International Journal of Integrative Pediatrics and Environmental Medicine, V6, 2020, 20-29

Original research

THE CONTENTS OF NITRATES IN FOOD AND THEIR IMPACT ON THE HEALTH OF THE POPULATION OF MELITOPOL

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Abstract: An important characteristic of the quality and safety of foods of plant and animal origin is the content of common contaminants such as nitrates and nitrites. From 2017-2019, we investigated the nitrates and nitrites in food of plant and animal origin in Melitopol, Ukraine. Plant foods were purchased from supermarkets and farmers' markets in May (spring) and September (autumn). Meats were analyzed one year (2019). The largest concentrations of nitrates were observed in spring vegetables and strawberries from the farms that used common agro-technological methods. The lowest nitrate levels were found in the vegetables and fruit grown on local farms and sold at farmers' markets. Safe nitrate levels were found in fresh meat, but the sausage products contained dangerous levels. The nitrate and nitrite pollution of food affect the health status of the population of Melitopol, which is most often manifested in morbidity of the cardiovascular or digestive systems, oncopathology, and food poisoning.

Keywords: Food, Nitrates, Nitrites, Contaminants, Melitopol, Diseases of the population

INTRODUCTION Nutrition is one of the most important factors affecting the physical and mental capacities of the human body. Man is a heterotrophic organism, who consumes both plant and animal foods to obtain the proteins, fats, and carbohydrates to support life. Each food is characterized by both its nutritional value and by the presence of foreign, potentially dangerous compounds of anthropogenic or natural origin [1] as contaminants. Of the food contaminants, nitrates and nitrites are among the most widespread and dangerous [2] and therefore, the study of their contents in food products continues to be of importance.

Nitrates are colorless crystalline substances, salts of nitric acid HNO_3, which are a normal product of metabolism of nitrogenous substances of any organism. They are formed by the interaction of nitric acid with the corresponding metals, or their oxides or hydroxides. Nitrites are colorless crystalline substances, salts of nitric acid HNO_2, which are formed from nitrogen oxides. Organic nitrites are part of all plant and animal organisms [3].

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In the human body, nitrates are converted to nitrites that react with amines and amides to form nitrosamines, which have carcinogenic properties [3]. The formation of nitroso-compounds occurs during the interaction of nitric acid with secondary amines in food in the process of their culinary processing and also inside the body [4].

Nitrates enter the human body primarily with fruits and vegetables, and nitrites enter the human body primarily with meat products [3,5-10]. In the case of a balanced nutritional ration, 70-80% of the daily dose of nitrates and nitrites are consumed as vegetables, 5-10% with fruit and berries, and the rest with water, meat, fish, and cheese products [11].

Research [3,12] has shown that the nitrate content of plants depends upon many factors such as the species and variety, the age, the agro-technical methods of growth, the climate, the soil type, the intensity and duration of light, the degree of maturation, and the mode of storage of the plant to be eaten. Nitrates are mainly accumulated in the roots, the stems, the footstalks, and the large veins of the leaves. Nitrates are highest in green vegetables (lettuce, parsley, fennel, spinach) and the root-crops (red beets, carrots, radishes). Relatively few nitrates exist in tomatoes and potatoes. Early vegetables contain more nitrates than late vegetables. Vegetables grown in green-

houses typically contain 2-12 times more nitrates than vegetables grown in open soil.

Table 1 contains the nitrate concentrations of some vegetables and fruits according to the Scientific-Research Center of the Ministry of Health of Ukraine [6,13]. Additional sources of nitrates and nitrites are milk, cheese, meat, and fish. They originate from contaminated water and feed (Table 2) or from compoundsT added as preservatives and color stabilizers [11].

Depending on a person's daily diet, consumption of nitrates can oscillate from amounts that are safe to levels that become a risk.

Research has shown that nitrates and nitrites are characterized by a wide spectra of toxic effects [14-17]. Children, pregnant women, seniors, and individuals with chronic diseases of the respiratory system, nervous systems, or hematopoietic organs are at high risk.

According to World Health Organization recommendations on nutritional additives, the allowable daily dose of nitrates for adults is 5 mg/kg/day and for children, 2.5 mg/kg/day [6]. They suggest intake for children 6-12 months, 1-3 years, and 4-6 years be limited to 45, 57, or 141 mg/day, respectively.

The research of the contents of nitrates and nitrites in food and their impact on human health has world significance, because they are conducted in many countries around the world such as Egypt [18], Poland [13], Belgium [19], Iran [20], Spain [21] and other countries. The different effects of nitrates and nitrites on the human body are researched, including on vegetarians [13,22,23], and also the positive effects of nitrates on patients with chronic kidney disease [21].

MATERIAL AND METHODS The purpose of the article is to determine the contents of nitrates in foods of plant

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Vegetables and fruits	Average nitrate contents, mg/kg
Beet	1049.7 ± 158.3
Onion	381.6 ± 31.4
White cabbage	337.7 ± 33.3
Carrots	253.2 ± 9.7
Onions	237.9 ± 41.3
Cucumbers in green houses	237.8 ± 41.3
Cucumbers in arable lands	165.5 ± 12.9
Tomatoes in green houses	144.5 ± 16.7
Potatoes	108.7 ± 6.5
Melons	83.3 ± 8.3
Tomatoes in arable lands	76.4 ± 3.1
Apples	39.7 ± 5.3
Watermelons	37.9 ± 12.8

Table 1 Average calculated nitrate contents of vegetables and fruits in Ukraine [6]

Food of animal origin	Range of Nitrates, mg/kg
Canned vegetables and meat	470−3200
Sea fish	14.0–21.0
River fish	3.0–4.3
Sausage	2.5–3.9
“Doktorska” sausage	2.4−5.8
Chicken	2.1–4.0
Pork	1.4–5.4
Cheese	1.5–2.0
Pasteurized milk	1.1–14.0
Dairy products	0.5–6.0
Fresh beef	0–40

Table 2 Average calculated nitrate concentrations of animal foods in Ukraine, mg/kg [6,23]

and animal origin, and to analyze the presence of nitrate and nitrite of nutritional additives (E 250-253) in foods of animal origin.

Our research was conducted from 2017-2019 in Melitopol, Ukraine. We gathered data on the presence of nitrates and nitrites in the food supply, as well as statistical data from the town’s department of healthcare about the health status of the population of Melitopol [24-26].

Melitopol is a town in southeast Ukraine with a population of more than 153,000 people. It is a city with well-developed industry specialized in mechanical engineering (production of internal combustion engines, cars, tractor turbines, refrigeration machines, and hydraulic equipment) and the processing of agricultural raw materials (oil extraction, food caning and packing, and baking plants).

The objective of the research was to sample vegetables (potatoes, onions, carrots, tomatoes, cucumbers) and fruit apples, strawberries), and foods of animal origin (meat, hard cheese, ham, sausage, meat pates) purchased at 4 outlets in Melitopol. These included three supermarkets ("A" - 1 point, "B" - 2 points) and the local central market (3 points of farmer’s sales and 4 points of private sales). It is a place of trade of owners of private farmsteads (mainly pensioners) who independently grow agricultural products in personal plots using organic farming and without the use of chemicals.

The research on the presence of nitrates and nitrites in food was conducted in the laboratory of the Department of Ecological Safety and Rational Natural Use of Bogdan Khmelnitsky Melitopol State Pedagogical University. Nitrates were determined in plant products in May and September of 2017-2019 for a total of 6 sampling periods. Foods of animal origin were sampled 3 times in 2019.

The quantification of nitrate-ions in the food samples was carried out using two methods. The express-method used a digital nitrate-tester (SOEKS ® Model NUC-019-1, Kyiv, Ukraine) and the potentiometric method using the Ionomer I-160MI (Kyiv, Ukraine) with the ion-selective electrode ELIS-121NO3 K80.7. The divergence of results between the 2 instruments never exceeded 7%. For the study, 864 samples of plant foods and 21 samples of

animal products were analyzed.

Information on the nitrate content and the nutritional additives (E 250-253) of cheese and meat products was taken from the ingredients lists on the packaging of 21 types of these products. The information about the health status of the population of Melitopol was obtained from the reports of the Melitopol department of healthcare from 2017-2019 [24-26].

Results and Discussion The quantitative determinations of the concentration of nitrate-ions in the foods of plant origin are summarized (Table 3) with the maximum allowable concentration (MAC) for each food [6,21].

Nitrate concentrations in the spring potatoes exceeded the MAC in 38.8% of the samples. All autumn production exceeded the MAC. All of the potatoes purchased at the central market in the spring had high nitrate contents and in 27.8% of the autumn samples high nitrate concentrations were found. Potatoes grown at home did not exceed the MAC.

Onions sold in the supermarkets exceeded nitrate MAC in 100% and 55.5% in the spring and autumn samples, respectively. Excesses over the allowable nitrate concentration were found in all onion samples sold at the farmers' markets. Onions grown at home had nitrate concentrations approximately 15% lower than retail spring samples and autumn samples had concentrations below the MAC.

In the harvests of 2017-2019, carrots sold in the supermarkets in the spring displayed nitrate that slightly exceeded the normative indicator 44.4% of the time, and all autumn carrots were below the MAC. Carrots from the farms exceeded MAC in 100% of the spring samples and in 50% of the autumn samples. Carrots grown at home did not exceed the normative indicator.

Tomatoes sold in the supermarkets exceeded MAC in the spring samples but were near MAC in the autumn.

In the harvests of 2017-2019, spring cucumbers slightly exceeded the MAC in 44.4% of the samples and all autumn cucumbers were close to MAC. Local farm products and home-grown cucumbers had nitrate concentrations near the MAC. Apples did not exceed the normative indicator. The lowest nitrate levels in apples were found in the spring, due to the lengthy storage of the fruit. The nitrate contents decreased with the ripening of the fruit.

In strawberries sold in the supermarkets, the nitrate contents in 100% of the spring and in 83.3% of the autumn samples were above the normative indicator from 0.2 to 0.9 times. In strawberries grown at home, the nitrate contents of all spring and autumn samples did not exceed the MAC.

The seasonal dynamics of plant food production (Figure 1) shows that the MAC for nitrates was exceeded in spring samples of tomatoes (100%), onions (89%), and strawberries (75%). The nitrate concentrations in carrots, cucumbers and potatoes exceeded MACs in 63.88, 58.33 and 44.44% of cases, respectively. In the autumn periods excesses were less frequent. Cases that exceeded the MACs of nitrates in the autumn samples included strawberries (63.89%), onions (58.33%), and tomatoes (41.67%). The nitrate MACs of potatoes, cucumbers, and carrots were exceeded 16.67, 16.66, and 11.11% of the time, respectively.

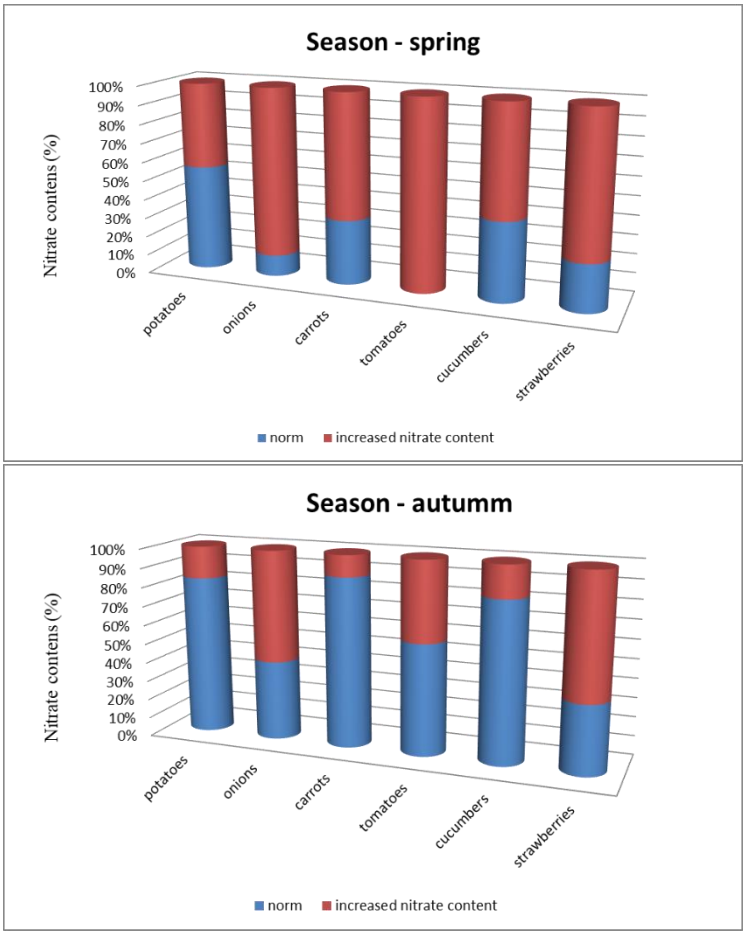

Figure 1 Part (%) of nitrate content in food products of plant origin of the Melitopol trade network (2017-2019)

Table 3 Nitrate concentration in food of plant origin in Melitopol (min-max) and mean, mg/kg

Name of point of sale	**POTATOES** (Maximum allowable nitrate concentration (MAC) = 250 mg/kg					
	2017		2018		2019	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Supermarket A	224-249 237.67	155-186 173.5	229-239 234.0	168-218 194.83	251-268 262.67	190-206 199.83
Supermarket B	242-278 263.67	169-197 182.67	216-245 234.33	187-211 198.33	246-271- 259.17	173-202 187.5
Central Market: Farming Producers	342-437 397.17	204-286 267.83	328-399 372.0	229-274 255.17	339-482 422.17	203-260 243.17
Central Market: Private Producers	164-189 179.33	105-138 124.83	149-202 188.33	129-160 145.17	167-215 196.67	131-196 164.0
ONIONS MAC=80 mg/kg						
Supermarket A	99-118 109.0	75-99 92.83	95-98 96.66	72-97 88.67	85-118 104.0	77-115 104.67
Supermarket B	98-126 117.5	70-107 98.17	96-116 111.33	79-98 89.17	88-105- 97.5	73-102 94.83
Central Market: Farming Products	152-337 269.5	124-166 143.0	164-309 252.83	119-184 167.5	183-336 276.67	113-169 150.33
Central Market: Private Products	79-88 83.33	32-59 50.17	81-92 87.83	49-57 53.83	77-102 94.33	48-64 57.17
CARROTS MAC=250 mg/kg						
Supermarket A	204-269 247.5	185-231 209.17	209-264 247.5	183-218 204.5	171-308 272.33	196-226 211,0
Supermarket B	232-298 269.17	189-217 205.33	198-279 246.0	164-201 189.33	205-263 254.33-	173-202 195.5
Central Market: Farming Products	289-349 325.33	194-248 225.83	284-367 343.5	209-274 254.17	211-391 348.5	239-282 228.5
Central Market: Private Products	184-240 221.33	87-160 139.83	189-252 199.33	109-187 166.17	160-205 189.5	71-144 118.5
TOMATOES MAC=50 mg/kg						
Supermarket A	185-219 204.5	114-164 142.33	196-214 205.5	89-118 107.17	201-228 216.33	90-106 100.33
Supermarket B	187-239 116.17	125-157 146.5	189-216 204.67	97-121 110.5	198-241 223.33	73-102 96.5
Central Market: Farming Products	192-341 285.67	144-216 196.67	204-309 271.5	119-154 126.5	171-302 244.67	133-160 149.17
Central Market: Private Products	174-210 199.17	59-108 95.5	181-202 193.17	44-92 76.67	191-225 206.33	76-99 90.5
CUCUMBERS MAC=150 mg/kg						
Supermarket A	146-159 151.0	110-144 132.67	149-154 151.5	120-139 131.67	125-160 146.67	114-146 135.33
Supermarket B	139-154 147.5	125-146 139.17	142-156 151.0	114-141 131.83	138-152 146.83	109-138 125.17
Central Market: Farming Products	152-171 163.5	134-166 154.0	154-183 171.0	119-154 138.67	161-193 180.0	133-179 156.67
Central Market: Private Products	114-130 124.0	59-108 76.33	121-147 134.0	44-92 63.83	119-135 126.33	76-112 96.33
STRAWBERRIES MAC= 60 mg/kg						
Supermarket A	91-116 106.5	54-77 68.17	79-106 94.17	56-76 67.0	85-100 92.83	60-86 73.17
Supermarket B	96-108 101.33	50-59 54.67	88-97 89.0	58-66 60.37	82-105- 96.17	53-74 68.83
Central Market: Farming Products	129-147 138.67	84-113 99.17	126-163 146.0	90-107 99.83	133-194 171.17	91-109 101.0
Central Market: Private Products	41-57 50.5	22-39 31.33	46-57 51.33	38-47 42,5	49-55 51.67	34-46 39.83

Our results confirmed the presence of high concentrations of nitrates in each plant food. Many of the spring vegetables and fruits were grown in a green house, with poor light, high moisture, and high concentrations of nitrogen-containing fertilizers in the soil.

Our analysis demonstrates that large concentrations of nitrates do not accumulate in fresh meat, so it is a safe food for the population. The sausage products contain the greatest concentrations of nitrates among the researched foods. The highest concentration of nitrates, which exceeded the MAC (200 mg/kg) 5.86 times is boiled-smoked sausage "Servelat", followed by the boiled sausage "Doktorska" (2.97 times), sausage "Molochni" (2.42 times), and sausage with cheese (1.65 times).
Nitrates were added during the productionn of these products in very high concentrations in the form of food additives (Table 4).
Modern food technology involves the wide use of food additives. Salts of nitrates and nitrites are added to the composition of meat products, sausage, and cheese to improve their shelf life and organoleptic characteristics (taste, color, odor, and feel). In Ukraine, the food additives, E 250-sodium nitrite, E 251-sodium nitrate, and E 252-potassium nitrate may be added to foods, but in other countries these additives are banned. Food labels on all 34 investigated samples of boiled and boiled-smoked sausage, ham, and meat pate listed the additives E 250 and/or E 252. The additives were used to improve shelf life and color. The food additive E 251 was not detected in any analyzed animal product.

Our results confirmed that nitrates are detected in sausage products at the highest and most dangerous levels. Consumption of food products in this category should be limited.

Foods of plant and animal origin are the main sources of nitrates and nitrites to the human body. Our research has shown that these contaminants are present in the nutritional ration of the population of Melitopol, at often dangerous levels. Therefore, we tried to analyze the medical-demographic situation for the people of Melitopol.

The toxicity of the nitrate and nitrite load is most often manifested in morbidity of the cardiovascular and digestive systems, oncopathology, and food poisoning [14-17]. According to annual statistics for Melitopol, the number of the diseases connected with the consumption of environmentally contaminated food increased rapidly [24-26].

Today in Melitopol, the main health issue faced by the population is cardiovascular disease. From 2017-2019 an increase in deaths caused by circulatory system disease was noted. Annual increases in the number of cases of

Food	**Nitrate content, mg/kg (MAC * nitrates = 200 mg/kg)**	**MAC for nitrates, times**
Chicken meat	87	0
Pork	102	0
Beef	123	0
The highest-grade sausage "Molochni"	684	2.42
The highest-grade sausage with cheese	530	1.65
Boiled sausage of the highest grade "Doktorska"	795	2.97
Boiled-smoked sausage of the highest grade "Servelat"	1372	5.86

Table 4 Nitrate concentrations in food from an animal source for the population of Melitopol in 2019 (n = 3 samples of each product)

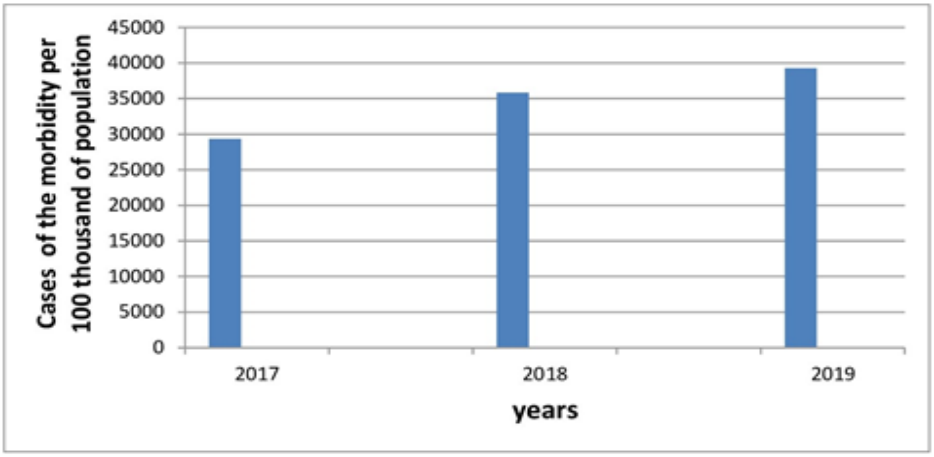

Figure 2 Cardiovascular Disease in Melitopol: 2017-2019

cardiovascular insufficiency, hypertension, myocardial infarction, coronary artery disease, stroke, atherosclerosis, and vegetative-vascular dystonia (Figure 2) were reported.

Of special concern is the increase in the number of strokes in young and middle-aged people in Melitopol (Figure 3). Strokes in children have been observed.

Among the known risk factors for cardiovascular pathology are the toxic effects of nitrates and nitrites. The high concentrations of these toxins in the sausage products and fresh vegetables, especially consumed by the children and the young people of Melitopol are particularly worrisome.

The origin and development of human cancer is a major consequence of pollution. Nutrition plays an important role in the development of cancer and accounts for 35% of cases [14]. Intestinal, rectal, and mammary gland cancers are closely related to nutrition [14]. Nitrates and nitrites are carcinogens [15]. Malignant tumors place second in the cause of morbidity and mortality in Melitopol. For 2017-2019, 2483 new cases of malignant tumors were reported in Melitopol [24-26]. During this period the frequency of cancer steadily increased (Table 5).

Oncopathology of the digestive system and the peritoneal organs was as high as 26.7% (Figure 4) which in many cases could be related to the nitrate and nitrite pollution of food.

Among the population of Melitopol, the number of cases of digestive organ disease increased every year. In 2019, 18.9 thousand cases of digestive diseases were reported per 100 thousand population (in 2017 it was 17 thousand

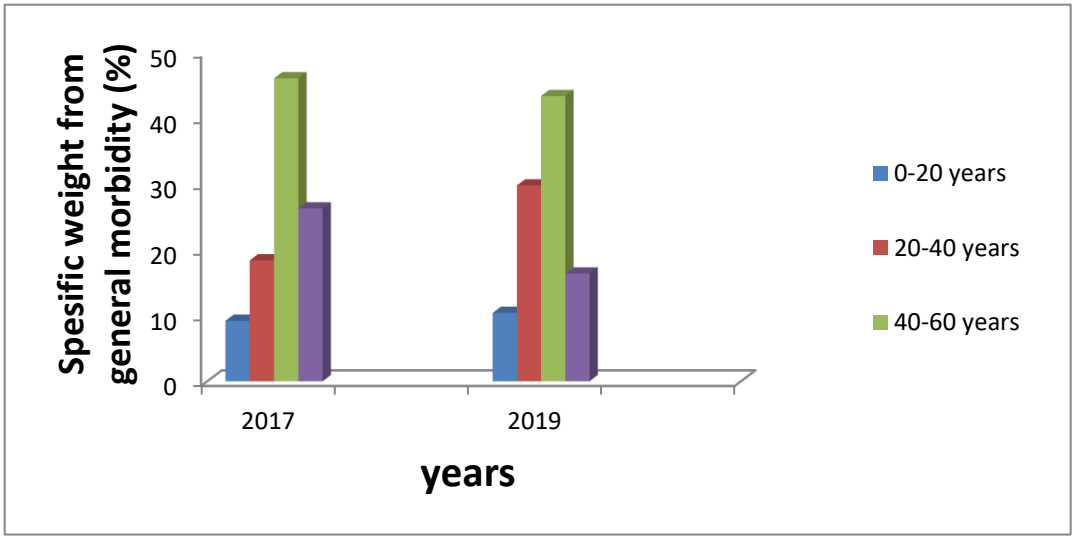

Figure 3 Cardiovascular disease by age in Melitopol (2017, 2019)

Year	2017	2018	2019
The absolute number	780	834	869
Frequency in population (%)	0.49	0.53	0.55

Table 5 Cancer of population of Melitopol (2017-2019)

per 100 thousand population). Among the digestive diseases, gastritis and duodenitis (24.3%), cholecystitis and cholangitis (19.8%), gastric and duodenal ulcer (14.9%), disease of pancreas (11.5%), and chronic hepatitis (5%) were the most common in the town. Digestive diseases were the fourth most common cause of death in the population. Cirrhosis of the liver, pancreatitis, and gastric ulcer were the most common causes in this group.

In Melitopol, the total number of food poisoning cases doubled for the period 2017-2019 and one of the reasons for the increase may be pollution of the food with nitrates and nitrites [26].

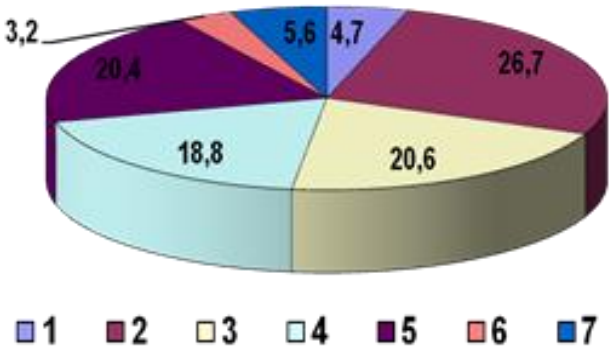

Figure 4 Frequency of oncopathology (%) among the population of Melitopol (2017-2019): 1 - mouth and throat; 2 - digestion and peritoneum; 3 - breathing; 4 - bones, connective tissues, skin, mammary gland, thyroid and other endocrine glands; 5 - urogenital system; 6 - nervous system and organs of sense; 7 - lymphatic and hematopoietic tissue.

Conclusion: High concentrations of nitrates in food of vegetable origin from the Melitopol trade network were observed. The greatest number of nitrates were found in spring vegetables and strawberries, which are produced by the farms, where modern agro-technological techniques were employed. The lowest concentrations of nitrates were found in the vegetables grown in the farmland using predominantly organic fertilizers.

We found that concentrations of nitrates were higher in the spring period than in the autumn. The levels dangerous to human health were the nitrate concentrations in the spring products such as tomatoes - 100%, onions - 88.89%, strawberries - 75%, carrots - 63.88%, cucumbers - 58.33%, and potatoes - 44.44%; and in autumn: strawberries – 63.89%, onions – 58.33%, tomatoes – 41.67%, potatoes – 16.67%, cucumbers – 16.66%, and carrots – 11.11%.

The nitrate contents of fresh meat indicated it was a safe food for Melitopol citizens, and the most dangerous foods of animal origin included the sausage products, which in all samples exceeded the MAC from 1.65 to 5.86 times.

The nitrate and nitrite pollution of food affected the health of the population of Melitopol, which was most often manifested in the increase of morbidity of the cardiovascular and digestive systems, oncopathology, and food poisoning. While it is difficult to eliminate the use of these contaminants in the food industry today, it is necessary to constantly control the quality of food products and to limit the consumption of the most nitrate-containing plant and animal products in the nutritional ration.

Disclosure: The authors have not conducted any research on animals or people for this article.

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