Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2017) 6 (2) 48-89
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2017) 6 (2) 48-89

NITRATES AND NITRITES, METABOLISM AND TOXICITY

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Review

Summary

Nitrates naturally occur in the environment and are involved in the nitrogen cycle. Nitrates and nitrites are chemical compounds used in fertilizers, rodenticides and as food preservatives. They can be found in the air, soil, water and food (especially in vegetables) and can be synthesized in the human body. Nitrates play a significant role in the diet and metabolism of plants. Nitrates are formed by oxidation of organic waste by the action of nitrogen-binding bacteria. Humans are exposed to nitrates and nitrites via consumption of vegetables and processed meat products, and to a lesser extent via water and other food. Consumption of drinking water with an increased concentration of nitrate may affect the human body in two ways: (i) acutely, most often manifested as methemoglobinemia (where nitrates in the digestive system are reduced to nitrites, which then oxidize iron in the hemoglobin of the red blood cells forming methemoglobin unable to transmit oxygen in the body), resulting in blue skin, and (ii) chronic, manifested by the occurrence of cancer as a result of organism exposure to nitrosamines (formed during the reaction of nitrates with amines in the body).

Introduction

Nitrogen is essential for all living beings as it is a component of proteins. Nitrogen exists in the environment in many forms and changes forms as it moves through the nitrogen cycle (Oram, 2015). There is a widespread concern over exposure to nitrate from dietary and environmental origins and its increased risk to negative impact on human health (Wagner et al., 1983; Rivett et al., 2008). Nitrates and nitrites are ubiquitous within environmental, food, industrial and physiological systems (Moorcroft et al., 2001; Santamaria, 2006). Nitrates and nitrites are present in different forms, but usually are white or crystalline powder (US EPA, 2007). These chemicals play a significant role in the nutrition and function of plants. Nitrates are formed by oxidation of organic waste by the action of nitrogen-binding bacteria (HAH, 2014). Nitrate is an important component of vegetables due to its potential for accumulation that can affect many biotic and abiotic factors (about 80% of nitrate in human nutrition comes from vegetables). Elevated levels of nitrate are usually found in leaves, while in small amounts they are found in seeds or bulbs. Humans are exposed to nitrates via consumption of vegetables, and to a lesser extent by water or other food, and in addition, nitrates are formed endogenously (Lundberg et al., 2008; EFSA, 2008; Hord et al., 2009).

Inorganic anions are the most common pollutants found in high concentrations in drinking water. In addition, there is always a need to find new and eco-friendly technologies to remove such pollutants. Elevated nitrate concentrations in drinking water can threaten human health because nitrates are reduced to nitrites in gastrointestinal tract. Furthermore, nitrates can cause methemoglobinemia, while nitrites and nitrates have the potential to form carcinogenic *N*-nitroso compounds (Katan, 2009; Rangabhashiyam et al., 2014). However, some studies have demonstrated that inert anions (NO_3^- and NO_2^-) can be recycled *in vivo* to form nitrogen oxide (NO), representing an important alternative source of NO to the conventional synthesis in L-arginine-NO-synthase pathway, especially in hypoxic states (Lundberg et al., 2008).

Nitrates and nitrites are naturally occurring substances in fruits and vegetables, which humans are encouraged to consume because of their beneficial health effects. On the other side, nitrates and nitrites are used as food additives such as sausages, ham and other processed meat. Such processed food is linked with gastric cancer risk in humans consuming it in high amounts (Song et al., 2015). Therefore, the objective of this

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work is to review the most common sources of nitrates and nitrites, their occurrence in food and water for human consumption, as well as explain their metabolism, and finally, to show the effect of ingested nitrate and nitrite on human health.

Sources of nitrates and nitrites

Nitrates and nitrites are the most common pollutants in the environment. The highest concentrations of these compounds have been found in areas of intensive agricultural production where they are accumulated in the soil and groundwater. By using nitrogen fertilizers, nitrates are irrigated from the groundwater because the nitrate ions do not bind to the soil adsorption complex and are prone to rinsing from the soil to groundwater (Sofilić, 2014). However, the total contamination of water with nitrates, contributes the fecal water from septic tanks and inadequately built sewage systems, the application of organic fertilizers and atmospheric deposition (Filipović et al., 2013).

Nitrates and nitrites in food

Plant species that can accumulate nitrates include Brassica plants, are green cereals grains (oats, wheat, rye and maize), sorghum and Sudan grass, corn, beets, sweet clover and raspberry (Wrathall, 2002).
Vegetables and cured meat products are the largest sources of nitrates and nitrites in food, but small amounts may be present in fish and dairy products. Meat products may contain <2.7-945 mg nitrate/kg and <0.2-6.4 mg nitrite/kg; dairy products usually contain <3-27 mg nitrate/kg and <0.2-1.7 mg nitrite/kg. Food processing, use of fertilizers and growth conditions (especially soil temperature and daytime light intensity), influence on the quantity of nitrate in vegetables. Crops such as beetroot, green salad, radish and spinach often contain nitrates at concentrations above 2500 mg/kg, especially when grown in greenhouses. The amount of nitrite in food is very low (generally below 10 mg/kg) and rarely above 100 mg/kg. An exception is noticed in vegetables that are damaged, inadequately stored or stored for a long time, as well as grilled or fermented vegetables. Under such conditions, up to 400 mg/kg nitrite was determined (WHO, 2011). The main intake estimates for nitrate and nitrite in the United States (US) and Europe differ by researcher, but are comparable. The international estimates of nitrate intakes from food is 31-185 mg/day in Europe and ≈40-100 mg/day in the US. Nitrate intake from foods other than vegetables, drinking water and cured meat products, has been estimated to be about 35-44 mg/person/ day for a human with 60 kg (Hord et al., 2009).
Nitrites are often added to meat products as an antimicrobial factor against *Clostridium botulinum* bacteria, and to obtain a characteristic red-pink color of meat (Abid et al., 2014). In the European Union, only certain processed foods (meat products) may contain added nitrites (European Council, 2011) in amounts up to 150 mg/kg (maximum amount that can be added during production). The maximum permissible concentration (MPC) for nitrites in "traditional" meat products is 50-175 mg/kg, and for nitrates 10-300 mg/kg (without added nitrite). Nitrates are also allowed in hard, semi-hard and semi-sweet cheeses, and MPC for cheeses is up to 150 mg/kg (HAH, 2014). Nitrates and nitrites from processed meat and smoked cheeses can lead to the formation of *N*-nitroso compounds that occur when the nitrogen reacts with secondary amines and *N*-alkylamides, a process that is primarily carried out endogenously. The International Agency for Research on Cancer concluded that nitrates and nitrates ingested under conditions that cause endogenous nitrosation are "probable human carcinogens (2A)". However, the formation of *N*-nitroso compounds can be changed by other nutritional factors. Heme iron, of which red meats are a rich source, can act as a catalyst in the formation of *N*-nitroso compounds in the gut (Abid et al., 2014).
In the NIH-AARP study (nutrition and health study developed at the National Cancer Institute, USA), the intake of nitrates and nitrites from processed meat products was associated with stomach cancer, esophagus, bladder, pancreas, thyroid (only in men), prostate and ovarian cancer, as well as with the death caused by chronic liver disease (Abid et al., 2014). The International Agency for Research on Cancer (IARC), clas-

sified processed meat as carcinogenic hazard to humans (Group 1), with the formation of carcinogenic nitrosamines as contributing factor (EFSA, 2017a).

In order to improve the health status by vegetable consumption, certain measures should be taken to reduce exposure to nitrates and nitrites, but to maintain the recommended amount of vegetables. Also, the excessive use of nitrogen fertilizers should be avoided as nitrates are incorporated into soil and then into vegetables. Vegetables should be stored and processed to prevent contamination caused by bacteria, and thus reduction of nitrates to nitrites. Removal of stems result in a reduction of nitrate content by 30-40% in lettuce and spinach. Peeling of potatoes and beetroot decreases the nitrate content by 20-62%. By cooking peas, cabbage, beans, carrots, potatoes, spinach and endives in water, nitrate levels decrease by 16-79% (Chan, 2011). Hord et al. (2009) conducted nitrate and nitrite analysis on a convenience sample of vegetables, fruit, vegetables and fresh and processed meats shown in Table 1.

Table 1. Nitrate and nitrite contents of a convenience sample of some fruits, vegetables and eat products* (Hord et al., 2009)

	Nitrates	**Nitrites**
	(mg/100 g)	
Fruits		
Apple sauce	0.3	0.008
Banana	4.5	0.009
Fruit mix	0.9	0.08
Orange	0.8	0.02
Vegetables		
Broccoli	39.5	0.07
Carrots	0.1	0.006
Cole slaw	55.9	0.07
French fries	2.0	0.17
Ketchup	0.1	0.13
Mustard greens	116.0	0.003
Salad mix	82.1	0.13
Spinach	741	0.02
Tomato	39.2	0.03
Vegetable soup	20.9	0.001
Desiccated vegetable dietary supplement**	27,890	10.5
Meats/processed meats		
Bacon	5.5	0.38
Bacon, nitrite-free	3.0	0.68
Ham	0.9	0.89
Hot dog	9.0	0.05
Pork tenderloin	3.3	0

*Nitrate and nitrite concentrations were quantified by ion exchange chromatography (ENO 20 Analyzer; Eicom, Kyoto, Japan). Analysis of food reflects the mean value from triplicate or quadruplicate analysis.
**Nature's Way Garden Veggies (1 capsule; 900 mg desiccated vegetables; Nature's Way Products Inc, Springville, UT)

L'hirondel et al. (2006) stated that although the MPC in the United States for nitrate in drinking water is 45 mg/L, nitrate concentration in vegetables can be 50 times higher (vegetables often contain between 2000-3000 mg of nitrate per kilogram). However, vegetables rich in nitrates are good for health. The authors pointed out that the intake of dietary nitrates have a lower tendency to increase nitrosation due to the presence of nitrosation inhibitors in vegetables.

Nitrates and nitrites in drinking water

Determination of nitrogen in the form of ammonia, nitrate or nitrites is very important because these compounds are generated by the decomposition of organic substances (proteins, urea, etc.) by spontaneous chemical reactions or by bacterial action.

In the last few years nitrate contamination of groundwater is an increasing problem all over the world. The highest nitrate concentrations in groundwater were recorded in areas of intensive agricultural production (Filipović et al., 2013). Natural levels of nitrate in groundwater depend on the type of soil and geology. Bacteria from the soil translate various forms of nitrogen into nitrate, which is a desirable process, because most of the nitrogen used by plants is absorbed in the form of nitrate. However, nitrate are highly leachable and easily moves with water through the soil profile. If the precipitation is strong, or if irrigation is frequent and abundant, the nitrate will leach below the plant's root zone and eventually reach groundwater (Oram, 2015). In Croatia, the limit value of nitrate in groundwater, prescribed by the Ordinance on compliance parameters and methods of analysis of water for human consumption (Ministry of Health of the Republic of Croatia, 2013), is 50 mg/L expressed as NO_3^-, which is also the limit value prescribed by the Nitrates Directive in the EU (European Commission, 1991). In the United States, nitrate concentrations are in range from 4 to 9 mg/L, while agricultural activities can result in increased levels (up to 100 mg/L). Drinking water rich in nitrates is associated with private wells, especially shallow wells (<15 m deep) in areas with permeable soils (Fewtrell, 2014).

Under aerobic conditions, nitrate can leach in relatively large amounts in the aquifer when there is no plant material absorbing the nitrate. Degradation or denitrification occurs only in a small extent in the soil and rocks forming the aquifer. Under anaerobic conditions, nitrate can be completely degraded to nitrogen. The presence of high or low groundwater level, the quantity of rainwater, the presence of other organic material with different physico-chemical characteristics are important in determining the fate of nitrate in the soil. In surface waters, nitrification and denitrification may also occur, depending on the temperature and pH. However, nitrate absorption by plants is the most common reason of nitrate reduction in surface waters (WHO, 2011).

Nitrates and nitrites in the body

Although nitrate and nitrite are present in food and therefore their intake into the body is unavoidable, their excessive amount in the body can cause harmful effects on health.

However, the metabolism of nitrates in the human body should be emphasized. Namely, nitrate in saliva (not in food), are converted to nitrite by oral microflora. In fact, plasma nitrate are extracted by the salivary glands and excreted in high concentrations in saliva. Healthy adults have a salivary conversion of nitrate to nitrite of 5 to 7% of the total nitrate intake, while infants and patients with gastroenteritis, who have a higher pH, can have a greater conversion rate (Alexander at al., 2008).

The primary harmful effect to human health from drinking water with nitrate nitrogen occurs when nitrate is converted to nitrite in the digestive system (Oram, 2015).

Nitrate and nitrite in humans are present due to diet or are produced by the action of endogenous L-arginine-NO synthase. Nitric oxide, generated by NOS (NO synthase) enzymes, is oxidized in the blood and tissue to form nitrate and nitrite. The reaction of NO with oxyhemoglobin produces nitrate and methemoglobin, while the oxidation of NO forms nitrite, a process catalyzed in plasma by the multi-copper oxidase and NO oxidase ceruloplasmin. The level of nitrate is considerably lower in populations lacking ceruloplasmin. The same was confirmed in experi-

mental mice. Regular exercise increases the expression and activity of endothelial NOS (eNOS) which results in higher circulating level of nitrate (Lundberg et al., 2008).
Lundberg et al. (2006) gave an overview of studies based on the correlation between fruit and vegetable intake and cardiovascular disease. It is supposed that a large intake of fruits and vegetables reduce the risk of coronary heart disease and stroke. Namely, this group of authors propose that the high content of inorganic nitrate is a major factor related to the positive health effects of certain vegetables via bioconversion to nitrite, nitric oxide, and other secondary reaction products (nitroso/nitrosyl compounds), all of which may have positive effect on the cardiovascular system. Although toxicological studies in rats have not confirmed that nitrate or nitrite are carcinogenic, these considerations have not diminished the public's concerns about current amounts in drinking water and food. However, numerous papers indicate that nitrite is the key physiological regulator of hypoxic vasodilation and mitochondrial respiration (Lunberg et al., 2006).

Metabolism of nitrate and nitrite in humans

There are two major sources of nitrate in human body. One is endogenous NO formation, which is spontaneously oxidized in blood and tissues forming nitrite, and the other is the diet. In the diet, nitrate exists mostly in the form of precursor nitrate. Only a small portion is taken up directly as nitrite via ingestion, for example, cured meat products (bacon and sausages), where it is used as a food preservative in combination with vitamin C (Lundberg et al., 2006).
Understanding the risk assessment of dietary nitrate and nitrite does not only imply description of the intake and removal of these ions, but also knowledge of their distribution in the body. The action of oxyhemoglobin in oxidizing nitrite to nitrate, maintains blood nitrite concentrations at very low levels. Hence, endogenous nitrosation is most likely to occur in the gastrointestinal lumen (Schultz et al., 1985).
The metabolism of inhaled, injected and endogenously formed NO, NO_2, N_2O_3, NO_2^- and NO_3^- in blood depends on diffusion, convection, distribution coefficient and chemical reactions between different compartments of human blood (Kelm, 1999).
Nitrates are rapidly absorbed in the stomach and small intestine. Orally administered nitrate salt reaches a peak plasma concentration within 1 hour. Approximately 25% of peroral nitrate is concentrated and excreted via the salivary glands, so that the concentration of salivary nitrate is about ten times that of plasma. Part of this salivary nitrate is reduced by facultative anaerobic bacteria found on the surface of the tongue. These bacteria use nitrate in the absence of oxygen and act as terminal electron acceptor and produce nitrite as a byproduct. It is important to note that the concentration of nitrite in the saliva is 1000 times higher than in plasma in fasting state. Furthermore, a small amount of the nitrite is reduced to NO by bacteria or peridontal acidity, but most will be swallowed and will react with stomach acid to form a complex mix of nitrogen oxides, including nitrous acid, nitrogen dioxide, dinitrogen trioxide and nitrogen oxide (EFSA, 2008; Gilchrist et al., 2010).
Ingested nitrate is absorbed from the small intestine into the circulatory system. Blood nitrate is either concentrated in the saliva glands where it is reduced to nitrite or removed by the kidney and excreted in the urine. Sodium nitrite is absorbed unchanged from the stomach of rats and mice. In blood, nitrite is rapidly and irreversibly oxidized to nitrate, which is then eliminated by excretion. Nitrite is unstable in acidic media and is spontaneously decomposes to nitrate and nitrogen dioxide. In acidic conditions and in the presence of food, nitrite disappeared with a half-life of 2.2 h and pH 4.5; 0.93 h and pH 3.5 and 0.42 h at pH 2.5. There is no data on the absorption, distribution, metabolism or excretion of sodium nitrite in humans (US Department of Health and Human Services, 2001).
Wagner et al. (1983) investigated the fate of nitrate (3.5 mmol $^{15}NO_3^-$) in 12 healthy young adults. Samples of urine, saliva, plasma and feces were collected over a period of 48 hours. The subjects received either 60 mg of ascorbic acid, 2 g of ascorbic acid, or 2 g of sodium ascorbate per day. About 60% of $^{15}NO_3^-$ appeared in urine as nitrate within 48 hours. Less than 0.1% appeared in the feces. The ^{15}N label

of nitrate was found in urine (3%) and feces (0.2%) in the form of ammonia or urea. The fate of the remaining 35% $^{15}NO_3^-$ is unknown. Also, the effect of ascorbic acid or sodium ascorbate on the level of nitrate and nitrite in plasma, saliva, urine or feces was not observed. Wagner et al. (1983) stated that the diet is not the only source of nitrate, but that nitrate is endogenously synthesized. Therefore, not the total amount of nitrate excreted in the urine is from food, although during the period of high nitrogen intake, the contribution of endogenous nitrate biosynthesis to urinary nitrate is small.

Toxicity of nitrate and nitrite

Concerns about nitrate emerged in 1940s when first report on infant methemoglobinemia ('blue child syndrome') was associated with high concentrations of nitrate in well water. Case studies of methemoglobinemia showed that such cases were rare when the concentration of nitrates in well water was below 44 mg/L. Nitrate alone is not the primary cause, and without bacterial contamination it is unlikely that nitrates will cause methemoglobinemia, therefore, a limit of 45 to 50 mg/L in drinking water is not necessary (Lundberg et al., 2008; Gilchrist et al., 2010).
In 2017, EFSA updated and improved the exposure assessment to dietary nitrate and nitrite used as food additives. Experts evaluated that the exposure to nitrates from food additives was less than 5% and did not exceed the safe levels. Unfortunately, the safe levels could be exceeded (for individuals if all sources of nitrates are considered, such as food additives, nitrates naturally occurring in foods, etc.), especially for individuals of all age groups. The exposure to nitrates used as food additives is higher for children, who might also exceed the ADI (EFSA, 2017a).

Acute toxicity

The primary health hazard of drinking water with an elevated nitrate concentration occurs when nitrate is transformed into nitrite in the digestive system. The nitrate ion oxidizes iron in hemoglobin of red blood cells forming methemoglobin that has no ability to transmit oxygen to cells in the body, resulting in blue color of the skin. The potential risk of cancer due to nitrates exists when nitrates react with amines in the body and thus produce nitrosamines that are known to be carcinogens. In this case, the nitrates must be converted into nitrites before nitrosamine is formed (Oram, 2015). Some studies confirmed the concern about cancer-causing potential of nitrates and nitrites used as preservatives and color-enhancing agents in meats. Elevated risk of non-Hodgkin's lymphoma and cancers of the esophagus, bladder, colon and thyroid have been reported. Additionally, an increase of stomach cancer was observed in workers manipulating with nitrate fertilizers. However, epidemiological and human toxicological studies have not shown and unambiguous relation between nitrate intake and the risk of cancer (ATSDR, 2013).
Plants absorb nitrates converted into nitrite by nitrate reductase and are incorporated into amino acids and proteins. Genetic or environmental factors that interfere with or inhibit the function of nitrate reductase, allow for nitrate to accumulate in the plant. In response to stress conditions, certain types of sorghum species reduced the activity of reductase. Drought and conditions with reduced sunlight will also reduce the activity of the nitrate reductase system. Despite the decrease in activity, the plants continuously absorb nitrate and accumulate them in abnormally high amounts. Ruminants, by consuming such plants, convert nitrate to nitrite and finally to ammonia. The nitrate toxicity is a function of the amount of consumed nitrate. When an animal consumes conventional feed with a large amount of nitrate, the conversion of nitrite to ammonia becomes a limiting factor that allows the accumulation of nitrite in toxic amounts. Toxicity occurs by absorption of nitrite into the blood that oxidizes iron to hemoglobin from ferrous (+2) to ferric (+3) state. The resulting methemoglobin has a low affinity for oxygen transfer capacity of red blood cells. Death due to anoxia may occur if 70-80% of hemoglobin is converted to methemoglobin (Wrathall, 2002). In Table 2 some methemoglobin-inducing substances are listed.

Table 2. Substances that promote the formation of methemoglobin (Knobeloch et al., 2000)

Substance	**Utilization**
Aniline dyes	Laundry inks, markers
Benzocaine, lidocaine	Local anesthetics
Chlorates	Matches
Isobutyl nitrite	Roomdeodorizers
Naphtalene	Moth balls
Nitrate/nitrite	Drinking water, fruits, vegetables, cured meats
Nitric oxide	Inhalant used to treat pulmonary hypertension in newborns
Nitrobenzene	Metal cleaners
Nitroethane	Nail care products
Nitrogen oxides	Auto emissions, wood smoke, gas-burning appliances
Nitroglycerine	Angina drug, explosives
Resorcinol	Antipruritic, over-the-counter medications
Sodium nitrite	Pickling salts, boiler conditioners, cleaning solutions
Sulfonamides	Antibiotics

Intoxication of nitrate is caused by the consumption of groundwater with high concentration of nitrate. Recorded lethal doses of nitrate ions for humans range from 67 to 833 mg of nitrate ions per kg of body weight. Toxic amounts - with the formation of methemoglobin as a criterion for toxicity - ranged from 33 to 350 mg of nitrate ions/kg of body weight. The oral lethal dose for humans is estimated to vary from 33 to 250 mg of nitrate ions per kg of body weight (lower doses are applicable for children and elderly). Doses of 1 to 8.3 mg of nitrate ions per kg of body weight lead to the induction of methemoglobinemia. Comparison of these results for humans with the results for experimental animals, shows that nitrite toxicity is similar for humans and experimental animals (Table 3). Compared to humans, rats are 10 to 100 times less sensitive to nitrate because they do not have mechanism to convert nitrate to nitrite (Boink and Speijers, 2001).

Table 3. Toxicity of nitrate/nitrite in rats and humans (Boink and Speijers, 2001)
Chronic toxicity

	Lowest observed effect level: nitrate
Rat	360-2000 mg/kg/day
Human*	30-150 mg/kg
	Lowest observed effect level: nitrites
Rat	10-200 mg/kg/day
Human*	1-250 mg/kg

As it is already mentioned, after nitrate is converted to nitrite in the body, it can react with amines from food thus forming nirosamines. Nitrosamine formation, however, can be inhibited by antioxidants from food (Vitamin C and E). Several long-term toxicity/carcinogenicity studies have been conducted on nitrate. The rats were given 0, 0.1, 1, 5 and 10% sodium nitrate through diet for two years, which is 0, 50, 500, 2500 and 5000 mg/ kg/body weight/day. A No observed effect level (NOEL) of 500 mg/kg/ body weight/day was determined for sodium nitrate based on a slight decrease in growth rate. No histological changes or increase in tumor frequency were observed. Furthermore, rats were given water with 0 or 0.5% sodium nitrate for 84 weeks, which is 0 and 500 mg/kg/ body weight/day, and no histopathological effects were observed at that time. Another two-year study was conducted where 0, 2.5 and 5% sodium nitrate in drinking water (0, 2500 and 5000 mg/kg body weight/day) were given to rats. This study found a no adverse effect level (NOAEL) of 2500 mg/ kg body weight/day. All studies have found low chronic toxicity of nitrate (EFSA, 2008).
L'hirondel et al. (2006) consider that the cancer risks of nitrate is unjustified because, if drinking water with 10-20 ppm of nitrate-nitrogen (NO_3-N) is toxic, then vegetables (with extremely high levels of nitrate) would also be toxic, in spite the presence of a known nitrosation inhibitor.

The effect of nitrite on carcinogenicity in rats was tested with nitrite in drinking water (doses were 0, 10, 100, 200 and 300 mg/kg body weight/day), where no significant differences were observed between control and treated groups for growth, mortality and total hemoglobin level. At the highest three doses, methemoglobin increased to 5, 12 and 22% and lung toxicity was observed. Focal degeneration, fibrosis of the heart and dilatation of coronary arteries were observed at the highest dose (EFSA, 2008).

Research on humans

An ecological study of nitrate in drinking water and non-Hodgkin lymphoma (NHL) and digestive and urinary tract cancer was conducted in the Slovak Republic. Data on nitrate in villages using public water supplies were correlated with the occurrence of cancer for the period 1986-1995. There was an elevated rate of colorectal cancer in women, as well as non-Hodgkin lymphoma in women and men. There were no indications of kidney and bladder cancer. In addition, exposure to nitrate from drinking water is associated with increased risk of colorectal cancer (EFSA, 2008).

Acceptable daily intake

The concept of acceptable daily intake (ADI) was defined by the Joint Expert Committee of the Food and Agriculture Organization of the United Nations/World Health Organization – JECFA for substances deliberately added to food or to contaminants (additives, residues of pesticides, etc.). Due to the lack of data on the possible effects of vegetable matrices on the bioavailability of nitrate, JECFA considered it inappropriate to compare the exposure to nitrate from vegetables with ADI. JECFA and the European Commission's Scientific Committee on Food (SCF) have set an ADI for NO_3 of 0 to 3.7 mg/kg body weight, which is actually 277 mg of nitrate per person (75 kg). The US Environmental Protection Agency (US EPA) has set a reference dose (RfD) for nitrate nitrogen ($N\text{-}NO_3$) of 1.6 mg/kg body weight/day, equivalent to 7 mg NO_3/kg body weight/day (Santamaria, 2006). In 2017, EFSA re-evaluated sodium and potassium nitrate as food additives. It was stated that even using the highest nitrate-to-nitrite conversion factor of 9% a dose corresponding to ADI of 3.7 mg/kg body weight/day will be converted to 0.25 mg nitrite ion/kg body weight/day. They recommended additional studies on humans measuring the excretion of nitrate into the saliva and its conversion to nitrites, as well as further studies on the levels of nitroso compounds formed in different meat products with known amounts o nitrates/nitrites added (EFSA, 2017b).

The European Union sets limits of 2500-3000 mg/kg of nitrates in spinach (fresh product), and 2000 mg/kg of processed product (frozen spinach). Taking into account the consumption of 2 liters of water per day and 100 g of vegetables per day, the total nitrate intake can vary between 200 and 400 mg. The statistical model of exposure has shown that ADI is usually exceeded by 15% and in children even by 45% (Boink and Speijers, 2001).

Conclusions

High levels of nitrate in drinking water and fruits and vegetables are frequently reported. The potential risk of nitrate poisoning is from its conversion to nitrite before and after ingestion. For prevention of unnecessary exposure to nitrate, regulatory standards and advisory levels have been established. Some efforts have been made in order to protect consumers from poisoning, for example, monitoring the water quality helps to ensure that nitrate levels are known, taking measures to reduce nitrate and nitrite from vegetables in order to maintain the recommended intake of fruits and vegetables (reduction of nitrogen fertilizers, appropriate storage and preparation of fruits and vegetables, etc.), and education and outreach to the public. The ADI concept gives a very high degree of protection to the consumers against exposition to nitrate and nitrite, except if high nitrate amount in vegetables or drinking water is exceeded over a longer period of time. Regarding this, it is highly desirable to produce and consume foods with reduced amounts of nitrate.

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NITRATI I NITRITI, METABOLIZAM I TOKSIČNOST

Pregledni rad

Sažetak

Nitrati i nitriti su kemijski spojevi koji se koriste kao gnojivo, rodenticidi ili konzervansi. Mogu se naći u zraku, tlu, vodi ili hrani (posebice povrću) a mogu se i stvarati u ljudskom tijelu. Nitrati imaju važnu ulogu u hranjenju i metabolizmu biljaka. Nastaju oksidacijom organskog otpada djelovanjem dušičnih bakterija. Ljudi mogu biti izloženi nitratima i nitritima preko konzumacije povrća i mesnih prerađevina, a manjim dijelom preko vode ili ostale hrane. Pijenje vode sa većom koncentracijom nitrata može djelovati na ljudsko tijelo na dva načina: (i) akutno, što se najčešće manifestira methemoglobinemijom (nitrati u probavnom sustavu se reduciraju u nitrite koji potom oksidiraju željezo u hemoglobinu crvenih krvnih stanica stvarajući methemoglobin koji nije sposoban prenositi kisik po tijelu), a koja se manifestira pojavom plave boje kožw, i (ii) kronično, što se manifestira pojavom karcinoma kao rezultata izloženosti nitrozaminima (koji nastaju tijekom reakcije nitrata sa aminima u tijelu).

Ključne riječi: nitrati, nitriti, toksikolokogija