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# RISK ASSESMENT OF NITRITE INTAKE TO HEALTH

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## Abstract

The aim of this study was to estimate dietary intake of nitrite in meat products. The content of nitrite in various categories of meat products that are on the market of Tuzla Canton. Data on concentrations of nitrite are associated with individual data on food consumption. The study included 200 respondents, aged 19 years and over. Dietary intake of nitrite was estimated by semi-quantitative questionnaire that included questions about the frequency and quantity consumed of certain types of meat products. To estimate the intake, combined data on the average concentration of nitrite in meat products and information on individual consumption. The average concentrations of nitrite in meat products analyzed samples was 7.44 mg/kg (range 0.6 to 23.59 mg/kg). Respondents consume certain types of meat products by an average 52 days/year, and the average daily amount of all types of meat products consumed was 168.92 g per day (per individual categories of meat products is between 15.29 to 22.27 g). Determined the average daily intake of nitrite from meat products is low and amounts to 13.41% compared to the acceptable daily intake (ADI) (for certain categories of meat products ranged from 0.02 to 0.9% of the ADI). The results suggest that a daily intake of nitrite in meat products by the population of the limits of acceptable daily intake, and exposure to nitrites in meat products in the study population does not represent a risk to health.

***Keywords:*** "nitrites"; "meat products"; "intake"; human health"; "risk assessment"

## 1. Introduction

The use of nitrates and nitrites in process of meat and similar products preservation has significant importance. In small amounts, nitrites reduce the growth of various microorganisms, particularly *Clostridium botulinum*, as well as *Listeria monocytogenes*, *Salmonellae* and *Staphylococcus aureus* (EFSA, 2003). The nitrites fix the color of meat and meat products. Nitrates are not toxic, but nitrites, their oxides and N-nitroso compounds can effect on human health. The experimental studies of acute toxicity of nitrites have been published recently. Acute effects include the muscles relaxation, vasodilatation and the reduction of blood pressure, as well as formation of methaemoglobin (MetHb). Effects of the long-term consumption of nitrite in water by experimental animals have shown vasodilatation, sedation, methemoglobinemia and hystopatological changes on heart muscle, lungs, liver, etc. Hypertrophy of the adrenal zona glomerulosa is the most sensitive indicator of toxicity and has important role in NOAEL value definition (Til *et al*., 1990). The nitrites in blood transform haemoglobin into methaemoglobin causing to methemoglobinemia with different clinical symptoms, such as cyanosis with concentration of MetHb of 3% with children, levels of 10% and higher are associated with clinical symptoms of the oxygen reduction, levels above of 20 % lead to hypoxia and cyanosis, and levels of 50% lead to death. Nitrites are more toxically for younger children (up to 3 month of age) compared to adults, because relative high concentration for methaemoglobin in blood and the lower activity of NADH cytochrome b5 MetHb reductases which transform methaemoglobin into haemoglobin. Under specific conditions (low pH,

 

high temperature), nitrates can lead to the formation of toxic N-nitrosamines and their carcinogenicity in animals has been proved, and there are possible cancer influence on man. It also shows mutagenic affects. The formation of nitrosamines during nitrite preservation of food is possible, as well as the formation of N-nitroso compounds in meat which has been preserved by nitrites has been proved (Tricker, 1997). The main source of N-nitroso compounds in food are meat products preserved by nitrites (Haorah *et al.*, 2001). Persons with lesions or disadvantages of gastric, particularly with atrophic gastritis, pernicious anemia and persons on cimetidine and antacid therapy present particular risk group with nitrite intake and approach of gastritis cancer cannot be avoided. Healthy persons and those who are in risk group, there is possible connection between nitrites and gastric cancer, that has been based on endogenic formation of cancer N-nitroso compounds.

The first international toxicological evaluation of risk connected to ingestion of nitrites has been performed by WHO/FAO scientific board for food additives (Joint FAO/WHO Expert Committee on Food Additives, JECFA) in 1961. The Scientific Committee for food of European Commission (Scientific Committee for Food, SCF) has defined ADI for nitrites from 0 to 0.06 mg/kg of body weight (SCF, 1995) and JECFA has defined the Acceptable daily intake (ADI) from 0 to 0.07 mg/kg of body weight (JECFA, 2002). In 2017. EFSA has redefined ADI for nitrites, and has concluded that the increase of methaemoglobin level with humans and animals, is relevant data for defining of ADI, and its value of 0.07 mg of nitrite ions by kg of body weight per day (EFSA, 2017a).

The determination of amounts of individual additives in food is of great importance in order to determine the maximum allowed concentrations. Based on the content of individual additives in specific foods, it is possible to conduct their daily intake with some populations, in order to determine eventual deviations from determined values for acceptable of daily intake. The assessment of human exposition by nitrites from its main dietary sources is very important because of possibility of forming of toxic compounds, nitrosamines. The main objective of this work is to evaluate possibility of negative impacts on health because of dietary exposure by nitrites from meat products.

## 2. Material and Methods

In 50 samples meat products, taken by random method on Bosnia and Herzegovina market, preserved by Sodium-nitrite (E 250), the level of residual amounts of nitrites, expressed as mg NaNO2/kg, has been estimated using referent laboratory method according to ISO 2918-1975 *(International standard ISO 2918-1975 (E): Meat and meat products-Determination of nitrite content).* The laboratory analyses have been performed in accredited laboratory „Herkon“ Mostar, Bosnia and Herzegovina.

The level of residual nitrites has been analyzed in meat products, as follows:

- Sausages (lasting, half lasting, thermally treated);
- Cans (pastries, meat cutlet),
- Dried meat products (beef ham, bacon, smoked turkey – chicken).

Method of questioning about 200 persons (127 male and 73 female), age more than 20 years, the amount and frequency of meat consumption has been estimated. The questions were divided into categories depending on frequency of consumption (daily, weekly and monthly) and kinds of meat products, above mentioned.

Daily intake of nitrites in meat products (presented as mg per kg of body weight per day) has been evaluated by calculation an average concentration of nitrites in meat products, including level and period of exposition, as well as an average weight of persons. It has been done using the following formula:

$$I = C \times CR \times EF / BW \times AT$$

Where,

I – intake (amount of intake of chemicals per kg of body weight) – mg per kg of body weight,
C – concentration of chemicals (an average chemical concentration) – mg /kg
CR – the part of contact (amount of consumed food in time unit) – mg/day
EF – frequency of exposure – days per year
BW – body weight (an average of body weight during period of exposition) – kg
AT – an average time (period of exposure) – number of days

Probability of negative healthy effects has been estimated on the base of relationship of daily estimated nitrite intake by consumption of meat products and reference values of acceptable daily intake (SCOOP, 1998; JECFA, 2009). Reference values for Acceptable Daily Intake (ADI) for nitrites is form 0 to 0.07 mg/kg of body weight per day (EFSA, 2017b).

 

## 3. Results

An average level of residual nitrites (estimated as $NaNO_2$) divided by meat categories was from 5.20 to 10.02 mg/kg. An average level of residual nitrites in all 50 samples of meat products, which were analyzed, was 7.44 mg/kg (Table 1).

Table 1: Residual level of nitrite in meat products samples

| Meat product | $NaNO_2$ (mg/kg) | | |
|---|---|---|---|
| | $\bar{x}$ | Range | Standard Deviation |
| *Lasting sausage* | 5.20 | 4.38-6.88 | ±0.89 |
| *Half lasting sausage* | 7.91 | 0.60-18.03 | ±5.62 |
| *Thermal treated sausage* | 8.20 | 1.82-23.59 | ±7.32 |
| *Pastry* | 10.20 | 2.96-18.79 | ±7.90 |
| *Meat cans* | 9.53 | 5.29-10.74 | ±2.68 |
| *Dry meat products* | 5.35 | 1.76-9.54 | |

Questioning have included 200 persons, of what 127 were male and 73 female, and they had an average weight of 73.99 ±14.44 kg. An average frequency of consumption of meat products from all analyzed categories was 52 days per year. Individual meat products were consumed from 46 to 81 day per year (Figure 1).

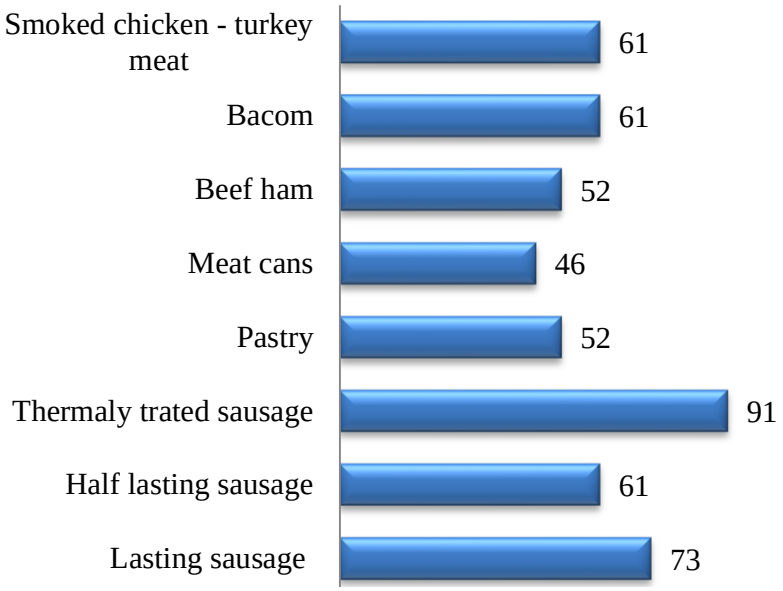


**Figure 1:** Frequency of consumption of meat products

An average daily consumption of all meat products was 168.92 g. An average consumption according to individual meat products was from 16.61 to 22.27 grams per day (Table 2).

Table 2: Amount of consumed meat products

| Meat product | Amount (g) | |
|---|---|---|
| | Range | Daily |
| *Lasting sausage* | 88.88-238.75 | 22.27 |
| *Half lasting sausage* | 105.00-232.14 | 22.14 |
| *Thermal treated sausage* | 106.00-200.00 | 20.60 |

 

| | | |
|---|---|---|
| *Pastry* | 56.66-180,00 | 15.29 |
| *Meat cans* | 100-210.83 | 16.61 |
| *Dry meat products* | 40-215.21 | 17.19 |

The estimated daily intake of nitrite through the consumption of meat products was 0.00001-0.0006 mg/kg of body weight per day. Estimated daily intake of nitrites through the consumption of meat products was 0.0094 mg/kg of body weight per day. Estimated daily intake of nitrite by the consumption of meat products was 0.02-0.9% according to acceptable daily intake (ADI) and estimated daily intake of nitrites in all meat products was 13.41% according to ADI (Table 3).

Table 3: Estimated daily intake of nitrites according to ADI

| **Meat product** | **Estimated intake values (mg/kg/day)** | **%ADI** |
|---|---|---|
| **Sausages** | | |
| *Lasting sausage* | 0.00001 | 0.02 |
| *Half lasting sausage* | 0.0004 | 0.56 |
| *Thermal treated sausage* | 0.0006 | 0.9 |
| **Cans** | | |
| *Pastry* | 0.0003 | 0.43 |
| *Meat cans* | 0.00028 | 0.4 |
| **Dried meat products** | | |
| *Beef ham, Bacon, Smoked chicken – turkey meat,* | 0.0002 | 0.28 |
| **ALL PRODUCTS** | **0.0094** | **13.41** |

## 4. Discussion

The use of preservatives in the technology of meat production has been known since years. Nitrates and nitrites, and food additives from preservative category, which has a long history of use, are considered as very efficient for meat preservation and products in order to provide microbiological safety for their use. As additives for meat production, are potassium nitrite (E 249) and sodium nitrite (E 250). Nitrites are particularly effective preservatives for protection against bacteria *Clostridium botulinum.* Nitrites are added because they stabilize products, its expiry value and protect against microbiological contamination.

In table 4, there are values of daily intake of nitrites all over the world by consumption of meat products, for different age populations (EFSA, 2017c; OEHHA, 1997; Health Canada, 2013; Hsu *et al*., 2009; FAO/WHO, 2009; IARC, 2006). The maximum value have been given for Australia, 5.3 mg/kg of body weight and age of 18 to 64 years. The lowest value according to literature date has Canada, 0.5 mg/kg of body weight.

Table 4: Estimated daily intake of nitrite from food in the world

| | EU (use only as food additives)[1] | U.S.[2] | Canada[3] | Australia[4] | African[5] | Latin American[5] | World[6] |
|---|---|---|---|---|---|---|---|
| | mg/kg bw/day | mg/day | | | | | |
| Infants (12 weeks-11 month) | 0.01-0.14 | | | | | | |
| Toddlers (12-35 month) | 0.01-0.36 | | | | | | |
| Children (3-9 years) | 0.03-0.27 | | | | | | |
| Adolescents (10-17 years) | 0.04-0.22 | | | | | | |
| Adults (18-64 years) | 0.06-0.18 | 0.3-2.6 | 0.50 | 5.3 | 1.7 | 1.6 | 0.74-2.2 |
| The elderly (≥ 65 years) | 0.04-0.13 | | | | | | |

*[1]EFSA, 2017; [2]OEHHA, 1997; [3]Health Canada, 2013; [4]Hsu et al., 2009; [5]FAO/WHO, 2009; [6]IARC, 2006*

 

Nitrites can react with proteins, lipids, pigments and other meat ingredients, and the fraction which has not reacted, appears in free form (as NO2- and $HNO_2$) and are called free or residual nitrites, and others are called connected nitrites. The amount of free (residual) nitrites depend on many factors such as pH, temperature of processing and storing. During storing, the concentration of nitrites has been reduced, and meat products on market contain from 5 to 30 mg/kg of free residual nitrites (Pegg *et al.*, 2004). When meat or meat products have been conserved by nitrites, there are reactions that produce the damage and cancer compounds N-nitrosamines, whose cancerogenity and toxicity has been proved (Jakszyn *et al.*, 2006). The reaction of N-nitrosamines production depend on many factors such as amount of amines, amount of added nitrites, pH, temperature, redox potential, and the presence of other chemical compounds and agents.

In this research, the estimated level of residual nitrites was from 0.60 to 23.59 mg/kg. The lowest value of nitrite was estimated in samples of lasting sausages (5.2 mg/kg), and the largest in samples of pastries (10.02 mg/kg). No any sample has had the level of residual nitrites than it is maximum permissible concentrations.

There is opinion on monitoring of residual nitrites in meat, products, that it has the limited value and do not give „safety" of products to possibilities of the growth of *Clostridium botulinum*, although till today, a significant contribution has been achieved in monitoring of residual nitrites. In research, which has been done in different meat products in many countries in world, including France, Belgium, Germany, and Great Brittan, etc., the content of nitrites was in allowed limits. The results have also showed bad production practices and the increased use of nitrites for preserving. In research, performed within SCOOP study (EU Scientific Cooperation) (EFSA, 2003b), an average values of nitrites content have been estimated. In France, in period of 1995 to 2002, the research of residual level of nitrites has been performed in various preserved meat products, and 3112 samples were tested, and the content of residual nitrites in 59%, respectively 74%, was from 0 to 0.9 mg/kg. In Germany, from 2001 to 2002 the level of residual nitrites was below 20 mg/kg for 85% tested samples. The research in Belgium, in period of 2002, the content of residual nitrites was below 20 mg/kg, for 76% of tested samples, but in 2003, the percentage was reduce to 71%. During 2001 and 2002, the similar research has been performed in Ireland, and there 147 samples of bacon and 386 sample other meat products were tested. The results of testing have showed the following; the content of residual nitrite in bacon, expressed as $NaNO_2$, were from 0-20 mg/kg in 36% sample, from 20 to 29 mg/kg in 20% tested samples; from 30 to 39 mg/kg in 12% samples and from 40 to 49 mg/kg in 7% samples. In research in Serbia, performed in 2006, 256 samples of preserved meat products were tested in order to determine the level of residual nitrite, and in all tested sample in was in the range which are allowed by regulations (Prica *et al.*, 2007). Pleadin and associates (2009) in Croatia, the results of determination of nitrite content in meat products have showed that the highest values were determined in samples of half lasting and thermally treated sausages, ranging from 38.02 to 64.44 mg/kg, but the lowest values were determined in lasting sausages 4.51-7.56 mg/kg. The content of nitrites in meat products in Estonia, in period of 2000 and 2001, as well as from 2003 and 2004, were in determined limits, which are allowed by regulations, as well as that the concentration are reducing continually (Reinik *et al.*, 2005).

In our research, the low values of nitrites, as dietary daily intake, originating from meat products (divided to categories) and they were from 0.00001 (lasting sausages) to 0.0022 mg/kg of body weight per day (half lasing sausages). The total daily intake of nitrites originating from all meat products was 0.0094 mg/kg of body weight per day.

In Ireland, an average daily intake of nitrites for adults from meat products was 0.2048 mg/kg of body weight (FSAI, 2001). An average daily intake of nitrites with adults in Finland was 1.74 mg/day, and meat and meat products provided about 69% of total nitrite intake (Laitinen *et al.*, 1993). From the preserved meat, an average daily intake of nitrites in France was, 1.88 mg/day, and it was 28% from preserved meat (Cornee *et al.*, 1992). The content of nitrites in Denmark was also monitored in period from 1996 to 2006, and an average for age 15 to 75, it was 0.007 mg/kg of body weight per day for male and 0.003 mg/kg of body weight per day for female (Penttilä *et al.*, 1990).

The estimated dietary nitrite intake originating from all consumed meat products in our research was 13.41% compared to ADI, and it is corresponding to similar research in the world.

In dietary nitrate intake, based on analytical data about consummation of food by different populations from Finland, an average dietary nitrite intake has been estimated, and it was 1.88 mg, mainly originating from meat products and it was 2.5% compared to ADI (Menard *et al.*, 2008). Within monitoring program in France, which was performed from 2000 and 2006, assessed dietary exposure to nitrites by meat consumption, was from 33 to 67% compared to ADI (Let *et al.*, 2008). In Belgium, dietary intake of nitrites was determined on

 

the base of chemical analyses of meat products about consumption of meat products, and daily intake nitrites from them was very low, 6% compared to ADI (Temme *et al.*, 2011). The study in Iran, in which the content of residual nitrites was monitored in 180 samples of preserved meat, have showed that daily average intake of nitrites from meat products was 0.0058 mg/kg, and it is 8.28% of ADI (Yarmohammadi *et al.*, 2015). Thomson and associates (Thomson *et al.*, 2007) in New Zeeland, have estimated an average daily intake of nitrites by food and drinking water, and value is 16% of ADI.

Taking into consideration, significant problem for people health can present N-nitrosamines. Many of studies, performed till today, that small amount of nitrites, added in meat as preservative, lately has showed in the form of volatile N-nitrosamines. Nitrites react in particular conditions with amine and amino acids from meat during heat treatment, and after that compounds, as N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) are produced. Experiments on animals have showed that they are cancerogenic, mutagenic and teratogenic (Mirvish, 1995). Available scientific literature give results about research of the risk on increased dietary exposition of toxic N-nitrosamines compounds by consuming of various food products, particularly vegetables, meat and meat products. Dietary intake of nitrites and nitrosamines as risk factor for stomach cancer, have been proved in many research in the world (Song *et al.*, 2015). In study, which was performed in the period of 1995 to 2006, has been proved, that dietary intake of nitrites by consumption of meat products could be the risk for cancer of pancreas with male population (Aschebrook-Kilfoy *et al.*, 2011). The high dietary intake of nitrites can lead to high exposure of endogen formed nitrosamines, and in that way the risk for formation of colorectal cancer (Dellavalle *et al.*, 2014). Because of all indirect proves about particular connecting with cancer appearance with humans, there is possibility about possible causal taking into consideration that 35% of all cancers with human caused by food consumption (Honikel, 2008).

## 4. Conclusion

It is very important to mention that results in this research have showed the following:

- Value of intake of nitrites from meat and meat products are less than values presented by various countries all over the world;
- Nitrites are very important in preservation of meat, but their content and adding through technological process is of great importance, therefore it should be monitored;
- It would be of great importance that companies and governmental institutions make regular assessment of nitrite intake by meat and meat products;
- The content of nitrite concentration in meat products is within limits which are given by valid regulations in Bosnia and Herzegovina, and EU.

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