

Nitrite, a Hidden Foe in Foods: Evaluation of Nitrite in Toxicological Perspective

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ABSTRACT

Nitrites and nitrates possess a unique position in human toxicology. They are both ubiquitous in the environment and can be formed from nitrogenous compounds. Water, vegetables and meat are important sources of nitrite exposure. Nitrites have several detrimental effects on health. The primary health concern regarding nitrate and nitrite exposure is the formation of methemoglobinemia. Baby food and infant formulas can be important sources of nitrite and infants younger than six months are the most susceptible population. Furthermore, nitrites react with primary, secondary, or tertiary amines in an acid medium and form nitrosamines many of which are known to cause cancer. Health care providers, governments and health authorities should be aware of the routes of nitrite exposure and must have knowledge to assess the hazards. This review will focus on the detrimental effects of nitrite exposure, the possible outcomes and standards put by regulatory authorities to prevent high nitrite exposure especially for susceptible populations.

Key words: nitrite, toxicity, methemoglobinemia, nitrosamines

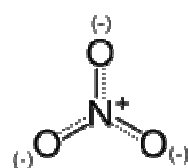
1. INTRODUCTION

Nitrate is a salt of nitric acid with an ion composed of one nitrogen and three oxygen atoms (NO_3^-) in inorganic chemistry (Figure 1). Besides, in organic chemistry the esters of nitric acid and various alcohols are called nitrates. The nitrite ion is NO_2^- . The anion is bent, being isoelectronic with O_3 . More generally, a nitrite compound is either a salt or an ester of nitrous acid [1].

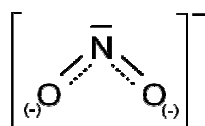
Nitrites and nitrates possess a unique position in human toxicology [2]. They are both ubiquitous in the environment and can be formed from nitrogenous compounds by microorganisms present in the soil, water, saliva and the gastrointestinal tract. Nitrates occur naturally in soil containing nitrogen-fixing

bacteria, decaying plants, septic system effluent and animal manure. Other sources of nitrate include nitrogenous fertilizers and airborne nitrogen compounds emitted by industry and automobiles [3]. Nitrate penetrates through soil and remains in groundwater for decades [4].

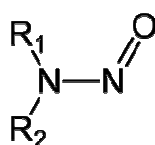
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Nitrate



Nitrite



Nitrosamine

Figure 1. The chemical formula of nitrate, nitrite and nitrosamine

Nitrites and nitrates are used in meat because they bind to myoglobin, inhibiting botulinum exotoxin production; however, these compounds can cause adverse cellular effects, although the extent and significance of health risk resulting from exposure of nitrites and nitrates remain unclear [2, 5]. The toxicity due to nitrate ingestion is relatively low. 5% to 20% nitrates taken from different sources including water and several foodstuffs are endogenously converted to nitrites, which have higher toxicity and are thought to be responsible of several adverse health effects [6-8].

2. NITRITE / NITRATE SOURCES

Nitrate or nitrite exposure can occur from water, different foodstuff and certain medications and volatile nitrite inhalants. Other medications implicated in cases of nitrate or nitrite toxicity are quinone derivatives (antimalarials), nitroglycerine, bismuth subnitrite (antidiarrheal), ammonium nitrate (diuretic), amyl and sodium nitrites (antidotes for cyanide and hydrogen sulfide poisoning), and isosorbide dinitrate/tetranitrates (vasodilators used in coronary artery disease therapy). Ammonium nitrate found in cold packs and nitrous gases used in arc welding are other possible sources of exposure. An ethyl nitrite folk remedy called “*sweet spirits of nitre*” has caused fatalities. Accidental exposures to nitrites in chemical laboratories and ingestion in suicide attempts have been reported. Deliberate abuse of volatile nitrites (amyl, butyl, and isobutyl nitrites) frequently occurs. [9].

2.1. Nitrite content of water

Drinking water is the major source of nitrate/nitrite exposure. Nitrate has steadily accumulated in the water resources largely due to nitrogen fertilizer use, human and animal waste, and air pollution [10]. To protect

infants from developing methemoglobinemia, the U.S. Environmental Protection Agency (EPA) set the maximum contaminant level in drinking water of 10 mg/L as nitrate-nitrogen (N) (equivalent to approximately 45 mg/L nitrate) [11]. Shallow, rural domestic wells are those most likely to be contaminated with nitrates, especially in areas where nitrogen-based fertilizers are in widespread use. Other nitrate sources in well water include seepage from septic sewer systems, or other contaminants. The first reported case of fatal acquired methemoglobinemia in an infant due to ingestion of nitrate-contaminated well water occurred in 1945 [12]. In the following 25 years, about 2,000 similar cases of acquired methemoglobinemia in young infants were reported worldwide; about 10% of such cases resulted in death [13]. Sporadic cases and occasional fatalities occurred through the 1980s and 1990s, most often resulting from ingestion of nitrate-contaminated well water by infants [14-16].

2.2. Nitrite content of food

Being acquainted with the origin and quality of agricultural products, as well as the quality of locally produced food, is getting more important for the consumers and health care providers. There are many factors influencing the nitrate and nitrite content of agricultural products. These factors may be listed as the kind of the product; time of harvesting (nitrite content may differ through seasons and years); ammonium containing fertilizer use; irradiation; temperature and humidity; type of growing (in open air or under protective conditions); length of the growth period; pedologic properties of soil (nitrogen content of soil) and contamination after harvesting [6, 17-21].

The high nitrite content of an agricultural product may be attributed to several reasons. High ammonium containing fertilizer use may be the first and most important reason of this high nitrite levels. Though chemical fertilizers are used extensively in modern agriculture, in order to improve yield and productivity of agricultural products, nutrient leaching from agricultural soil into groundwater resources poses a major environmental and public health concern [22]. High humidity in the storage after harvesting may be another reason. Besides, the type of soil, type of water used in the watering and type of growing may be other factors affecting the total nitrite content of the plant [23, 24]. Contamination of samples with several nitrogen containing compounds may be another important cause of these high levels [25, 26]. Furthermore, contamination of soil with airborne nitrogenous compounds emitted from industry and automobiles might have an effect of the total nitrite content of the soil and therefore this may be effective in the total nitrite content of the plant [3].

In human diet, vegetables are considered to be one of the major sources of dietary exposure to nitrates and nitrites [20]. Green leaf vegetables (e.g. watercress, celery, collard greens, lettuce, broccoli, parsley, fennel and spinach) are thought to highest nitrite containing vegetables. Although vegetables are seldom a source of

acute toxicity, as green-leaf vegetables, carrots and beets accumulate high amounts of nitrite, their consumption below the age of 6 months may not be appropriate. Besides, cauliflower, radish, potatoes and carrots may contain high amounts of nitrite. Nitrite content of fruits is low. Strawberries seem to have the highest content of nitrite while apple and pear seem to have the lowest content [6].

Thompson et al. performed a study on 200 foodstuff and 1021 water samples from 561 different supplies. They found that green vegetables had the highest nitrite content as expected. Watercress had the highest nitrate content (mean 1640 $\mu\text{g g}^{-1}$), whereas celery samples had the second place (mean 1610 $\mu\text{g g}^{-1}$). They also worked on other vegetables including potatoes, carrots and pumpkins. Potatoes had 48-240 $\mu\text{g g}^{-1}$, with a mean nitrate content 129 $\mu\text{g g}^{-1}$ and carrots had <5-290 $\mu\text{g g}^{-1}$, with a mean nitrate content=58.3 $\mu\text{g g}^{-1}$ [27].

Mozolewski and Smoczynski reported a study on nitrate and nitrite levels in different potato tubers. They found that nitrite levels of Ibis potato tubers were the highest among groups (4.3 $\mu\text{g NO}_2 \text{ g}^{-1}$). Culinary processing also reduced the nitrite levels. It was indicated that potato peeling and thermal processing raised the hygienic quality of potato meals [28]. Another study performed by Chung et al. on the nitrate and nitrite content of vegetables grown in Korea showed that winter and summer nitrite content of vegetables show differences. Among the vegetables analyzed *Allium tuberosum* had the highest content (5.3 $\mu\text{g g}^{-1}$) in winter while Chinese cabbage had the highest nitrite content in winter (14.3 $\mu\text{g g}^{-1}$). In our study we found that vegetable-based soups had the fifth place in the nitrite content among other groups (mean 87 $\mu\text{g g}^{-1}$) [29]. Other vegetables, meat and fast-food also contribute to the nitrite and nitrate intake [27, 30]. In meat industry, sodium and potassium nitrites in combination with salt are used for the delay of botulinal toxin development. Nitrites also stabilize the red meat color and retard the development of oxidative rancidity and off-odors and off-flavors during storage [31-33]. "Residual nitrite" is the phrase used to describe the remaining nitrite after the processing of meat with nitrite and it is suspected as also being a health risk [34]. Thompson and co-workers also worked on processed meat products (bacon, ham, salami, beef sausages, hamburger, beef mince) [27]. Hams had the highest nitrite content 35.3 $\mu\text{g g}^{-1}$ among processed foods and among other foodstuff analyzed. Reinik et al. studied Estonian origin meat products produced by different companies [35]. They found that cooked sausages contained <5-90 $\mu\text{g g}^{-1}$ nitrite, smoked sausages contained <5-75 $\mu\text{g g}^{-1}$ nitrite and hams contained <5-100 $\mu\text{g g}^{-1}$ nitrite through 2000-2004. Ellen et al. analyzed 16 kinds of cured meats for contents of residual nitrite. In six samples no nitrite was detectable, the remaining samples contained 1-140 $\mu\text{g g}^{-1}$ nitrite, median value 6.8 $\mu\text{g g}^{-1}$ nitrite [36].

Another study performed on processed foods marketed in Korea measuring nitrite levels was by Hee Lee et al. [37]. The study was done on meat products classified as processed fish products, bacon, ham, sausage, dried

processed meat products, comminuted meat products, salt fermented fish products, and other processed meat products. Among the following groups, dried meat products had the highest nitrite concentration (11.33 $\mu\text{g g}^{-1}$). They also gave the average consumption of nitrite-permitted foods by age and sex groups, and estimated the daily intake of nitrite for average consumers by age and sex groups. In conclusion they found that nitrite intake in all age-sex groups were well below acceptable daily intake (ADI) in Korea. They also gave the estimated daily intake of nitrite for average consumers in different countries. Among the given countries, Finland and UK had the first rank with an estimated daily intake value of 1.4 mg per person per day. In our work, we found that the least nitrite containing meat-based soup had 184 $\mu\text{g g}^{-1}$ nitrite and the highest nitrite content in meat-based soups was 1229 $\mu\text{g g}^{-1}$ [38].

There is limited number of studies on nitrite levels of different foodstuff in our country. We have recently reported the nitrite content of meat- and chicken-based bouillons. The mean nitrite concentration of the samples was 2.955 $\mu\text{g g}^{-1}$ with a maximum level of 10.530 $\mu\text{g g}^{-1}$. After a simple comparison, it was observed that the bovine-based bouillon group had 20% higher nitrite contamination than the chicken-based samples [39]. Türkdoğan et al. found that herb-enriched cheese contained 4.14 $\mu\text{g g}^{-1}$ nitrite, while bread baked by wood fire contained 0.82 $\mu\text{g g}^{-1}$ nitrite and bread baked by animal manure contained 3.02 $\mu\text{g g}^{-1}$ nitrite in Van, Turkey. Furthermore, it is suggested that a nitrite and nitrate rich diet can significantly affect the development of endemic upper gastrointestinal cancers in the city [40].

3. UNSAFE INTERACTIONS BETWEEN NITRITE AND HEMOGLOBIN

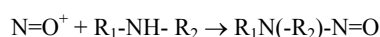
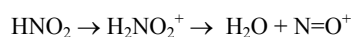
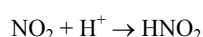
Nitrite exposure has serious outcomes and some of the issues in nitrite exposure still remain unclear. One of the most important effects of nitrites is on hemoglobin (Hb). Hb's function in oxygen carriage is so overwhelmingly important that it has obscured some of the other functions that Hb plays in physiology. The transport of oxygen requires reversibly bound to ferrous Hb, $\text{Hb}(\text{Fe}^{2+} \text{O}_2)$. Many oxidizing agents including nitrites oxidize ferrous Hb to ferric Hb, $\text{Hb}(\text{Fe}^{3+})$, which is unable to bind oxygen. The resulting compound is methemoglobin and the condition is called as "acquired methemoglobinemia", which presents clinically with symptoms and signs of tissue hypoxia. [21, 41, 42]. Signs and symptoms of methemoglobinemia include shortness of breath, cyanosis, mental status changes, headache, fatigue, exercise intolerance, dizziness and loss of consciousness. Arterial blood with elevated methemoglobin levels has a characteristic chocolate-brown color as compared to normal bright red oxygen containing arterial blood [43]. Moreover, acquired methemoglobinemia is also induced by other oxidizing agents such as chlorates, aniline dyes and dapsone and benzocaine [44, 45].

Under normal physiological conditions methemoglobin reduction is provided by erythrocyte NADPH-

methemoglobin reductase (also known as cytochrome-b₅ reductase) in a very efficient manner that there is only <1 % methemoglobin in the circulation, but in hereditary methemoglobinemia this enzyme is deficient and methemoglobin is not sufficiently reduced to hemoglobin [21]. Methemoglobinemia can also arise in patients with pyruvate kinase deficiency due to impaired production of NADH- the essential cofactors for diaphorase I. Nitrites also have a vasodilatory effect that can further contribute to the problem of nitrite-induced anoxia [46]. Infants younger than 5-6 months of age are especially susceptible to nitrate exposure through topical silver nitrate used in burn therapy. Besides, pregnant women, nursing mothers, and elderly are more susceptible to nitrite-induced methemoglobinemia [47]. Patients with glucose-6-phosphate dehydrogenase deficiency, a common condition in some Asian and Mediterranean populations, may develop methemoglobinemia and intravascular hemolysis following exposure to oxidants such as nitrites [48, 49].

4. WHAT ABOUT CANCER AND NITROSOAMINES?

Nitrosamines ($R_1N(-R_2)-N=O$) are ubiquitous in our environment and diet. They are formed from the reaction of nitrite with primary, secondary, or tertiary amines in an acid medium and many of which are known to cause cancer [50-52]. Besides, nitrates can react with amino acids to form nitrosamines, which have been reported to cause cancer in animals [53]. Under acidic conditions the nitrite forms nitrous acid (HNO_2), which is protonated and splits into the nitrosonium cation $N=O^+$ and water. The nitrosonium cation then reacts with an amine to produce nitrosamine:



A high dietary intake of nitrite and nitrate has been implicated as a risk factor for human cancer, and formation of nitrosamines (NOCs) in the stomach is correlated with nitrate/nitrite levels in food and water [52, 54]. There is thought to be an association between gastric cancer and nitrosamines. Diet can be the major determinant of risk [55]. Many nitroso compounds are carcinogenic in animals and most probably in man. International Agency for Research on Cancer (IARC) has classified four NOCs as probable, and another fifteen NOCs as possible carcinogenic in humans [56]. As there is a genotoxic mechanism of action, no safe level has been determined for NOCs [57].

Nitrosamines are found in many foodstuffs, especially beer, fish, and fish byproducts, and also in meat and cheese products preserved with nitrite pickling salt. The U.S. government established limits on the amount of nitrites used in meat products in order to decrease cancer risk in the population. There are also rules about

adding ascorbic acid or related compounds to meat, because they inhibit nitrosamine formation [58]. There is a clear correlation between nitrite added for curing meat and the formation of volatile nitrosamines [59-62]. The mean dietary intake of nitrosodimethylamine was estimated to be between 0,1-1,1 $\mu\text{g}/\text{day}$ in the last decade [63]. The dietary levels of carcinogenic NOCs found in recent studies are in the range of 0,3-0,5 $\mu\text{g}/\text{day}$ in Europe. These low levels are the achievement of regulations set by Europe countries and EU.

Data from epidemiologic, experimental, and animal studies indicate that diet plays an important role in the etiology of cancer. Studies on 130 different NOCs obtained from several foodstuffs, from the environment and from the interactions between drugs and nitrite, have shown that 80% of these NOCs are carcinogenic in laboratory animals [64]. 39 species of animals including 5 primate species have been tested against the carcinogenicity induction of NOCs and nitrosamides and none of them has been found resistant. Tumors have been found in great variety of organs and tissues and induction of tumor formation has been found to be in dose-dependent manner. After metabolic activation, NOCs have been found to be genotoxic in several gene mutation, chromosomal aberration, DNA-interaction and DNA-repair tests. Toxicity symptoms, preneoplastic changes and genotoxic effects have been well-documented in several animal experiments and in accidental human exposure and poisoning. NOCs are carcinogenic to animals and they can also be considered as human carcinogens [65].

Some NOCs induce oxidative stress and lipid peroxidation. There is thought to be an association between gastric cancer, esophageal cancer and hepatocellular carcinomas and the endogenous formation of mutagenic NOCs, from secondary amines and nitrites by the catalyzation of thiocyanate, which is found in human saliva and stomach. NOCs were found to cause induction of gastric cancer in several animal studies and there is thought to be a possible relationship between esophageal cancers and nitrite and nitrosamine intake [43, 44]. An increased incidence of was observed in one group of workers with occupational exposures to nitrate fertilizer; however, the weight of evidence for gastric cancer causation is mixed [57, 66, 67]. In a review, Jakszyn and Gonzalez studied on all the published cohort and case-control studies from 1985-2005, and analyzed the relationship between nitrosamine and nitrite intake and the most important related food intake and cancer risk. The paper covered 11 cohorts and 50 case-control studies. A high proportion of case-control studies found a positive association with meat intake for both tumors (11 of 16 studies on gastric cancer and 11 of 18 studies on esophageal cancer). A relatively large number of case-control studies showed quite consistent results supporting a positive association between processed meat intake and gastric cancer and esophageal cancer risk (10 of 14 studies on gastric cancer and 8 of 9 studies on esophageal cancer). Almost all the case-control studies found a positive and significant

association between preserved fish, vegetable and smoked food intake and gastric cancer though the evidence regarding esophageal cancer risk was more limited [68]. Furthermore, elevated risk of non-Hodgkin's lymphoma and cancers of the nasopharynx, bladder, brain, and prostate have been reported in association with the exposure to nitrosamines [69-73].

5. OTHER TOXIC EFFECTS OF NITRITES

Hypotension is the main cardiovascular effect seen with nitrate and nitrite medications. It is not commonly seen with ingestion of nitrates and nitrites in food and water [74]. Maternal exposure to environmental nitrates and nitrites may increase the risk of pregnancy complications such as anemia, threatened abortion/premature labor, or preeclampsia [75, 76].

Recent epidemiologic data have suggested an association between developmental effects in offspring and the maternal ingestion of nitrate from drinking water; however, a definite conclusion on the cause-and-effect relationship cannot be drawn [77]. The maternal transfer of nitrate, nitrite, and NOCs, and the potential effect on fetal death and malformation have been described [78].

A few studies have hinted at a role for nitrate intake in the risk for developing diabetes mellitus in childhood [79-81]. All of these reproductive and developmental effects require further study.

A number of biochemical changes, functional impairments and histopathological lesions have been observed in nitrite-treated rats. Orally administered 25–100 mg of sodium nitrite kg^{-1} diet to Balb/c mice for 21 days results in a dose dependent decrease in lymphocyte percentages, concanavalin A- and lipopolysaccharide-induced lymphocyte proliferation, natural killer cell activity against WEHI-164 target cells, as well as IgM and IgG titers against injected sheep erythrocytes, and the immunosuppressive effect of sodium nitrite is reversible after cessation of exposure [82]. Based on the incidence of hypertrophy, zona glomerulosa of the adrenal in rats treated with nitrite for 13 weeks, the no-effect level is lower than 100 mg $\text{KNO}_2 \text{L}^{-1}$ in the drinking water, which is equivalent to a level lower than 10 mg KNO_2 per kg body weight day^{-1} [83]. While nitrates are not as toxic as nitrites, ingestion of large amount of nitrates may cause severe gastroenteritis. Methemoglobinemia, anemia and nephritis caused by prolonged exposure to small amounts of nitrates are likely resulting from its reduction to nitrite [84].

Though the detrimental effects of nitrites receive wide acceptance, there are reports that dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Nitrite is an important acute marker of nitric oxide (NO) flux and formation and it is the major storage form of NO in blood and plasma. An experiment was performed on mice receiving nitrite supplementation (50 mg/L) in drinking water for 7 days. Nitrite supplemented mice exhibited significantly high plasma myocardial levels of nitrite and displayed a 48% reduction in the infarct size after myocardial ischemia-reperfusion injury. Though higher levels of nitrite are

harmful, dietary nitrite intake from nitrite/nitrite-rich foods is required to maintain adequate levels of NO [85].

6. AN IMPORTANT ISSUE: NITRITE CONTENT OF BABY FOOD AND INFANT FORMULAS

Infant formula, like no other food, is regulated by its own law, the Infant Formula Act of 1980 as amended in 1986 [86]. EU Commission has set regulations (Commission Regulation 466/2001 and 466/2005 Amendment) on the maximum levels of nitrates, mycotoxins (aflatoxins, ochratoxins, patulin, fusarium toxins), heavy metals and polycyclic aromatic hydrocarbons (PAHs) in infant food [87-89].

Human milk and cow milk can be a source of nitrite and nitrate [90, 91]. For infants who are bottle-fed, however, the major source of nitrate exposure is drinking water used to dilute formula. Home-prepared infant foods containing vegetables are potential causes of methemoglobinemia. There is one report in literature concerning 2 infants, aged respectively 2 and 1 months, suffering from methemoglobinemia, fed with a formula that was reconstituted with a high concentration of courgette soup to resolve constipation. They developed a severe cyanosis with methemoglobinemia (respectively 30.4 and 27 %) and were hospitalized and treated with methylene blue at 1 %. After 12 h the syndrome was completely resolved. It is important not to feed infants with vegetables having a high nitrate content (e.g., courgette, spinach, beets and green beans) to resolve constipation since, particularly in the first months of life, they may cause severe methemoglobinemia [92]. On the other hand, the other exposure route is through different foodstuff prepared at home or bought as baby food/infant formula or baby puree. As time is very important for mothers today, they chose to buy baby food and baby purees rather than preparing them at home. Though generally commercially prepared infant foods are thought to be safe, sanitary, and nutritious alternatives for a caregiver to use when not preparing an infant's foods at home, several contaminants or several detrimental ingredients that come from the nature of the product which it is prepared from, may be present. The important thing is to give baby a balanced diet that has vegetables and fruits, during the first years of life. When introducing infants to complementary foods, it is essential for the caregivers to introduce the right food for the right month and determine the infant's acceptance to each food. Though the manufacturers put the right month to be chosen on the label, mothers may still buy an inappropriate food or puree by mistake. For instance, consumption of contaminated foodstuffs, prepared baby foods having high green vegetable content, and sausage preserved with nitrates and nitrites have caused exposure in children and are the potential cause of acquired infant-methemoglobinemia. This may cause serious consequences and there are reports in the literature that these babies are hospitalized and needed medical help [93, 94]. The nitrite content of baby foods and infant formulas has been in concern of public and researchers for a long time. Though several methods

have been implanted for the nitrite content of baby foods, there are not many studies in literature concerning the nitrite content of baby foods. In a study performed by us, the nitrite content of several baby foods and infant formulas was determined. We randomly collected 42 baby food and infant formula samples and divided them into 4 groups: milk-based, cereal-based, vegetable-based and fruit-based. The vegetable-based samples had the highest nitrite content ($341.88 \mu\text{g g}^{-1}$) whereas fruit-based ($243.71 \mu\text{g g}^{-1}$) and cereal-based samples ($166.14 \mu\text{g g}^{-1}$) had the second and third place, respectively [95]. We also performed another study on commercially available baby purees. The mean nitrite levels in the tested purees were found 23.05 and $39.29 \mu\text{g g}^{-1}$ in fruit- and vegetable-based purees, respectively. It was observed that the group of vegetable-based purees has 59 % higher nitrite than fruit-based samples [96]. From the performed studies, we could see the high nitrite content of food available for infants and babies.

7. WHAT ARE THE STANDARDS FOR NITRATE/NITRITE LEVELS?

EPA has set an enforceable standard called a maximum contaminant level (MCL) for nitrates at 10 ppm, and for nitrites at 1 ppm. EPA believes that exposure below this level is not expected to cause health problems. Given present technology and resources, this is also a level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water. All public water supplies must abide by these regulations. Once a water source is contaminated, the costs of protecting consumers from nitrate exposure can be significant. Nitrate is not removed by conventional drinking water treatment processes; its removal requires additional, relatively expensive treatment units [97]. Some published results suggest an association between nitrate in drinking water and human malformations. However, a review of the toxicology in relation to possible adverse effects on reproduction and development offers no evidence for teratogenic effects attributable to nitrate or nitrite ingestion. The present maximum contaminant level appears to adequately protect even sensitive populations from nitrate-induced toxicity [77].

The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the United Nations/World Health Organization and the European Commission's Scientific Committee on Food have set an ADI for nitrate of 0–3.7 mg nitrate ion/kg body weight. This intake appears to be safe for healthy neonates, children, and adults. The same is also true of the EPA reference dose (RfD) for nitrate of 1.6 mg nitrate nitrogen/kg body weight per day (equivalent to about 7.0 mg nitrate ion/kg body weight per day). JECFA has proposed an ADI for nitrite of 0–0.07 mg nitrite ion/kg body weight. EPA has set an RfD of 0.1 mg nitrite nitrogen/kg body weight per day (equivalent to 0.33 mg nitrite ion/kg body weight per day) [26, 98].

In Turkey, according to the regulations about food additives stated that non-heated, cured, dried meat

products could have $150 \mu\text{g g}^{-1}$ sodium nitrite as an indicative ingoing amount and $50 \mu\text{g g}^{-1}$ sodium nitrite as a residual amount; other cured meat products and canned meat products could have $150 \mu\text{g g}^{-1}$ sodium nitrite as an indicative ingoing amount and $100 \mu\text{g g}^{-1}$ sodium nitrite as a residual amount and cured bacon could have $175 \mu\text{g g}^{-1}$ sodium nitrite as a residual amount as maximum levels, as also expressed in European Parliament and Council Directive 95/2/EC of 20 February 1995 [99, 100].

8. FUTURE CONCERNS

As water and food are the major sources of nitrite exposure, several measures have to be taken to prevent high exposure to nitrite especially for susceptible populations through these ways. Health care providers should be aware of the routes of nitrite exposure and must have knowledge to assess the hazards. Mothers should be very careful in preparing or buying food for infants who are <6 months of age. Moreover, governments and health authorities must take serious measures to prohibit the serious outcomes of nitrite exposure and strict law enforcement in our country should be taken in the conservation of soil and nutrient management issues as the contamination of waterways and wetlands by nitrogenous compounds and the exposure of soil to high nitrogenous fertilizers and to industrial wastes/compounds increase day by day. Furthermore, farmers should be warned and educated by regulatory authorities as the toxicity of several toxic compounds that may be decreased by good agricultural practices. New soil management guidelines that respond to pollution of environment should be established as there are growing concerns for food safety and its' implementations on human health. Several foodstuffs must be checked for nitrite contamination continuously to prevent the threat to human health.

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