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Research article

HEALTH RISK ASSESSMENT TAKING INTO ACCOUNT N-NITROSAMINES' CONCENTRATIONS IN FOOD PRODUCTS

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N-nitrosamines formed in meat products, especially during heat treatment, are potentially hazardous to human health. Smoked sausages are a product with a high content of N-nitrosamines, including those not regulated in Russia, which can cause health risks for population.

The aim of this study was to perform health risk assessment considering ratios of N-nitrosamine concentrations in food products.

The data on N-nitrosamines' concentrations were analyzed using MS Excel and Statistica 10.0 programs. The relationship between these concentrations was evaluated using Spearman correlation coefficient. Parameterization of dependencies was carried out by regression analysis with evaluation of model significance by Fisher's criterion. Differences at the level of $p \leq 0.05$ were considered statistically significant. Risk assessment was performed in accordance with the Guideline R.2.1.10.3968-23.

Correlation analysis between N-nitrosamines in smoked sausages revealed a significant correlation between concentrations of NDMA and NEMA ($r_s = 0.77$) and NDMA and NPyRA ($r_s = 0.81$) at $p < 0.05$. The level of total carcinogenic risk ($\sum CR$) derived from data based on calculated concentrations of NEMA and NPyRA was determined to be unacceptable for the adult population. It should be noted that the contribution to $\sum CR$ value (11.0 %) is related to NEMA and NPyRA that are not regulated in Russia.

Our study results support the findings of earlier EFSA publications indicating that not only NDMA and NDEA, but also other N-nitrosamines may form a health risk upon simultaneous exposure.

Correlation and regression analyses allowed us to assess the concentrations of NEMA and NPyRA in sausages. Use of both these concentrations and actual NDMA and NDEA concentrations established unacceptable carcinogenic risk for certain consumer groups in case N-nitrosamines concentrations were above regulated level. The contribution of NEMA and NPyRA in $\sum CR$ value was 11.0 %.

Keywords: *correlation analysis, regression analysis, meat products, smoked sausages, N-nitrosamines, risk assessment, carcinogenic risk, consumers' health, NDMA.*

N-nitrosamines are food contaminants able to produce multiple negative effects on the human body. However, carcinogenic effects are considered the most hazardous among them upon oral introduction of N-nitrosamines. This is due to the fact that most N-nitrosamines upon oral introduction are transformed into carcinogens under in-

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fluence of enzyme systems of cytochrome p450[1] [1–6]. N-nitrosamines enter the human body predominantly with food products [1, 2]. Meat products, including boiled, smoked and summer sausages, are major sources of these chemicals since technologies of their production involve adding nitrites to them to achieve better properties (the mass concentration should not exceed 0.005 % per 100 grams of a product)[2]. Nitrites support nitrosation and, consequently, formation of N-nitrosamines[3] [7–9]. In addition, meat products are sources of biogenic amines, which are precursors of N-nitrosamines [10]. Use of up-to-date methods for chemical identification in food products allows identifying a wide range of N-nitrosamines [7]. Thus, at present, it is possible to quantify nine nitroso compounds in food products; all of them are classified by the IARC as strong carcinogens for humans[4] [1, 2, 11–13].

Nevertheless, the federal state statistic forms contain data on contamination in food products only for two compounds, N-nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA)[5] [14]. International organizations that deal with food safety highlight the necessity to estimate safety of all identifiable N-nitrosamines that can occur in food products [1] including estimations based on assessing health risks for consumers.

Given that, assessment of health risks for consumers associated with N-nitrosamines intake with smoked sausages seems quite relevant. It should rely on using levels of exposure to N-nitrosamines, which are calculated considering their actual contents in analyzed food products.

The aim of this study was to assess additional health risk considering ratios of N-nitrosamine concentrations in food products on the example of smoked sausages.

Materials and methods. The study relied on using data on estimated levels of N-nitrosamines in smoked meat products taken from previous publications [14].

Results obtained by quantification of N-nitrosamines in the examined food products were analyzed with standard MS Excel 2014 and Statistica 10.0 applied statistical software package. Data that deviated from normal distribution (established by using the Shapiro – Wilk test) were described with median (Me), upper and lower quartiles ($Q_{25} - Q_{75}$).

Associations between regulated N-nitrosamines (NDMA and NDEA) and other nitrosamines identified in smoked sausages were estimated by using the Spearmen nonparametric correlation coefficient (r_s). The obtained relationships were parameterized by regression analysis together with assessing significance of models per the Fischer's test (F) stating the model parameters (b_0), regression coefficient (b_1) and determination coefficient (R^2). Differences were considered significant at $p \leq 0.05$.

The linear regression model in general is as follows:

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[1] Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – N-Nitrosodimethylamine. Ottawa, Ontario, Health Canada, 2011, 45 p.; Toxicological profile for N-nitrosodimethylamine. Athlanta, Agency for Toxic Substances and Disease Registry (ATSDR), 2023, 231 p.

[2] GOST R 55455-2013. Boiled-smoked meat sausages. Specifications: State Standard. *Baza GOSTov*. Available at: https://allgosts.ru/67/120/gost_r_55455-2013 (August 15, 2024) (in Russian); GOST 16351-86. Semi-smoked sausages. Specifications: Inter-State Standard. *The library for regulatory documents*. Available at: https://files.stroyinf.ru/Data2/1/4294836/4294836073.pdf (August 15, 2024) (in Russian).

[3] Nitraty, nitrity i N-nitrozosoedineniya [Nitrates, nitrites and N-nitrosamines]. Geneva, World Health Organization, 1981, 120 p. (in Russian).

[4] Agents Classified by the IARC Monographs, Volumes 1–136. *IARC*. Available at: https://monographs.iarc.who.int/agents-classified-by-the-iarc/ (June 05, 2024).

[5] Formy federal'nogo gosudarstvennogo statisticheskogo nablyudeniya [The federal state statistic forms]. Available at: https://47.rospotrebnadzor.ru/content/формы-федерального-государственного-статистического-наблюдения (August 13, 2024) (in Russian).

$$Y_i = b_0 + b_1 \cdot X_i , \quad (1)$$

where Y_i is the dependent variable; X_i is the independent variable; b_0 and b_1 are the parameters of the mathematical model.

Validity of differences between actual and calculated concentrations of associated chemicals was estimated using the Mann – Whitney test (*U*-test).

Levels obtained by using regression models considering the 90th percentile were employed to assess exposure for subsequent assessment of health risk caused by associated chemicals. Health risk was assessed in conformity with the Guide R.2.1.10.3968-23 Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment[6]. According to the Guide, the value typical for malignant neoplasms of the digestive organs was taken as a severity measure ($g = 0.495$).

Results and discussion. Earlier chemical and analytical identification of quantitative levels of N-nitrosamines in the analyzed sausages established their actual levels to be within a range from 0.0002 to 0.35 mg/kg of a product (Table 1).

Analysis of pair correlations between actual NDMA and NDEA levels and other N-nitrosamines in sausages established authentic correlations between levels of two pairs of N-nitrosamines: NDMA → NEMA ($r_s = 0.77$) and NDMA → NPyRA ($r_s = 0.81$) at $p < 0.05$.

Table 1

Levels of N-nitrosamines in smoked sausages identified by chromato-mass-spectrometry (mg/kg of a product)

Sample No.	Levels in smoked sausages			
	NDMA	NEMA	NDEA	NPyRA
1	0.0147	0.0014	0.0018	0.0073
2	0.23	0.062	0.0003	0.042
3	0.09	0.024	-	0.012
4	0.35	0.105	0.0009	0.086
5	0.189	0.043	0.0003	0.0278
6	0.043	0.0021	0.0007	0.0047
7	0.0006	0.0116	-	0.0031
8	0.0359	0.0037	0.0002	0.0012
9	0.0006	0.0005	-	0.0029
10	0.0003	0.0003	-	0.0018
11	0.0003	0.0014	-	0.0007
12	0.0008	0.0003	0.0003	0.0032
13	0.0732	0.0323	-	0.0073
14	0.0165	0.0014	-	0.0016
15	0.075	0.0004	0.0003	0.0044
16	0.0916	0.022	0.0003	0.0044
Me	0.0395	0.0029	0.0003	0.0044
[Q_{25}]	0.0008	0.0012	0.0003	0.0026
[Q_{75}]	0.0904	0.0261	0.0007	0.0085

Note: NPyRA is for N-nitrosopyrrolidine amine; NEMA is for N-nitrosoethylmethylamine.

[6] Guide R.2.1.10.3968-23. Rukovodstvo po otsenke riska zdorov'yu naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh sredu obitaniya [Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2023, 221 p. (in Russian).

Table 2

Parameters of significant regression models

Independent variable (X)	Dependent variable (Y)	b_0	Error	b_1	Fischer's test (F)	Model authenticity (p)	Determination coefficient (R^2)
NDMA	NEMA	-0.0019	0.002	0.282	172.4	$p < 0.001$	0.93
NDMA	NPyRA	-0.0029	0.007	0.211	119.6	$p < 0.001$	0.89

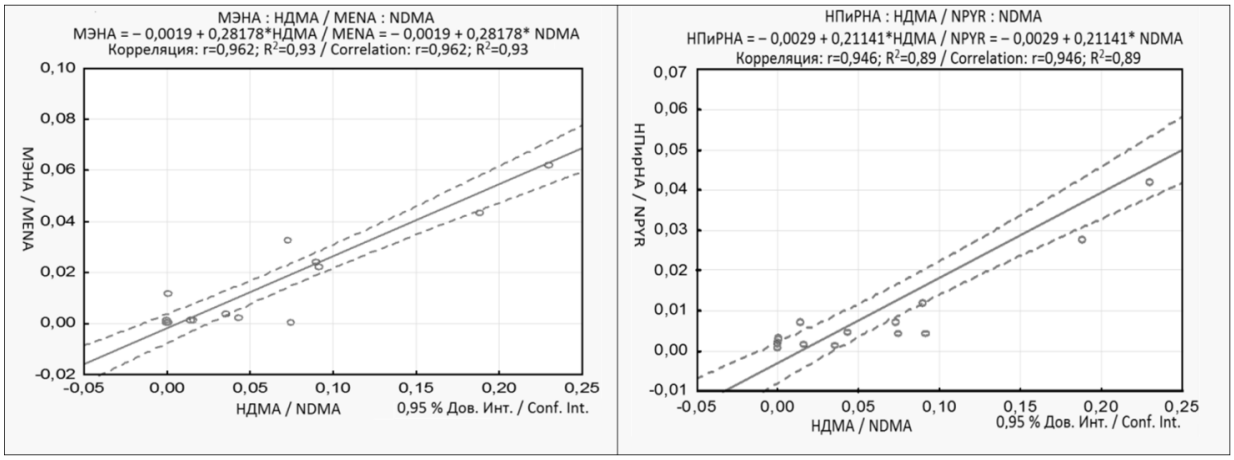

Figure. The results obtained by using the regression model of th eraltisnhios between levels of N-nitrosamines

A strong positive correlation ($r_s \geq 0.7$) between levels of these chemicals may indicate their probable co-occurrence in the analyzed food products. The obtained relationships were parameterized by using regression analysis and this showed obvious associations between these two n-nitrosamines and NDMA levels. Table 2 provides the parameters of regression models.

The models that describe the relationships between two of the analyzed N-nitrosamines and NDMA levels make it possible to calculate their levels in smoked sausages (Figure).

Calculated levels, 90th percentile taken into account, were as follows: NEMA, the actual level 0.053 mg/kg of a product (the calculated one, 0.057 mg/kg of a product); NPyRA, the actual level, 0.035 mg/kg of a product (the calculated one, 0.043 mg/kg of a product). The calculated levels, which were used in subsequent exposure assessment, did not have any authentic differences from the actual ones as confirmed by the Mann – Whitney test (the value of the U-Mann – Whitney test was 120 and 115 for NEMA and NPyRA respectively at the critical U-test value being equal to 83).

Exposure was assessed within carcinogenic risk calculation by using an average volume of consumed sausages equal to 0.002 kg per day for the whole population. The figure was established in previous publications [15]. However, according to a study by E.E. Keshabyants with colleagues [16], smoked meat products are consumed regularly by 18.8 % of the adult population and average intake of all types of sausages amounts to 56.4 grams per day. Bearing in mind that smoked sausages account for 7.9 % of the total sausage consumption [17], we can estimate average daily consumption of smoked sausages as equal to 0.005 kg per day. Given that, volumes of smoked sausage consumption were estimated per two scenarios to assess exposure: for the total popula-

tion (scenario 1) and basing on data obtained for adults who consume smoked sausages regularly [16] (scenario 2).

Carcinogenic risk assessment (*CR*) that considered contributions made by specific N-nitrosamines established that the permissible risk level was surpassed under the exposure scenario 2 ($CR \geq 1.0 \cdot 10^{-4}$) (Table 3).

Carcinogenic risk assessment under the scenario 1 established that the total carcinogenic risk (∑CR) was equal to $7.39 \cdot 10^{-5}$, which is considered permissible (acceptable). At the same time, the total carcinogenic risk for adults who consume smoked sausages regularly was established to be alerting ($\sum CR = 1.85 \cdot 10^{-4}$) and this is unacceptable. NDMA contribution to carcinogenic risk amounted to 87.6 %. NEMA and NPyRA each contributed 10.3 % into carcinogenic risk levels.

In Russia, levels of two N-nitrosamines, namely the sum of NDMA and NDEA, are monitored in sausages[7]. In its turn, no strict regulations are introduced for such chemicals in food products in the European Union [18]. Nevertheless, we should not rule out probable unacceptable health risks caused by intake of all detected potentially hazardous N-nitroso compounds. This is confirmed by a recent study conducted by the European Food Safety Authority (EFSA), which highlights the necessity to assess risks for human health caused by all detected N-nitrosamines in food products and to develop safe standards of their contents in foods [1, 2].

In some countries, normative documents have been already adopted that stipulate maximum permissible levels (MPLs) of N-nitrosamines in meat products. Thus, the Canadian Food Inspection Agency (CFIA) established the following MPLs for N-nitrosamines in dried meat: 0.01 mg/kg for NDEA, N-nitrosodipropylamine (NDPA), NDMA, N-nitrosodibutylamine (NDBA) and 0.015 mg/kg for NPyRA. In Chile, the MPL for N-nitrosodimethylamine (NDMA) in meat is fixed at 0.03 mg/kg[8] [18].

Table 3

Carcinogenic risk levels caused by NDMA, NDEA, NEMA and NPyRA intake with smoked sausages

Calculated values	NDMA	NEMA	NPyRA	NDEA	∑CR
Cancer slope factor, Sfo	51	22	2,1	150	
Scenario 1					
Average daily dose (carcinogen), mg/kg of body weight	$2.57 \cdot 10^{-6}$	$7.00 \cdot 10^{-7}$	$5.23 \cdot 10^{-7}$	$1.35 \cdot 10^{-8}$	$7.39 \cdot 10^{-5}$
Carcinogenic risk, *CR* considering disease severity	$6.48 \cdot 10^{-5}$	$7.62 \cdot 10^{-6}$	$5.43 \cdot 10^{-7}$	$1.00 \cdot 10^{-6}$	
Scenario 2					
Average daily dose (carcinogen), mg/kg of body weight	$6.41 \cdot 10^{-6}$	$1.75 \cdot 10^{-6}$	$1.31 \cdot 10^{-6}$	$3.37 \cdot 10^{-8}$	$1.85 \cdot 10^{-4}$
Carcinogenic risk, *CR* considering disease severity	$1.62 \cdot 10^{-4}$	$1.90 \cdot 10^{-5}$	$1.11 \cdot 10^{-6}$	$2.50 \cdot 10^{-6}$	
Contribution to carcinogenic risk level, %	87.6	10.3	0.7	1.4	100

[7] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: Tekhnicheskii reglament Tamozhennogo soyuza (s izmeneniyami na 23 iyunya 2023 goda) [CU TR 021/2011. On Food Safety: Technical Regulations of the Customs Union (as of June 23, 2023)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320560 (June 24, 2024) (in Russian).

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The findings reported in this study highlight the importance to take a complex approach to assessing health risks caused by N-nitrosamines in food products. It is necessary to consider not only specific chemicals but their cumulative effect as well. The established correlations between levels of various N-nitrosamines prove the necessity to consider them in combination in health risk assessment as well as the possibility and even necessity to regulate their levels in food [19–21]. In its turn, assessment of only a limited number of N-nitrosamines in foods may result in underestimating adverse effects of chemicals on the human body. Moreover, when assessing health risks, it is necessary to consider not only levels of analyzed chemicals in foods but also a share of population who consumes an analyzed product regularly ant not the population as a whole, which is confirmed by our findings.

Conclusion. Correlation-regression analysis of data on NDMA levels made it possible to calculate NEMA and NPyRA levels in smoked sausages. Considering these results, the total carcinogenic risk was established to be unacceptable ($\sum CR = 1.85 \cdot 10^{-4}$) for adults who consume smoked sausages regularly. It should be noted that two N-nitrosamines (NEMA and NPyRA) that are not regulated in Russia account for 11.2 % of the total carcinogenic risk. At the same time, it was established that using data on volumes of consumption calculated for the whole population in exposure assessment reduced the total carcinogenic risk down to $7.39 \cdot 10^{-5}$. This highlights how important it is to consider actual consumption patterns in health risk assessment.

Therefore, the results of this study emphasize that it is relevant to consider all N-nitrosamines detected in food products in health risk assessment relying both on actual data and calculations to identify exposure levels more precisely. It is also very important to consider actual consumption patterns in health risk assessment.

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