

# Nitrite Toxicity: Chemical Analysis, Metabolism, and Health Effects

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**Abstract.** Nitrites can be formed from and away by the nitrate-nitrite-nitric oxide pathway. The properties of nitrite oxidation and metHb formation are carefully studied in mechanism, forming either N-binding or O-binding structures. Apart from nitrate and nitric oxide, nitrites can also form carcinogenic nitrosamines in acidic environments. MetHb can cause hypoxia and vasodilation, while symptoms are revealed in different degrees under recalled or present hypoxic conditions. The study thoroughly studied nitrite's metabolic properties, chemical pathways, and dosage effects on health. The cancer risks of consuming dietary nitrite need more statistical support, while its metabolite N-nitrosodimethylamine and NDMA concentration are well considered with increasing cancer risks. ED50 of human vasodilation is identified, and lethal doses on juvenile pike-perch can be further utilized to predict related doses for humans. More studies should be done to investigate relative nitrite doses to boost utilization and studies about this chemical.

**Keywords:** Nitrite Toxicity; Metabolism; Hemoglobin.

## 1. Introduction

Nitrite compounds can be used for color fixatives and antiseptics for meat products, while nitrite ions can cause severe food poisoning when consumed by people. In April 2013, three cases of nitrite poisoning were reported by Suzhou University affiliated No. 2 hospital, with each of patients experiencing nausea, tachycardia, unconsciousness, etc. These cases were caused by the incautious addition of nitrite-rich ingredients into their meals, containing 714,286 mg of nitrites per kilogram [1]. Apart from food additives, nitrites may also exist in leftover dishes, causing potential risks of nitrite poisoning.

Since consuming leftover food is common in Chinese families for economic reason, the severe consequences brought by nitrite toxicity have drawn much social concern. To further improve the public health system and reduce unnecessary medical resources consumption, studies on nitrite toxicity and mechanism should be investigated.

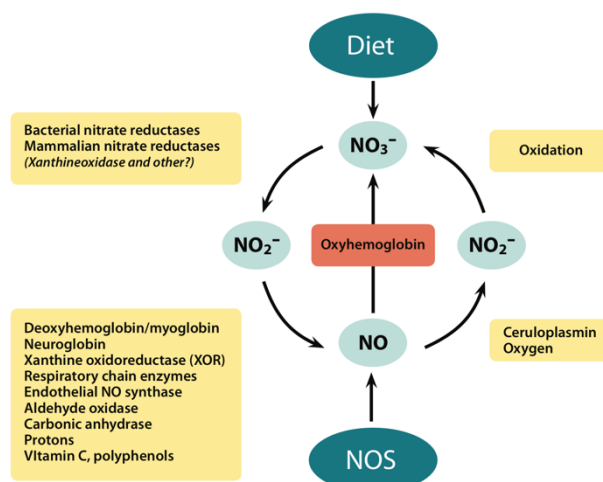
## 2. Nitrite Metabolism: Nitrate-Nitrite-Nitric Acid Oxide Pathway

While nitrite ions are toxic in the form of  $\text{NO}^{2-}$  once reaching a certain dose, it is one of the metabolites in the human nitrate-nitrite-nitric oxide pathway. A typical mammalian nitrogen cycle (shown in Figure 1) has been generated from the results reported by Weitzberg et al. [2]. Although nitric oxide (NO) can be straightly converted to nitrate in the presence of oxyhemoglobin, nitrite is still the essential intermediate substance connecting the conversion between nitrate and nitric oxide. Intake of nitrite can be achieved from straightly consuming food with nitrite ions, or converted from nitrate or nitric oxide through the nitrate-nitrite-nitric oxide pathway.

Nitrates from food can increase the concentration of nitrites in the human body. When ingesting food with nitrate, bacterial nitrate reductase secreted by salivary glands would reduce nitrate into nitrite through the removal of oxygen in the molecule. Jones's study points out the fact that beetroot is highly enriched with nitrate, containing more than 2500mg of nitrate per kg. Most dietary nitrate would join the plasma system, while about a quarter (25%) of them would stay in saliva and possibly be processed by nitrate reductase. Together with other common green leafy vegetables, beetroot can significantly increase the nitrate density in body [3].

Nitrite concentration in the human body can also be elevated by nitric oxides. When exposed to an oxygen-rich environment, excessive surrounding oxygens would bind to nitric oxide, and form negatively charged nitrite ions. The study by Cosby et al. shows that under room temperature (25°C) and a neutral environment (pH 7.0), the reduction of nitrite in forming nitric oxide has a rate constant of  $2.9 \text{ M}^{-1}\text{s}^{-1}$ , oxidizing deoxyhemoglobin ( $\text{HbFe}^{2+}$ ) into methemoglobin ( $\text{HbFe}^{3+}$ ) [4]. In the study of Kennedy et al., ceruloplasmin is recognized for its NO oxidizing characteristic, achieving this ability through catalytically using up NO. Under oxygen-deficient circumstances, ceruloplasmin, a nitric oxide oxidase secreted by the liver, would be activated and oxidized NO into  $\text{NO}_2^-$  [5].

The amount of nitrite in the system is not constantly stable, since it can be converted into nitrates or nitric oxides as well. The study of DeMartino et al. indicates that under acidic environments, nitrites are possible to be convert into nitric oxides without the interference of enzymes [6]. While nitrites arrive in the stomach from direct ingestion, protons from gastric acid would interact with nitrite, also possible in forming nitric acid. This can be further reduced to form negatively charged nitric oxides. If nitrites weren't reduced before it reaches the circulatory system, there is still a high possibility for them to be reduced by deoxyhemoglobin or myoglobin in the blood or tissue. Moreover, nitrite can be oxidized to nitrates under suitable conditions. In figure 1, a circular diagram from Weitzberg et al.'s study illustrates a general summary of the nitrate-nitrite-nitric oxide pathway and possible routes of conversion of nitrites.

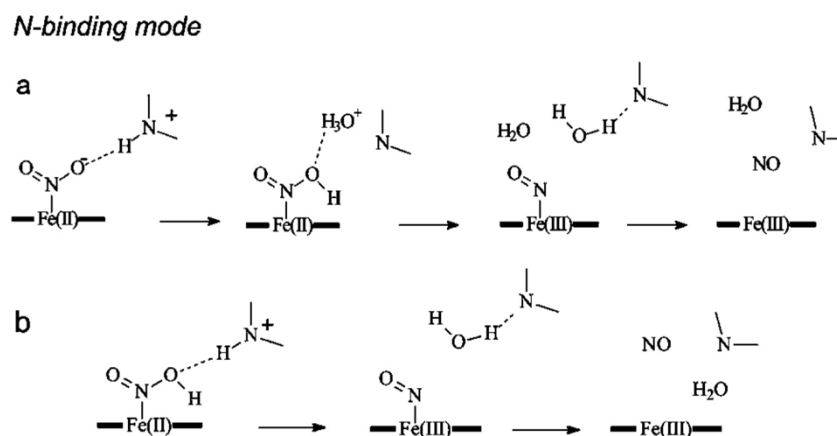


**Fig. 1** A brief overview summarizing different stages of the nitrate-nitrite-nitric acid pathway by Weitzberg et al. [2].

### 3. Nitrite Oxidation of Hemoglobin (Hb) Mechanism

In blood vessels, nitrites can bind with hemoglobin (Hb) and myoglobin (Mb) due to their high electronegativity and attraction towards the positive-charged binding atom of deoxy-Hb and Mb. It was shown that nitrogen-bonding on nitrite with Hb is a thermodynamically favorable and dominating product in this reaction, but the study of the structure in Mb in horse heart indicates an exception case, where oxygen-binding between Hb and nitrite is favored. Therefore, Perissinotti et al.'s study concluded that there are two possible binding routes during the oxidation of Hb, N-bonding, and O-bonding, in which the latter is considered to be more energetically favored [7].

During the process, nitrite binds to ferrous Hb, gaining hydrogen from the environment, and releasing water and nitric acid as products. During this process, ferrous Hb (containing  $\text{Fe}^{2+}$ ) is oxidized to ferric metHb (containing  $\text{Fe}^{3+}$ ), which losses the ability to carry or transport oxygen, and couldn't release present oxygen molecules attached to it due to its increased oxidation state. Figure 2 shows an enlarged view presenting the mechanism of heme iron oxidation through binding with nitrite.



**Fig. 2** Illustration of two processes the heme iron in hemoglobin could be oxidized [7].

As has been shown in Figure 2, both oxidations initiated from nitrogen atom on nitrite bonding with ferrous iron in Hb. In route a, negative-charged oxygen on nitrite attracts hydrogen from environment nitrogen compounds, forming a hydroxyl group attached to nitrite. The lone pair electrons on the oxygen of the hydroxyl group then attack the hydrogen on environmental  $\text{H}_3\text{O}^+$ , breaking the N-O bond and forming two water molecules. The ferrous heme iron ( $\text{Fe}^{2+}$ ) was oxidized to ferric heme iron ( $\text{Fe}^{3+}$ ) while the hydroxyl group is detached from nitrogen on nitrite. Donating a lone pair to central nitrogen, oxygen forms a coordinate bond with nitrogen, detaching from the ferric heme atom, and is reduced to nitric oxide (NO).

Route b is very similar to route a, while in this case the hydroxyl group forms a water molecule before it was attacked by lone pair of environmental nitrogen compounds and detached from nitrogen on nitrite. Further investigating the rate of nitrite oxidation under different environments, Kim-Shapiro et al. concluded that the maximum rate of nitric oxide generation from nitrite oxidation is 1 nanomolar per second, under 0.01M heme, 100nM nitrite and assuming a bimolecular rate constant of  $1 \text{ M}^{-1}\text{s}^{-1}$  [8].

In 2008, the research of Lundberg indicates the auto-catalyzing characteristics of nitrites under R-state or allosteric conditions, which further introduced a positive correlation between the increase of oxygen concentration (and saturation of oxyhemoglobin) and bimolecular rate constant for the reduction of nitrite [9]. After one year of further investigations, Lundberg et al. came up with a conclusion in 2009's research that the oxidation reaction of nitrite into NO can be catalyzed through either enzymatic or non-enzymatic ways, while multiple enzymes may be involved in catalyzing the reaction, and numerous factors including different levels of environmental pH, oxidation states and oxygen tension [10].

#### 4. Nitrite forming Nitrosamines

Nitrites may form carcinogenic nitrosamines through nitrosation in the stomach. In gastric environments, nitrites can form  $\text{N}_2\text{O}_3$  from binding with nitric oxides, causing it to be chemically active in reaction with amines. It would possibly undergo nitrosation, where it is bonded to amines to form nitrosamines.

Ascorbates are involved in this reaction and would be converted to dehydroascorbates after the reaction. The most common product of nitrosamine from this reaction is N-nitrosodimethylamine (NDMA), releasing nitric oxides and hydrogen into the environment. Apart from the intake of dietary nitrites, atmospheric nitrogen dioxide ( $\text{NO}_2$ ) concentration increase may also cause increased production of NDMA in the human body. Early in 1991, a study by Tannenbaum et al. pointed out that experiments have indicated a positive correlation between atmospheric  $\text{NO}_2$  concentration and urinary level of NDMA as excretion [11].

## 5. Methemoglobin-induced hypoxia and vasodilation

When heme iron is oxidized by nitrite, hemoglobin is converted to methemoglobin, which contains ferric heme and is no longer capable of transporting oxygen to cells of the body. Kim-Shapiro et al. concluded that two general phases are involved during the conversion of ferrous and ferric Hb: the initial phase is proportional to the concentration of nitrite and environmental proton, and the second phase requires a conversion between oxyhemoglobin and ferric Hb bonded with nitrite [12].

During this process, the body would experience oxygen deficiency, which may cause different levels of concentration difficulties, visual disturbance, dizziness, and limited cognitive functions. Certain organs will also experience varying degrees of damage under hypoxic conditions caused by methemoglobin. The percentage of people experiencing these symptoms presents small differences between people with recalled hypoxia exposure and current hypoxia exposure. Tu et al. conducted an experiment in 2020, investigating different portions of symptoms of hypoxia under both recalled and current hypoxia conditions. Surveys were used to collect necessary data. From their results, 8 of 14 symptoms investigated were observed more frequently in the current hypoxia condition (hot flashes, dizziness, visual disturbances, numbness, air hunger, paresthesia, fatigue, anxiety), while the sensation of dizziness and anxiety are significantly observed more frequently in current hypoxia: 1.88 times more cases of dizziness (107) and 1.96 more cases of anxiety (55) are observed in current hypoxic conditions than recalled conditions (57 cases of dizziness and 28 cases of anxiety) [13].

The binding of nitrite to hemoglobin can cause vasodilation in the body. Practicing exercises can stimulate promoting characteristics of nitrite in blood transportation, expanding blood vessels and increasing blood flows. Studies have shown that the injection of nitrite in forearm blood circulation causes immediate vasodilation, resulting in an increase in blood flow and expansion of blood vessel radius. When red blood cells are present, the effects of vasodilation caused by nitrites are more distinct, since the tension for oxygen has decreased.

Such vasodilation by nitrogen chemicals brings positive effects to human health. Rocha's research indicates that vasodilation caused by dietary nitrate can effectively increase the degree of vasodilation on areas of the dorsolateral prefrontal cortex, enhancing the executive functioning of the body. Moreover, vasodilation caused by nitrates may support extensive nutrition and oxygen supply to process complex cognitive tasks [14].

## 6. Cancer Risk

Studies and experiments on animal models indicate that nitrites and N-Nitroso compounds (NOC) are carcinogens. It remains inconclusive on the oncogenic properties of nitrites in epidemiological studies, and very limited NOC in food is well studied to examine further characteristics of pathology. So far, only N-nitrosodimethylamine was well considered in case studies. Eichholzer et al. compared the association of common NOCs and risks of stomach cancer between case-control studies in various areas, and the results indicate that the amount of nitrogen amines is strongly and positively correlated with the risk of stomach cancer. Cases from the Greater Milan area and France all presented a strong association between stomach cancer risk and N-Nitrosodimethylamine level. Cases from Spain indicated stomach cancer risk is greatly increased by increasing the level of nitrosamine, while the risk significantly decreases under increasing nitrate. Moreover, the result indicates an overall decreasing trend of gastric cancer risk with increasing nitrate concentration, and there aren't significant data indicating a clear association between gastric cancer risk and an increase in nitrite concentration [15].

There still lacks strong epidemiological evidence in constructing a clear association between dietary intake of nitrites and gastric, cerebral, or esophagus cancers, while higher exposure to NOC can increase the risk of getting nasopharyngeal or esophageal cancer. Moreover, a higher concentration or deposition of NDMA increases the risk of colorectal cancer. Dich et al. summarized in their study that salted fish and cured meats, consumed by 51.9% and 48.1% of the population, were the most common sources of dietary NDMA [16]. Further studies by Knekt et al. indicated that the

risks of cancer at different proportions of the body, where NDMA showed a higher relative risk of cancer at the lower quartile in sites of head, neck, and stomach, while presented a positive correlation between relative cancer risk and quartile at colorectum [17]. Further studies could be executed to examine the possible correlation between risks of gastric cancer and other common NOC or nitrosamines in food.

## 7. Dose

Considering the possible lethal effect of nitrites and ethical issues, knowledge and data directly from clinical studies in measuring doses of different effects are limited.

Dejam et al. conducted a series of experiments on humans, investigating details about nitrite doses that reflect kinetics, process mechanisms, and potentials of this chemical. The experiment is separated into two parts. In the first part, the rate of dose spreading are measured. 5 volunteers were injected with sodium nitrate would be infused at 0, 7, 14, 28, 55 and 100  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 5 minutes each dose level. Blood samples of these volunteers were collected regularly among 3 hours after doses were infused. 28  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  nitrite injection within 5 minutes was then performed after the previous measurements, followed by a repeat measurement. The second part measures different degrees of vasodilation. Nitrites of 0.07, 0.140, 0.350, 0.700, 1.400, 3.500, 7, 14, and 28  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  were injected into 15 volunteers, split into 3 groups of 5 volunteers for each and injected with saline of 0.9%, oxypurinol of 600 $\mu\text{g}/\text{min}$ , and ascorbic acid of 24mg/min [18].

Among the effect of vasodilation, Dejam et al. conducted experiments on cynomolgus, expressing a decrease in mean arterial blood pressure for  $12 \pm 4$  mm Hg when ingested 12000  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  dietary nitrite. In humans, the lowest dose infused to observe nitrite vasodilation is 0.07  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , where blood flow increased for  $0.4 \pm 6$   $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{mL}^{-1}$  tissue. The ED<sub>50</sub> of nitrite vasodilation for humans observed is 18.6  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  [18].

At higher doses, nitrite presents toxic effect on animals. In the case of juvenile pike-perch, Wuertz et al. investigated lethal doses for nitrites. Results have shown that within 120 hours, LC<sub>50</sub> was 6.1mg of nitrites per liter, and a potential LC<sub>99</sub> when nitrite exceeds 14mg/L [19]. With suitable conversion and calculation with parameter values for humans, it is possible to predict the potential effective and lethal doses for humans [20].

## 8. Summary

Nitrite compounds are constantly involved in the nitrate-nitrite-nitric oxide pathway as metabolites of the human body. In the form of nitrite anions, it can oxidize hemoglobin through either N-binding or O-binding pathways, converting ferrous hemoglobin into ferric methemoglobin (metHb). Nitrites could form carcinogenic nitrosamines under acidic environments, with NDMA being the most studied nitrosamine. MetHb produced during nitrite oxidation can lead to possible hypoxia for the incapability of metHb in carrying oxygen and can cause vasodilation when nitrites are bonded with metHb. ED<sub>50</sub> for human vasodilation is successfully conducted from early studies, and high doses of nitrite are identified to be lethal to specific animals.

As a common chemical and important metabolite in the human body, this paper studies and discusses important aspects of nitrite as metabolism, kinetics, and doses. Through reviewing these characteristics, further studies could be built on the current study, investigating more details in doses for humans and further toxicity mechanisms.

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