosed in 59% of the children, with levels between 3 - 47%. In the second case, six workers were found to have methaemoglobin levels of between 6 - 16% after drinking coffee contaminated with 300 ppm nitrite.
Reliability: (2) valid with restrictions
22-JUL-2005 (33)

Type of experience: Direct observation, poisoning incidents

Remark: A case of nitrite poisoning after ingestion of spinach in a 2 year old boy is reported. He developed severe methaemoglobinaemia of 53%. In 100 g of the spinach, which had been stored in a refrigerator after preparation for 3 days, 150 mg of nitrite ion was found. He had been fed in unusually great amount because of obstipation.

A congenital methaemoglobinaemia (haemoglobin-anomaly or deficiency of reducing enzymes) was excluded. After injection of Thionin there was quick recovery and the level of methaemoglobin returned to normal.

22-JUL-2005 (157)

Type of experience: Human - Exposure through Food

Remark: Spinach often contains a high concentration of nitrate. Nitrate is non-toxic but nitrite, into which nitrate is converted, is toxic. Nitrite poisoning after the consumption of spinach occurs virtually exclusively in children younger than 1.5 years. For purposes of prevention, spinach intended for consumption by children younger than 1.5 years should have a low nitrate concentration.

22-JUL-2005 (74)

5.11 Additional Remarks

Type: Neurotoxicity

Remark: Auxiliary information: THE INTEGRATED EVALUATION BY NTP (2001)
After a single subcutaneous dose of 55 mg sodium nitrite/kg body weight, locomotor, exploratory, and grooming activities were suppressed in 3-month-old male rats; complete recovery was observed after 24 hours. Exposure of rats to sodium nitrite at 100 to 2,000 mg/L in drinking water for 2 months produced changes in the pattern of brain electrical activity.

The electroencephalogram pattern of four dosed rats differed from those for controls and remained different after sodium nitrite withdrawal.

Reliability: (2) valid with restrictions

25-JUL-2005 (134)

Type: other: Toxicological Effects

Remark: Auxilary information: THE INTEGRATED EVALUATION BY NTP (2001)
The primary acute effect of sodium nitrite in rats and mice is methemoglobinemia. A study was reported methemoglobin concentrations ranging from 9.5% to 72.1% in female CD-1 mice following intraperitoneal injection of 0.5 to 2.8 nmol/kg (35 to 193 mg/kg). Methemoglobin concentrations in Sprague-Dawley rats increased to 45% to 80% 1 hour after an oral dose of 150 mg/kg and returned to normal within 24 hours in surviving rats. Sodium nitrite administered in drinking water at 1,000 to 3,000 mg/L (1,000 to 3,000 ppm) for 2 years elevated methemoglobin concentrations in male rats (unspecified strain) throughout the 2-year period.

The secondary effects of acute nitrite intoxication in animals are vasodilation, relaxation of smooth muscle, and lowering of blood pressure and a decrease in D-xylose absorption in the intestinal mucosa. Other nitrite-induced toxic effects include abdominal pain, diarrhea, atrophied intestinal villi, and apoptotic cell death in the intestinal crypts.

Reliability: (2) valid with restrictions

25-JUL-2005 (134)
Type: other: species difference in vitro

Remark: Sodium nitrite of 0.3 mmol/kg was given i.v. to rats, rabbits and cats. In cats the ferric hemoglobin formation strongly increased with a maximum with 90 min. In rats the induction of the ferric hemoglobin formation was only half as much and after 30 min the maximum had exceeded, with rabbit only a very weak induction had been measured and after few (5-10) minutes their maximum had already. In vitro test with erythrocyte of different animals, the highest ferric hemoglobin formation was observed in cattle and cats with 2.5 mmol/l at the initiaton of incubation, followed by those by with the erythrocyte of dogs. The ferric hemoglobin formation with erythrocyte of humans corresponded about that from cats. Also in this attempt the ferric hemoglobin formation was only moderate with erythrocyten of rabbits. Sodium nitrite is given as antidote with cyanide poisonings due to the ferric hemoglobin formation.
Reliability: (4) not assignable

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