

Assessment of potential hazards of the inadvertently present chemicals in food

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Abstract

During the 71st session of the Executive Committee of the Codex Alimentarius Commission, New Zealand proposed draft Guidelines for risk analysis of chemicals inadvertently present in food at low levels, noting that regulatory documents do not currently cover this group of substances. Methodological approaches to detecting and identifying chemical substances inadvertently present in foodstuffs were proposed in the Russian Federation. The developed methodological approaches include four stages: analytical identification of chemical substances; integrated assessment of chemical hazards employing additional selection criteria followed by the application of a score and summation of points; categorizing chemical substances with the assignment of potential hazard categories and final stage - health risk assessment for selected chemicals based on the integrated index. The presented methodological approaches were tested on the example of canned meat for infant nutrition consumed in the Russian Federation and the Socialist Republic of Vietnam. N-nitrosamines were assessed as a priority potentially hazardous inadvertently present chemical substance in samples of canned meat for infant nutrition for health risk assessment using methodological approaches developed in the Russian Federation. Assessment of the health risk to infants when consuming canned meat for infant nutrition containing N-nitrosamines on the example of products sold in the Russian Federation and the Socialist Republic of Vietnam revealed no excess hazard quotients ($HQ < 1.0$) in both territories. However, the calculation of carcinogenic risks has shown that when canned meat for infants sold in the Russian Federation from 6 months to 3 years of age is consumed, a carcinogenic risk may be formed in infants of the corresponding group. This difference between the Socialist Republic of Vietnam and the Russian Federation is due not only to the difference in the content of N-nitrosamines in the examined product but also to the greater volume of canned meat consumption in Russia.

Keywords: *nitrosamines, inadvertently present chemicals, contaminants, risk assessment.*

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1. INTRODUCTION

An increasing number of chemical compounds have been recently synthesized and brought into industrial circulation, which can inadvertently get into food (through packaging, containers, etc.) and pose a potential health hazard.

During the 71st session of the Executive Committee of the Codex Alimentarius Commission, New Zealand proposed draft Guidelines for risk analysis of chemicals inadvertently present in food at low levels, noting that regulatory documents do not currently cover this group of substances. The unintentional presence of chemicals means accidental contamination of food not regulated by current national or international safety standards in food or raw materials [1]. However, clear criteria for selecting these substances in the draft Guidelines were not proposed. Also, identifying and assessing the potential hazards of inadvertently present chemicals are necessary for further health risk assessment.

In the Russian Federation, methodological approaches to detecting and identifying chemical substances inadvertently present in foodstuffs have been proposed as a development of the provisions outlined in the draft Guidelines. The developed methodological approaches include four stages: analytical identification of chemical substances; integrated assessment of chemical hazards employing additional selection criteria followed by the application of a score and summation of points; categorizing chemical substances with the assignment of potential hazard categories and final stage – health risk assessment for selected chemicals based on the integrated index.

The aim of the investigation is the approbation of the methodological approaches by the example of canned meat for infants available in retail chains in Russia and the Socialist Republic of Vietnam.

2. MATERIALS AND METHODS

The analytical identification stage can be accomplished by:

- Gas chromatography-mass spectrometry (Gas chromatography-mass spectrometry for the identification of volatile organic compounds, pesticides, pharmaceuticals, persistent organic pollutants, et al.);
- Inductively coupled plasma mass spectrometry or atomic emission spectrometry/optical emission spectroscopy for the identification of metals and other elements;
- Recovery methods, including extraction, over-extraction, solid-phase extraction methods, affinity column chromatography, concentration, dilution, cineration, rectification, distillation methods, et al.

According to the results of analytical identification, all chemical substances detected in food products the content level of which is not regulated by sanitary and epidemiological requirements are ranked by several criteria. The criteria are the following: frequency of detection; potential danger; potential natural content, and the possibility of their entry into

food products and food raw materials in the process of their production. This is necessary to select the priority inadvertently present chemicals in the health risk assessment.

An inadvertently present chemical detected in a food product is considered potentially hazardous if it meets the following conditions:

- 90% or higher probability of matching with the mass spectra library with confirmation, using a standard substance sample;
- The presence of identified potentially hazardous chemicals in at least 50% of samples of food products or food raw materials of the same type;
- Availability of relevant sources of information on the possibility of developing adverse effects (non-carcinogenic, carcinogenic, embryotoxic, mutagenic, et al.) or the ability of the chemical to produce products with higher toxicity than the original substance.

It is advisable to use the toxicological characteristics of the identified substances as hazard quotients, for example, lethal dose (LD₅₀).

At the stage of integrated assessment, it is proposed to use the following criteria: toxicological, to a greater extent determining their potential danger depending on the toxicity class of the substance; the criterion characterizing the possibility of receipt of potentially hazardous substances at the stages of production and sale of products. These criteria include the possibility of entry during the preparation of food intended for consumption, migration from packaging, containers, reservoir, etc., as well as entry into the food product with the raw material.

In order to identify priority substances, toxicity scores were conducted according to the classification adopted by the Oxford Handbook of Hazardous Chemicals according to LD₅₀ for oral intake [2] (Table 1).

Table 1. Score according to the class of toxicity of substances established by LD₅₀ value (rats, intragastric, mg/kg)

<i>LD₅₀</i>	<i>TC</i>	<i>Description</i>	<i>Score</i>
≤1	I	Extremely toxic	6
1-50	II	Highly toxic	5
50-500	III	Moderately toxic	4
500-5,000	IV	Slightly toxic	3
5,000-15,000	V	Practically non-toxic	2
>15,000	VI	Relatively harmless	1

Note: TC - toxicity class

When assessing the priority of inadvertently present chemicals, criteria are used that take into account the possibility of migration in the preparation of food for consumption or the formation of new substances in the product during technological exposure (yes - 1 point;

no - 0 points), the possibility of migration from packaging, containers, reservoir, etc. (yes - 1 point; no - 0 points), the likelihood of their entry into the food product with the raw material (yes - 1 point; no - 0 points).

For the integrated assessment, the categorization is conducted according to the total score using Formula 1.

$$II = \frac{\sum_{n1+n4} n}{n1+n4}, \text{ where } (1)$$

II - integrated index;

n1...n4 - number of points according to the criteria.

At the categorization stage, depending on the value of the integrated index, the potential hazard category (PHC) of inadvertently present chemical substances is determined. These categories are the basis for making decisions on the choice of priority substances to assess the health risk (Table 2).

Table 2. Potential hazard categories of inadvertently present chemicals for health risk assessment and potential regulation

<i>PHC</i>	<i>Integrated index value</i>	<i>Potential hazard characteristics</i>
III	≤ 2	Low
II	3 - 5	Average
I	6 - 9	High

PHC - potential hazard category.

The inadvertent presence of chemicals with a high potential hazard in food products is considered a priority for risk assessment. The presented methodological approaches were tested on the example of canned meat for infant nutrition since this product is intended for the most sensitive group of the population.

3. RESULTS AND DISCUSSION

At the stage of analytical identification, the inadvertently present chemical substances in the selected product were determined by gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS). As a result, the identification revealed 20 inadvertently present chemical substances in 15 samples of the food product under study (Table 3).

At the stage of analytical identification, using the selection criteria (the probability of mass spectra matching with the library was 90% and the presence of more than 50% of food samples) allowed us to identify two groups of compounds that meet the selection criteria: N-nitrosamines and the phthalates group.

Table 3. Results of identification of unintentional chemical contaminants in 15 samples of canned meat for infant nutrition

<i>No</i>	<i>Substance</i>	<i>The frequency of occurrence in the samples, %</i>	<i>Mass spectrum library matching, %</i>
1	<i>N-nitrosodimethylamine</i>	93.3	> 90
2	<i>N-nitrosodiethylamine</i>	13.3	> 90
3	<i>Dibutyl phthalate</i>	93.3	> 90
4	<i>Diethyl phthalate</i>	93.3	> 90
5	<i>Phenol, 2,4-bis (1,1 - dimethyl ethyl)</i>	33.3	< 90
6	<i>Furfural</i>	26.6	> 90
7	<i>Ethyl thiocyanate</i>	33.3	< 90
8	<i>Acetic acid</i>	26.6	> 90
9	<i>Histamine</i>	13.3	< 90
10	<i>2-Butenoic acid (E570-crotonic acid)</i>	6.7	< 90
11	<i>Thymol</i>	6.7	> 90
12	<i>Pyridine-4-amine</i>	6.7	> 90
13	<i>Hexahydropyridine</i>	6.7	> 90
14	<i>Tuaminoheptane</i>	13.3	< 90
15	<i>Amiphenazole</i>	6.7	> 90
16	<i>Nimorazol</i>	6.7	> 90
17	<i>Pentobarbital</i>	6.7	> 90
18	<i>Amyl alcohol</i>	6.7	> 90
19	<i>Naphthalene</i>	6.7	> 90
20	<i>Ethosuximide</i>	6.7	> 90

At the integrated assessment stage, using additional criteria for selecting priority substances for further risk assessment, taking into account the LD₅₀, we calculated the integrated index and established the category of potential hazards of inadvertently present chemical substances (Table 4).

Table 4. Selection of priority potentially hazardous inadvertently present chemical substances in samples of canned meat for infant nutrition

No	Name of element/substance	CAS	Assessment of the probability of presence in the product (score)			Toxicity assessment				II PHC	
			Entry in raw materials	Probability of migration from packaging, containers, etc.	Probability of entry of substances during the preparation of a food product for consumption	LD ₅₀ , mg/kg	Reference	TC	Score		
1	<i>N-nitrosodimethylamine</i>	62-75-9	1	0	1	41	3	II	5	7	I
2	<i>N-nitrosodiethylamine</i>	55-18-5	1	0	1	280	4	III	4	6	I
3	<i>Dibutyl phthalate</i>	84-74-2	1	1	0	8000	5	V	2	4	II
4	<i>Diethyl phthalate</i>	84-66-2	1	1	0	8600	6	V	2	4	II

When selecting potentially hazardous inadvertently present chemical substances in samples of canned meat for infant nutrition for health risk assessment using methodological approaches developed in the Russian Federation, the study found that according to the PHC, N-nitrosamines (PHC- I) posed the highest potential hazard.

A health risk was assessed on the example of N-nitrosamines consumed with canned meat for infant nutrition sold in the Russian Federation and the Socialist Republic of Vietnam following the risk assessment methodology developed in Eurasian Economic Commission countries [7] and harmonized with generally accepted methodology [8].

At the stage of analytical identification, we conducted a quantitative study of the content of N-nitrosamines in canned meat for infant nutrition available in retail chains in Russia (20 samples) and the Socialist Republic of Vietnam (8 samples).

The analytical identification of N-nitrosamines was conducted using methods approved in Russia for determining N-nitrosamines in food products [9], which allow the determination of N-nitrosamines at low levels (LOD - 0.0002 mg/kg of product).

The results of chemical and analytical identification of N-nitrosamines in canned meat for infant nutrition are presented in Table 5.

Table 5. Concentrations of *N*-nitrosamines (mg/kg) in canned meat for infants produced in the Russian Federation and the Socialist Republic of Vietnam

<i>Substance</i>	<i>Russian Federation</i>	<i>Socialist Republic of Vietnam</i>
	<i>Concentration $\mu\text{g}/\text{kg}$</i>	
DMNA (N-dimethyl-nitrosamine)	4.9 \pm 0.8	0.6 \pm 0.1
MENA (N-methyl ethyl-nitrosamine)	0.76 \pm 0.12	1.0 \pm 0.16
DPNA (N-dipropyl-nitrosamine)	2.7 \pm 0.48	3.0 \pm 0.5
DBNA (N-dibuthyl-nitrosamine)	15 \pm 2.8	5.5 \pm 1
PIPNA (N-piperidin-nitrosamine)	3.2 \pm 0.6	0.4 \pm 0.07
PYRNA (N-pirrolidin-nitrosamine)	4.5 \pm 0.8	4.2 \pm 0.8
MORNA (N-morpholine-nitrosamine)	272.0 \pm 50	33.0 \pm 6.0
DPHNA (N-diphenyl-nitrosamine)	0.37 \pm 0.07	1.3 \pm 0.2

Note: n/d – not detected.

As a result of the toxicological characterization of the substances identified, the International Agency for Research on Cancer (IARC) [10] found that one *N*-nitrosamine was classified as a probable carcinogen, six as a possible carcinogen, and one as an unclassifiable carcinogen for humans (Table 6).

Table 6. Classification of carcinogenic hazards of chemical compounds according to IARC

<i>No</i>	<i>Compound</i>	<i>IARC classification</i>	<i>SFo</i>
1	<i>N</i> -methylethyl-nitrosamine (MENA)	2B	22
2	<i>N</i> -dipropyl-nitrosamine (DPNA)	2B	7
3	<i>N</i> -dibuthyl-nitrosamine (DBNA)	2B	5.4
4	<i>N</i> -piperidin-nitrosamine (PIPNA)	2B	9.4
5	<i>N</i> -pirrolidin-nitrosamine (PYRNA)	2B	2.1
6	<i>N</i> -diphenyl-nitrosamine (DPHNA)	3	0.0049
7	<i>N</i> -morpholine-nitrosamine (MORNA)	2B	6.7
8	<i>N</i> -dimethyl-nitrosamine (DMNA)	2A	0.000008

The non-carcinogenic effects of *N*-nitrosamines were manifested mainly as liver dysfunction.

An analysis of the oral intake reference dose (RfD) data for N-nitrosamines showed that DMNA, DPHNA, and DPNA had reference values of 0.000006, 0.02, and 0.25 mg/kg, respectively. Critical organs and systems for the adverse effects of these N-nitrosamines have been identified in several studies as liver, visual organs, blood, and developmental processes [11].

In evaluating the exposure of infants to N-nitrosamines from canned meat for infant nutrition, Vietnamese and Russian researchers conducted a sociological study and found that the average daily consumption of canned meat varies from 52 to 128 g per day in the Russian Federation (Table 7) and from 19.8 to 27.1 g per day for children in the Socialist Republic of Vietnam, depending on age [12] (Figure 1).

Table 7. Average daily consumption of canned meat (grams) by infants aged 6 months to 3 years in the Russian Federation

<i>Age</i>	<i>All types of meat products For the whole period of nutrition (grams)</i>
<i>6-12 months</i>	52
<i>13-24 months</i>	85
<i>25-36 months</i>	128
<i>Average for 2.5 years</i>	88

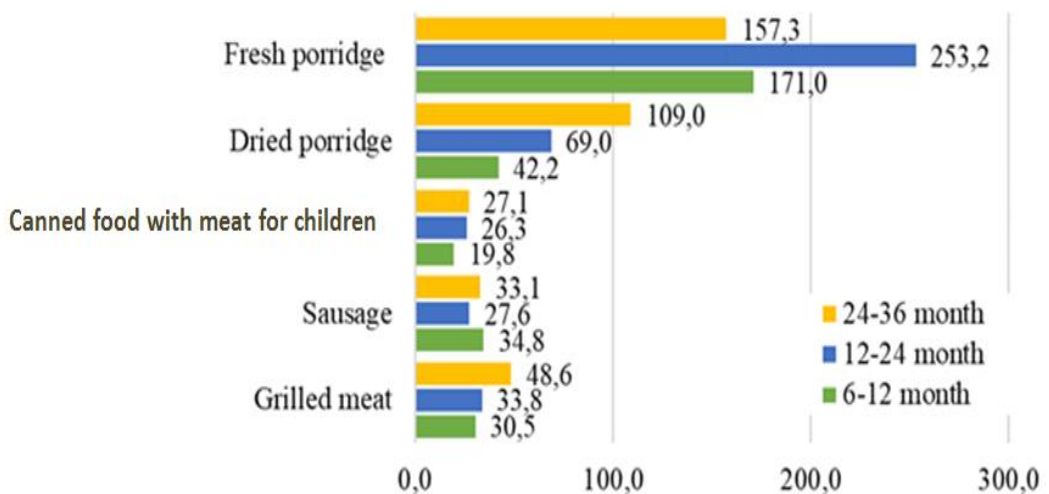


Figure 1. Average daily consumption of canned meat (grams) by infants aged six months to 3 years in the Socialist Republic of Vietnam

The specifics of calculating health risks for infants are the short period of consumption of canned meat and the body weight of infants (Table 8).

Table 8. The body weight of infants and the consumption volume of canned meat for infant nutrition in the Russian Federation and the Socialist Republic of Vietnam (SRV), depending on age

Age, months	6	7	8	9	10	11	12	18	24	30	36	Average for 2.5 years
Body weight according to WHO, kg	7.6	8	8.3	8.6	8.9	9.1	9.3	10.6	11.9	13	14.1	9.9
Consumption of canned meat in the SRV, g/day	20	20	20	20	20	20	20	26	26	27	27	20
Consumption of canned food in Russia, g/day	50	50	50	50	50	50	50	110	110	60	60	64

For the period of canned meat consumption (2.5 years), the average daily consumption of canned meat by infants in the Russian Federation and the Socialist Republic of Vietnam amounted to 0.064 kg and 0.02 kg per day, respectively.

As a result, lifetime average daily doses (LADD) associated with carcinogenic and non-carcinogenic effects were calculated. The doses obtained made it possible to assess the non-carcinogenic and carcinogenic risks due to the intake of N-nitrosamines with canned meat for infant nutrition.

Hazard quotients for oral intake of N-nitrosamines with canned meat for infant nutrition are given in Tables 9 - 10.

Table 9. Hazard quotients (non-carcinogenic (HQ) and carcinogenic (CR) for oral intake of N-nitrosamines with canned meat for infant nutrition in the Socialist Republic of Vietnam

	DMNA	MENA	DPNA	DBNA	PIPNA	PYRNA	MORNA	DPHNA
LADD non-carcinogenic	1.0×10^{-7}	2.0×10^{-7}	5.1×10^{-7}	9.3×10^{-7}	6.7×10^{-8}	7.1×10^{-7}	5.6×10^{-6}	2.2×10^{-7}
LADD carcinogenic	4.3×10^{-8}	8.6×10^{-8}	2.2×10^{-7}	4.0×10^{-7}	2.9×10^{-8}	3.0×10^{-7}	2.4×10^{-6}	9.4×10^{-8}
HQ	0.013	–	0.000002	–	–	–	–	0.00001
CR	2.2×10^{-6}	1.9×10^{-6}	1.5×10^{-6}	2.1×10^{-6}	2.7×10^{-7}	6.4×10^{-7}	1.6×10^{-5}	4.6×10^{-10}

Table 10. Hazard quotients for oral intake of N-nitrosamines with canned meat for infant nutrition in the Russian Federation

	DMNA	MENA	DPNA	DBNA	PIPNA	PYRNA	MORNA	DPHNA
LADD non-carcinogenic	2.6×10^{-6}	4.1×10^{-7}	1.5×10^{-6}	8.1×10^{-6}	1.7×10^{-6}	2.4×10^{-6}	1.5×10^{-4}	2.0×10^{-7}
LADD carcinogenic	1.1×10^{-6}	1.8×10^{-7}	6.2×10^{-7}	3.5×10^{-6}	7.4×10^{-7}	1.0×10^{-6}	6.3×10^{-5}	8.5×10^{-8}
HQ	0.3	–	0.00001	–	–	–	–	1.0×10^{-7}
CR	5.8×10^{-5}	3.9×10^{-6}	4.4×10^{-6}	1.9×10^{-5}	7.0×10^{-6}	2.2×10^{-6}	4.2×10^{-4}	4.2×10^{-10}

Assessment of the health risk to infants when consuming canned meat for infant nutrition containing N-nitrosamines on the example of products sold in the Russian Federation and the Socialist Republic of Vietnam revealed no excess hazard quotients (HQ < 1.0) in both territories.

However, the calculation of carcinogenic risks has shown that when canned meat for infants sold in the Russian Federation from 6 months to 3 years of age is consumed, a carcinogenic risk may be formed in infants of the corresponding group (CR > 1.0×10^{-4}).

Nevertheless, the risk assessment for N-nitrosamines consumed with canned meat for infant nutrition have several uncertainty factors. So, consumption of canned meat was assessed according to sociological study but not statistical data. Doses of consumed N-nitrosamines were calculated using standard values of body weight and average consumption data for 2.5 years. Also there were no reference doses for several N-nitrosamines. All these uncertainty factors can lead to under- or overestimation of risk and should be taken into account.

4. CONCLUSION

During the approbation of proposed methodological approaches to detecting and identifying chemical substances inadvertently present in foodstuffs it was found that N-nitrosamines have the highest potential hazard and they need further health risk assessment.

During the risk assessment by the example of canned meat for infant nutrition retailed in the Russian Federation and the Socialist Republic of Vietnam it was revealed that there was no excess hazard quotients in both territories. However, the calculation of carcinogenic risks has shown that when canned meat for infants retailed in the Russian Federation from 6 months to 3 years of age is consumed, a carcinogenic risk may be formed in infants of the corresponding group (CR for MORNA was calculated as 4.2×10^{-4}). The calculation of carcinogenic risk for canned meat retailed in Socialist Republic of Vietnam has shown the acceptable risk level (CR < 1.0×10^{-4}).

This difference between the Socialist Republic of Vietnam and the Russian Federation is due not only to the difference in the content of N-nitrosamines in the examined product but also to the greater volume of canned meat consumption in Russia.

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Đánh giá nguy cơ tiềm ẩn của các hóa chất không mong đợi có trong thực phẩm

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Tóm tắt

Trong phiên họp lần thứ 71 của Ủy ban điều hành Codex Alimentarius Commission, New Zealand đã đề xuất dự thảo Hướng dẫn phân tích rủi ro hóa chất không mong đợi có trong thực phẩm ở mức độ thấp, lưu ý rằng các văn bản quy định hiện nay không đề cập đến nhóm chất này. Phương pháp tiếp cận để phát hiện và xác định các chất hóa học không mong đợi có trong thực phẩm đã được nghiên cứu ở Liên bang Nga. Hướng tiếp cận của phương pháp được phát triển bao gồm bốn giai đoạn: Phân tích xác định các chất hóa học; Đánh giá tổng hợp các mối nguy hóa chất sử dụng các tiêu chí lựa chọn bổ sung, sau đó áp dụng cho điểm và tổng hợp điểm; Phân loại các chất hóa học với việc ấn định các chất có nguy cơ tiềm ẩn và giai đoạn cuối cùng - Đánh giá rủi ro sức khỏe đối với các hóa chất được lựa chọn dựa trên chỉ số tổng hợp. Các phương pháp tiếp cận phương pháp được trình bày đã được thử nghiệm trên ví dụ về thịt hộp để cung cấp dinh dưỡng cho trẻ sơ sinh được tiêu thụ ở Liên bang Nga và Việt Nam. N-nitrosamine được đánh giá là chất hóa học có khả năng gây nguy hiểm, có mặt không mong muốn trong các mẫu thịt hộp để làm dinh dưỡng cho trẻ sơ sinh được nghiên cứu để đánh giá rủi ro sức khỏe bằng cách sử dụng các phương pháp luận được phát triển ở Liên bang Nga. Đánh giá rủi ro sức khỏe đối với trẻ sơ sinh khi tiêu thụ thịt đóng hộp để bổ sung dinh dưỡng cho trẻ sơ sinh có chứa N-nitrosamine trên ví dụ về các sản phẩm được bán ở Liên bang Nga và Việt Nam cho thấy không có chỉ số nguy cơ vượt quá ($HQ < 1,0$) ở cả hai nước. Tuy nhiên, tính toán rủi ro gây ung thư đã chỉ ra rằng khi tiêu thụ thịt đóng hộp cho trẻ sơ sinh từ 6 tháng đến 3 tuổi ở Liên bang Nga, nguy cơ gây ung thư có thể hình thành ở trẻ thuộc nhóm tương ứng. Sự khác biệt này giữa kết quả đánh giá tại Việt Nam và Liên bang Nga không chỉ do sự khác biệt về hàm lượng N-nitrosamine trong sản phẩm được kiểm tra mà còn do khối lượng tiêu thụ thịt hộp lớn hơn ở Nga.

Từ khóa: nitrosamine, hóa chất vô tình xuất hiện, chất gây ô nhiễm, đánh giá rủi ro.

Note: Tiêu đề và tóm tắt tiếng Việt do Ban Biên tập biên dịch với sự đồng ý của tác giả / The Vietnamese title and abstract is translated by the Editorial Board with the agreement of the Author.