AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 3, pp. 559-577
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.1:632.4:615.9

doi: 10.15389/agrobiology.2021.3.559eng
doi: 10.15389/agrobiology.2021.3.559rus

MULTI-MYCOTOXIN SCREENING OF FOOD GRAIN PRODUCED IN RUSSIA IN 2018

M.G. KISELEVA[1, ✉], I.B. SEDOVA[1], Z.A. CHALYY[1], L.P. ZAKHAROVA[1], T.V. ARISTARKHOVA[1], V.A. TUTELYAN[1, 2]

[1]*Federal Research Centre of Nutrition, Biotechnology and Food Safety,* 2/14, Ust'yinskii per., Moscow, 109240 Russia, e-mail mg_kiseleva@ion.ru (✉ corresponding author), isedova@ion.ru, chalyyz@list.ru, zaharova@ion.ru, t.aristarkhova@yandex.ru, tutelyan@ion.ru;
[2]*Sechenov First Moscow State Medical University,* 8/2, ul. Trubetskaya, Moscow, 119992 Russia
ORCID:
Kiseleva M.G. orcid.org/0000-0003-1057-0886
Sedova I.B. orcid.org/0000-0002-6011-4515
Chalyy Z.A. orcid.org/0000-0002-9371-8163
Zakharova L.P. orcid.org/0000-0001-7355-5259
Aristarkhova T.V. orcid.org/0000-0001-9496-8626
Tutelyan V.A. orcid.org/0000-0002-4164-8992

The authors declare no conflict of interests.
Acknowledgements:
The authors thank the employees of the Federal Service for Surveillance on Consumer Rights Protection and Human Welfare, its regional Centers for Hygiene and Epidemiology for many years of cooperation, collection and provision of grain and grain products. The authors also express their gratitude to the reviewer for comments and suggestions which greatly contributed to the structuring of the results and a more complete discussion.
The study was carried out within the framework of research program 0529-2018-0113 "Development of the methodological and regulatory framework to ensure modern requirements for the food quality and the development of technologies for assessing the effectiveness of specialized food products"
Received January 21, 2021

Abstract

Accumulation and analysis of data concerning mycotoxins in food grain, their co-occurrence and concentration are essential for health risk analysis and management. Mycotoxins were analyzed in 162 samples of food wheat, barley, maize, oat and rye harvested in seven Federal Districts: Central, Volga, Urals, Siberian, Far Eastern, Southern and Northern Caucasus in 2018. High-performance liquid chromatography coupled to tandem mass spectrometry was used to detect 28 analytes: regulated mycotoxins deoxynivalenol (DON), T-2 toxin (T-2), zearalenone (ZEA), fumonisins B_1 and B_2 (FB_1 and FB_2), aflatoxin B_1 (AFL B_1), ochratoxin A (OTA), their derivatives 3- and 15-Acetyl-DON, nivalenol (NIV), fusarenone X (FUSX), HT-2 toxin (HT-2), T-2 triol, neosolaniol (NEOS), α- and β-zearalenol (α- and β-ZEL), aflatoxins B_2, G_1, G_2 (AFL B_2, G_1, G_2), sterigmatocystin STC; *Alternaria* mycotoxins tentoxin (TE), altenuene (ALT), alternariol (AOH), its methyl ether (AME); citrinin (CIT), citreoviridin (CTV), mycophenolic acid (MPA) and cyclopiazonic acid (CPA). Most wheat samples from Central, Volga, Urals and Siberian Federal Districts were positive for Alternaria toxins, while deoxynivalenol (DON) was discovered in the wheat from the Krasnodar region. ZEA, T-2 and HT-2, OTA, CIT and MPA were present in wheat samples also. FB_1 or FB_1 + FB_2 and DON (DON or DON + 15-AcDON) prevailed in corn from the Southern and the Northern Caucasus regions. MPA and NEOS were detected in a third of studied corn samples, while *Alternaria* toxins were absent. Barley from the South of Russia was mostly contaminated with T-2 and HT-2 alongside FB_1. Like wheat, most barley samples from Central, Volga, Urals and Siberian Federal Districts were positive for *Alternaria* toxins. The occurrence of *Alternaria* toxins in rye and oat samples was high regardless of region of origin. T-2 and HT-2, NEOS and CIT were detected in these samples also. However, DON was not found in any sample of barley, rye, or wheat. To the best of our knowledge, we are the first to report CTV in food grain of wheat, barley and corn from Russia. Thus, the detected mycotoxins pattern of food grain proved to depend on the crop and the grain origin. The results correlate well with reported data on fungal contamination of cereals and mycotoxins found in feed. High OTA occurrence (7.4 % of all samples) with 45 % positives over maximum level should be noted concerning safety assurance.

Keywords: food grain, wheat, barley, corn, rye, oat, mycotoxins; deoxynivalenol, T-2 and HT-2 toxins, zearalenone, fumonisins, aflatoxins, ochratoxin A, nivalenol, fusarenone X, T-2 triol, neosolaniol, zearalenols, sterigmatocystin, tentoxin, altenuene, alternariol, citrinin, citreoviridin, mycophenolic acid, cyclopiazonic acid, co-contamination, HPLC-MS/MS

Mycotoxins, the secondary metabolites of microscopic fungi are inevitable natural contaminants of agricultural products. Cases of mycotoxycoses in humans and animals have been reported in Japan, Brazil, the USA, Europe, China, the Soviet Union, and African countries [1]. Several hundreds of mycotoxins have been described. They have different toxic effects and differ in incidence and the levels in substrates. The most hazardous to human health are fusariotoxins deoxynivalenol (DON), T-2 toxin, zearalenone (ZEA), fumonisins B_1 and B_2 (FB_1 and FB_2), as well as metabolites of *Aspergillus* and *Penicillium* fungi — aflatoxin B_1 (AFL B_1) and ochratoxin A (OTA). Many countries have established maximum allowable levels (MALs) for these mycotoxins in food grains and grain-based products. In the countries of the Customs Union, there are technical regulations for food grain [2, 3] which establish the MALs for DON in wheat and barley at 700 and 1000 μg/kg, respectively, and for T-2 toxin in any grain at 100 μg/kg. The content of ZEA in the wheat, barley and corn grain cannot exceed 1000 μg/kg, and the total amount of FB_1 and FB_2 in corn cannot exceed 4000 μg/kg. The MAL for AFL B_1 and OTA is 5 μg/kg.

In the last decade, the development of analytical methods have significantly expanded the range of detectable mycotoxins. Universal, sensitive and selective method of high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) simultaneously recognizes tens [4-6] and even hundreds [7, 8] secondary metabolites of microscopic fungi.

Depending on the structure, incidence and danger to humans, mycotoxins are conventionally assigned to i) legally regulated mycotoxins and their structural derivatives, ii) Alternaria toxins, and iii) other mycotoxins the prevalence of which in plant-derived food products has not been fully studied. Structural derivatives are usually associated with regulated mycotoxins, and since most of them also have toxic effects, group MALs or conditional tolerable daily intake (CTDI) are used as the food safety parameters. For example, in the EU countries, both AFL B_1 and also the total AFL (AFL B_1 + B_2 + G_1 + G_2) in foodstuffs are under regulation [9]. CTDI values have been established for DON and its derivatives (3-AcDON, 15-AcDON and DON-3-glucoside) [10], T-2 toxin and its derivatives (HT-2 toxin, diacetoxyskirpenol, T-2 triol and tetraol, neosolaniol NEOS, their glycosides) [11], ZEA and its derivatives (glycosides and ZEA sulfates, α- and β-zearalenols — α-ZEL and β-ZEL, etc.) [12], fumonisins (FB_1, FB_2, FB_3 and FB_4) [13]. Alternariol (AOH) and its methyl ester (AME), tentoxin (TE), altenuene (ALT), tenuazonic acid, altertoxins, and their modified forms are the common secondary metabolites of *Alternaria* fungi in grain. The HPLC-MS/MS detects both the above-mentioned and little-studied mycotoxins, e.g., moniliformin, enniatins, beauvericin, sterigmatocystin (STC), mycophenolic acid (MPA), citreoviridin (CIT), cyclopiazonic acid (CPA). etc.

The incidence and levels of mycotoxins in grain directly depends on the prevalence of the mycotoxin producers. In the Russian Federation, *Fusarium* and *Alternaria* infections of cereals are most common [14]. In Russia, the grain levels of DON, T-2 toxin, ZEA, FB_1 and FB_2 (the secondary metabolites of the *Fusarium* micromycetes) are under regulation. DON derivatives include its acetyl derivatives, nivalenol (NIV), fusarenone X (FUSX), as well as glycosides and sulfo derivatives. Their main producers are *F. graminearum*, *F. culmorum*, and *F. cerealis*, and, depending on the geographic location, strains predominantly synthesizing NIV or DON + 3-AcDON or DON + 15-AcDON are dominant, a mixed chemotype is also possible [15-17]. A study of grain harvested in 2005-2010 showed that, on average, in Russia, the contamination for DON is much lower than the maximum allowable level (MAL) [18, 19], grain from the Southern

Federal District and North Caucasian Federal District is the most contaminated [20]. These territories, along with the Far Eastern Federal District, are traditionally the areas of grain fusarium in Russia [17, 21]. However, due to climate change and the seed exchange, more aggressive pathogens are gradually moving northward. For example, *F. graminearum*, a producer of DON and ZEA, appears in grain from northwestern Russia [22]. Since 2003, in the grain-producing regions of Western Europe, *F. culmorum* has also been replaced by a more thermophilic and toxinogenic species *F. graminearum* [16, 23].

The T-2 toxin group includes HT-2 toxin, T-2 triol, diacetoxyskirpenol, NEOS and their derivatives. Their main producers are *F. sporotrichioides*, *F. langsethiae* and *F. poae*. Contamination with these mycotoxins is especially typical for oats and barley (up to 75-95%), to a lesser extent for wheat, and for corn grain the proportion of positive samples is less than 22% [24]. The mycological studies indicate the widespread prevalence of T-2 group toxin producers in grain from the Krasnodar and Stavropol Territories [25], the Central and North-Western Federal Districts [26], in the Trans-Urals (on the example of Kurgan, Sverdlovsk, Tyumen, Chelyabinsk regions) [27]. For T-2 and HT-2 toxins in oats, barley and wheat grain, the rate averaged 59% [27]. T-2 triol and diacetoxyscirpenol (DAS) were also identified. Moreover, contamination with derivatives of T-2 toxin turned out to be more typical for oats and barley than for wheat. This is consistent with data from Croatia [28] and the results obtained for the domestic food grain harvests of 2008-2010 where T-2 toxin and HT-2 toxin contamination rates were 5-18% for wheat, 0-57% for rye, 21-27% for barley, and 13-50% for oats [18].

ZEA and its derivatives are metabolites of *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. crookwellense*, and *F. semitectum* [27]. In Russia, ZEA was detected in 7% of maize food grain samples in 2000-2016, with toxin levels ranging from 5 to 315 μg/kg [29]. In 2005-2010, 2-8% wheat samples, 0-39% barley samples, and 12-67% oat samples were contaminated with ZEA [18].

F. verticillioides and *F. proliferatum*, the main FB producers, mainly affect maize and sorghum [13]. FB_1 and FB_2 abundance in corn harvested in 2000-2016 in Russia exceeded 85% and 50%, respectively, and in 10% of 271 samples the FB level exceeded the MAL [30]. There is also information about the detection of FB_1 in the fodder grains of barley and wheat [31].

Secondary metabolites of *Aspergillus* and *Penicillium*, the AFL and OTA pose the highest risks to human health. During the monitoring of mycotoxins in Russian food grain in 2013-2016, three of 49 studied maize samples (6%) contained AFL B_1, with exceeding the MAL in two samples [30]. Survey of wheat, rye, barley and oats food grain harvested in 2003 and 2004 showed OTA contamination of 6%, 34%, 16% and 8% of the samples, respectively. In 2.5% of 272 samples tested, the OTA level exceeded the MAL [32]. In grain of 2012-2014 harvests, only two samples (1%) contained this toxin [20].

Alternaria metabolites are the second most abundant in cereals after fusariotoxins [33]. They exhibit an immunomodulatory effect, AOH and AME are genotoxic agents [34]. The ubiquitous abundance of *Alternaria* micromycetes in cereals in Russia was shown using samples from the republics of the North Caucasus [35], Stavropol and Krasnodar Territories, regions of the Central Federal District [36], and the Urals [37]. In wheat, oats, and barley grain from the regions of the Ural Federal District, TE, tenuazonic acid, AOH, and AME were identified.

Information on the prevalence of other mycotoxins in domestic grain is extremely sketchy. In wheat, barley and oats (56 samples in total) from the Trans-Urals, moniliformin and beauvericin were detected (12.5% and 34.0% of samples, respectively) [27]. In Russian food grains and grain-based product, the

contamination rates for STC (a biogenic precursor of AFL B_1) reached 8% at 150 μg/kg [38]. The European Food Safety Authority (EFSA) has estimated that no more than 1.5-8.0 μg/kg of STC in cereals and grain products is safe [39]. Compared to AFL B_1, STC is less toxic but much more common. Wheat from China had six times more samples with STC contamination than with AFL B_1 [40]. It is also of interest to determine the little-studied mycotoxins CIT, CPA and MPA in food grains. CIT and OTA are often detected together, both mycotoxins are nephrotoxic, and their synergistic effect is possible. Previously, a high rate of CIT and OTA co-contamination in feed was shown [41]; data on the food grain contamination are not available. MPA, a common contaminant of plant products, can reach several milligrams per kilogram. MPA has no pronounced toxic properties, but in high concentrations is an immunosuppressant [42]. CPA is cytotoxic, capable of suppressing the immune system and often found together with AFL [43].

Simultaneous measurements of regulated mycotoxins, their derivatives, secondary metabolites of *Alternaria* and poorly studied secondary metabolites of micromycetes in grain allows assessing the compliance with current safety standards, the level of co-contamination, and a range of probable mycotoxin producers. Such information will, on the one hand, allow a more complete assessment of the risks to human health caused by grain co-contamination with regulated and non-regulated mycotoxins, and, on the other hand, identify potential threats caused by a change in a potential of mycotoxicogenic species.

This paper is the first to report the abundance of contamination with 28 mycotoxins for food grain of five crops (wheat, corn, barley, oats and rye) from the Central, Southern, Volga, Ural, Siberian, North Caucasian and Far Eastern federal districts (21 subjects in total). The analytes were regulated mycotoxins deoxynivalenol (DON), T-2 toxin (T-2), zearalenone (ZEA), fumonisins B_1 and B_2 (FB_1 and FB_2), aflatoxin B_1 (AFL B_1), ochratoxin A (OTA), their derivatives 3- and 15-Acetyl-DON, nivalenol (NIV), fusarenone X (FUSX), HT-2 toxin (HT-2), T-2 triol, neosolaniol (NEOS), α- and β-zearalenol (α- and β-ZEL), aflatoxins B_2, G_1, G_2 (AFL B_2, G_1, G_2), sterigmatocystin STC; *Alternaria* mycotoxins tentoxin (TE), altenuene (ALT), alternariol (AOH), its methyl ether (AME); citrinin (CIT), citreoviridin (CTV), mycophenolic acid (MPA) and cyclopiazonic acid (CPA). This is the first systemic assessment of food grain co-contamination with 3- and 15-AcDON, NIV, FUSX, T-2 triol, NEOS, α- and β-ZEL, TE, ALT, AOH, AME, MPA, CPA, CIT and CTV in Russia. For the first time, CTV was revealed in wheat, barley and corn food grains.

The study aimed at the analysis of contamination of the main types of food grains from the regions of Russia with regulated mycotoxins, their structural derivatives, secondary metabolites of *Alternaria* fungi, and poorly studied mycotoxins.

Material and methods. Food grain harvested in 2018 (162 samples in total) were provided by branches of the regional Centers for Hygiene and Epidemiology of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare of the Central (Tula, Tambov, Kursk, Voronezh, Lipetsk, Belgorod and Orel provinces), Southern (Krasnodar Territory and Rostov Province), Volga (Penza, Saratov, Orenburg, Samara provinces and the Republic of Tatarstan), Ural (Tyumen and Chelyabinsk provinces), Siberian (Novosibirsk, Omsk provinces and Altai Territory), North Caucasus (Stavropol Territory) and the Far Eastern (Amur Province) Federal Districts of Russia. Samples were collected according to GOST R ISO 24333-2011 (Grain and products of its processing. Sampling. Moscow, 2013) from homogeneous batches stored at grain receiving and processing enterprises. In total, 114 samples of wheat grain, 18 samples of barley, 14 samples of corn, 8 samples of oats, and 8 samples of rye were analyzed.

Mycotoxins were determined by HPLC-MS/MS using an Agilent 1100 chromatographic system and an Agilent TQ 6410 triple quadrupole mass spectrometric detector (Agilent Technologies, USA; positive electrospray ionization at atmospheric pressure with the multiple reaction monitoring MRM mode). The voltage on the capillary of the detector ion source was 4000 V, the source temperature was 100 °C, the temperature of the drying gas (nitrogen) was 350 °C; the nebulizer pressure 60 psi (4.14 bar). The analytes were chromatographically separated (a Zorbax SB-C18 column, 150×4.6 mm, sorbent particle diameter 3.5 μm; 25 °C; the gradient elution mode). Mobile phase A was water:acetonitrile (95:5), phase B was acetonitrile. Both phases are acidified with formic acid (0.1 vol.%). Gradient scheme: start at 0% B, linear increase to 95% B over 30 min, 95% B up to 36 min, linear decrease to 0% B over 1 min; column equilibration for 6 min. The total chromatography time was 43 min, the sample volume was 20 μl. Each sample was analyzed twice. The analytes were identified by the coincidence of the retention time, the detection of characteristic product ions, and the ratio of the intensity of their signals. For quantification, the external calibration method was used. For accounting the effect of the matrix on the analytical signal of T-2 triol, HT-2 and T-2 toxins, isotopically labeled internal standards were used (the [$^{13}C_{22}$]-HT-2 for HT-2 toxin and [$^{13}C_{24}$]-T-2 for T-2 triol и T-2 toxin). Mycotoxins in grains of corn, barley, rye and oats was quantified by the method of external calibration "on a pure matrix". A series of eight multicomponent standard solutions was used for calibration. In standards for solvent, 50 μl of extractant, 100 μl of mobile phase A, and 10 μl of a mixture of internal standards were added to 50 μl of the standard solution. In standards for matrix, 50 μl of the "pure" matrix extract and 100 μl of mobile phase A were added to 50 μl of the standard solution. Detection limit (DL) and quantification limit (QL) were calculated as 3S/N and 7S/N criteria, respectively, where S/N is the signal-to-noise ratio.

Samples were prepared as described (MVI 410/4-2020 "Method of multidetection of mycotoxins in grain and primary products of its processing". Approved by Rospotrebnadzor). A 100 g portion of the sample was crushed in a laboratory mill to a homogeneous state, 5.0 g of a homogeneous ground sample was placed in a 50 ml centrifuge tube added with 5 ml of acetonitrile:water (80:20 vol.%) acidified with formic acid (0.5 vol.%). Extraction was carried out for 30 min alternately on a shaker (twice for 10 min) and in an ultrasonic bath (10 min). The resulting extract was filtered, a 200 μl aliquot was diluted with 600 μl of mobile phase A, centrifuged at 4000 rpm the least, degreased with hexane if necessary. The diluted extract (200 μl) was placed to a chromatographic vial added with 10 μl of a mixture of internal standards ([$^{13}C_{24}$]-T-2 and [$^{13}C_{22}$]-HT-2) (for wheat samples). The analysis was performed in duplicate.

Results. Table 1 shows the MRM parameters, retention time, recovery rates (average values for the studied crops), detection limits (DL) and quantitation limits (QL) for mycotoxins. For calculation, the first of mother ion—daughter ion transition indicated in Table 1 was used.

Wheat grain contamination. In wheat from Russian regions, DON, T-2, and HT-2 toxins, Alternaria toxins (AOH, AME, and TE), OTA, CIT, MPA, and ZEA were more common compared to other mycotoxins (Table 2). Acetyl derivatives of DON (3- and 15-AcDON) were found only in two samples of wheat from the Amur Province. In single cases, STC (1.3 μg/kg, a sample from the Lipetsk Province), β-ZEL (366 μg/kg, a sample from the Amur Province) and CTV (56 μg/kg, a sample from the Krasnodar Territory) were detected. NIV, FUSX, NEOS, T-2 triol, AFL, FB, ALT, α-ZEL and CPA were not detected.

The main contaminants of the wheat grain from the Central, Volga and Ural federal districts were *Alternaria* mycotoxins among which TE dominated. It

was detected in 62-88% of samples. This data is well consistent with the results of Orina et al. [37, 44] for wheat harvests of 2017 and 2018 from the Ural Federal District who reported TE detection (from 2.9 to 79.9 μg/kg) in all 36 tested samples. No more than 7% and 11% of the samples we tested were contaminated with T-2 + NT-2 and DON toxins, respectively. In none sample the content of the regulated mycotoxins exceeded the MAL.

In wheat from the Siberian Federal District, TE also prevailed (91%). In 20% of the samples, we found T-2 + NT-2 and DON; the content of regulated mycotoxins, with the exception of OTA, was significantly below the MAL. OTA was detected in 4 out of 11 samples (36%), and in one sample with a more than 4-fold excess of the MAL. On average, in all regions, the rate of OTA in wheat was 6%, or 7 samples. Interestingly, three of these samples (one sample from the Krasnodar Territory and two from the Omsk Province) were co-contaminated with OTA and CIT. High co-contamination of wheat with OTA and CIT was previously noted for feed grains from the central regions of the European Russia (16 out of 30 OTA-contaminated samples contained CIT) [41].

Another feature of mycotoxin contamination in wheats from the Central, Volga, Ural and Siberian districts was the MPA rate of 3-13% (at the level ranging from 40 to 3700 μg/kg). This is partly consistent with the reports on the MPA rate < 6% at 63-1255 μg/kg wheat fodder grain [45]. This mycotoxin was not found in wheat from other regions. The cases of high MPA accumulation in food grain revealed by us (3500 and 3700 μg/kg in wheat from the Tyumen and Saratov regions, respectively) deserve attention. Previously, it was reported that violated storage conditions led to self-warming and an increase in the MPA content in sunflower seeds from 53 to 2630 μg/kg [46]. MPA does not have pronounced toxic properties, but is widely used in transplantology as a strong immunosuppressant. The standard daily therapeutic dose of MPA is about 1.5 g [47], which is two orders of magnitude lower than its content in a kilogram of the most contaminated wheat grain samples. However, given the prevalence of MPA in mass-produced foods on the Russian market [48, 49], the possibility of chronic dietary intake of this mycotoxin is of concern.

Wheat from the main grain-producing regions of Russia — the Southern Federal District and North Caucasian Federal District was less contaminated with Alternaria mycotoxins than samples from the Central, Volga, Urals and Siberian regions. The tests did not reveal alternariatoxins in grain from Rostov Province. Of them, only TE was detected (in 13% of samples from the Krasnodar Territory while in other regions its rate could reach 100%. TE was detected in three of 21 samples (14%) from the Stavropol Territory, and AME was detected in the other three samples (14%). We did not detect AOH in wheat from the Southern Federal District and North Caucasian Federal District. According to the special literature, the co-infection of *Alternaria* and *Fusarium* micromycetes can suppress the synthesis of AOH [50]. Indeed, the main contaminant of wheat from these regions is traditionally fusariotoxin DON. In samples from the Krasnodar Territory, the rate for DON was 40%, with two samples exceeding the maximum permitted levels. In one sample, along with DON, we detected ZEA, OTA, and CIT, with the OTA content almost 2 times higher than the MAL. Only 10% of wheat samples from the Stavropol Territory were DON-positive, all below the MAL. Acetyl derivatives of DON were not detected in the samples from the Southern Federal District and North Caucasian Federal District. Wheat grain from the Rostov Province (Southern Federal District) turned out to be the least contaminated (only one sample contained CIT).

1. Mycotoxin detection parameters and their characteristics in the analysis of food grain by high performance liquid chromatography–tandem mass spectrometry (an Agilent 1100 chromatographic system, Agilent TQ 6410 mass spectrometric detector, Agilent Technologies, USA)

Analyt	t_R, min	Mother ion, m/z		Daughter ions, m/z	F, V	CE, V	Degree of extraction, %	DL, μg/kg	QL, μg/kg
NIV	10.1	$[M+Na]^+$	313.3	175.3; 247.2	90	10; 2	85.1	100	200
DON	12.0	$[M+H]^+$	297.1	249.2; 203.1; 175.2	90	5; 10; 18	98.7	20	40
FUSX	13.8	$[M+H]^+$	355.2	175; 247	100	20; 4	116.3	20	40
NEOS	14.0	$[M+H]^+$	383.2	305.1; 185.1	116	2; 14	88.3	0.3	1
15-AcDON	16.2	$[M+H]^+$	339.3	261.3; 231.1	90	5;5	107.2	20	40
3-AcDON	16.6	$[M+H]^+$	339.3	231.1; 212.8; 261.3	90	5; 8; 5	97.9	10	20
FB_1	17.6	$[M+H]^+$	722.4	334.2; 352.5; 316.4	165	40; 40; 40	89.9	5	20
T-2 triol	18.1	$[M+H]^+$	405.2	303.0; 124.8;	100	12; 12	94.4	30	80
AFL G_2	18.5	$[M+H]^+$	331.1	245.2; 257.2	150	30; 30	90.7	1	2,5
FB_2	18.6	$[M+H]^+$	706.5	336.0; 354.0; 318	165	35; 30; 38	94.9	5	10
ALT	18.8	$[M+H]^+$	293.1	257.1; 239.1	45	8; 16	114.6	2	5
AFL G_1	19.5	$[M+H]^+$	329.1	243.2; 200.0; 283.0	135	25; 46; 20	92.9	0.5	1
AFL B_2	19.5	$[M+H]^+$	315.3	287.0; 259.1; 231.2	135	22; 30; 30	92.4	0.5	1
AFL B_1	20.5	$[M+H]^+$	313.2	128.0; 241.1; 285.2	135	80; 35; 20	94.3	0.5	1
HT-2	20.6	$[M+Na]^+$	447.3	345.2; 285.2	190	16; 16	90.3	2	5
$[^{13}C_{22}]$-HT-2	20.6	$[M+Na]^+$	469.2	362.0	150	30	–	–	–
AOH	21.1	$[M+H]^+$	259.1	128.0; 185.1	153	40; 30	110.7	2	5
TE	21.1	$[M+H]^+$	415.2	132.0; 312.2	107	42; 8	113.6	0.5	1
α-ZEL	22.4	$[M+H]^+$	303.2	285.2; 267.0	110	5; 10	109.2	4	10
MPA	23.6	$[M+H]^+$	321.1	303.2; 207.1	73	4; 16	101.5	4	10
β-ZEL	24.0	$[M+H]^+$	303.2	285.2; 267.0	110	5; 10	118.3	4	10
CTV	24.1	$[M+H]^+$	403.2	285.0; 297.0;	45	10; 10	94.7	4	10
CIT	24.8	$[M+H]^+$	251.2	233.1; 205.0; 115.2	93	16; 24; 52	91.5	2	3
T-2	24.8	$[M+Na]^+$	489.3	245.1; 387.1; 327.2	165	24; 17; 20	99.2	0.5	2
$[^{13}C_{24}]$-T-2	24.8	$[M+Na]^+$	513.5	344.2	190	20	–	–	–
OTA	25.5	$[M+H]^+$	404.2	239.1; 358.2; 221.0	105	20; 10; 34	96.1	0.5	1
ZEA	26.8	$[M+H]^+$	319.2	185.0; 283.2; 301.2	90	22; 5; 5	104.6	2	5
AME	26.8	$[M+H]^+$	273.1	258.0; 230.0	156	24; 30	108.6	2	5

Continued Table 1

STC	28.2	$[M+H]^+$	325.1	281.1; 310.1	120	35; 22	108.5	1	2
CPA	28.5	$[M+H]^+$	337.2	196.1; 182.1	45	16; 12	82.8	30	80

N o t e. DON — deoxynivalenol, ZEA — zearalenone, FB_1, FB_2 — fumonisins B_1 and B_2, AFL B_1 — aflatoxin B_1, OTA — ochratoxin A, 3-AcDON and 15-AcDON — 3- and 15-Acetyl-DON, NIV — nivalenol, FUSX — fusarenone X, NEOS — neosolaniol, α- and β-ZEL — α- и β-zearalenol, AFL B_2, G_1, G_2 — aflatoxins B_2, G_1, G_2, STC — sterigmatocystin, TE — tentoxin, ALT — altenuene, AOH — alternariol, AME — methyl ether of AOH, CIT — citrinin, CTV — citreoviridin, MPA — mycophenolic acid, CPA — cyclopiazonic acid. Positive electrospray ionization at atmospheric pressure in multiple reaction monitoring (MRM) mode; t_R — retention time, F, V — fragmentator voltage, CE, V — collision cell voltage, DL — detection limit, QL — quantification limit. For the degree of extraction, the average values for the studied crops are given. Dashes indicate that internal standards were added to the prepared sample before analysis.

2. Rates and levels of mycotoxins in wheat food grain from Russian regions (2018, $n = 114$; HPLC/MS-MS)

Region	Contaminated samples/total number of samples (%)	Level (min-max; averaged), μg/kg (rate, %)									
		DON	ZEA	T-2	HT-2	OTA	CIT	AOH	AME	TE	MPA
CFD	19/29 (66 %)	100 (3 %)	10 (3 %)	4 and 17 (7 %)	35 and 45 (7 %)	0.4 (3 %)	–	< QL-13; 11 (21 %)	< QL-10; 6 (21 %)	2-37; 9 (62 %)	40 (3 %)
VFD	16/19 (84 %)	120 and 370 (11 %)	16 (5 %)	15 (5 %)	5 and 45 (11 %)	–	–	< QL-10 (16 %)	< QL-21 (21 %)	1-90; 11 (74 %)	3700 (5 %)
UFD	7/8 (88 %)	–	–	–	–	2.9 (13 %)	–	–	–	5-49; 14 (88 %)	3500 (13 %)
SiFD	10/11 (91 %)	180 and 250 (18 %)	–	2 and 3 (18 %)	5-70; 34 (27 %)	0.8-22; 7.8 (36 %)	15 и 97 (18 %)	< QL (9 %)	-	6-83; 27 (91 %)	380 (9 %)
Amur Province (FEFD)	3/3 (100 %)	430 and 530 (67 %)	–	5 (33 %)	13 (33 %)	–	–	26 (33 %)	< QL (33 %)	5-90; 38 (100 %)	–
Krasnodar Territory (SFD)	8/15 (53 %)	120-1270; 500 (53 %)	5 (7 %)	–	5 and 8 (13 %)	9 (7 %)	118 (7 %)	–	–	5 and 6 (13 %)	–
Rostov Province (SFD)	1/8 (13 %)	–	–	–	–	–	2.5 (13 %)	–	–	–	–
Stavropol Territory (NCFD)	6/21 (29 %)	100 and 730 (10 %)	–	–	–	–	–	–	< QL-20 (14 %)	7-197; 72 (14 %)	–

N o t e. DON — deoxynivalenol, ZEA — zearalenone, OTA — ochratoxin A, CIT — citrinin, AOH — alternariol, AME — methyl ether of AOH, TE — tentoxin, MPA — mycophenolic acid. See Table 1 for detection protocol. If the number of mycotoxin-contaminated samples was less than 3, the analyte content in the sample is indicated instead of the concentration range and average. DL — detection limit, QL — quantification limit; a dash means that mycotoxin was not detected (the content is below DL, < DL). CFD — Central Federal District, VFD — Volga Federal District, UFD — Ural Federal District, SiFD — Siberian Federal District, FEFD — Far Eastern Federal District, SFD — Southern Federal District, NCFD — North Caucasus Federal District.

In the world, data on mycotoxin abundance in wheat grain vary considerably. It should be noted that in Russia, wheat is less DON-contaminated compared to other leading grain exporters [51]. In the EU countries, the rate for DON in wheat crops of 2005-2012, according to Alexander et al. [10], ranged from 60 to 100%, and the maximum content reached 4130 μg/kg. In durum wheat from Italy (n = 74), the contamination rate was 16% for DON at 48-2267 μg/kg, 8% for T-2 and HT-2 toxins at 10-149 μg/kg, 31% for AOH at 8-121 μg/kg, and 26% for AME at 9-48 μg/kg [52]. In 2001-2010, in winter wheat from Germany, AOH and AME reached 832 μg/kg with the rate ranged from 0-77% and 905 μg/kg at 0-33% [53].

Corn grain contamination. Mycotoxins were studied in 14 samples of corn grain (Table 3), 11 samples from the Southern Federal District and North Caucasian Federal District, two from the Central Federal District, and one from the Volga Federal District. The last three samples were slightly contaminated, FB_1 40 μg/kg + NT-2 7 μg/kg and FB_1 220 μg/kg, or ten times lower than the MALs. In grain from the Southern Federal District and North Caucasian Federal District, the main contaminants were FB_1 or FB_1 + FB_2, DON or DON + 15-AcDON. ; approximately a third of the samples contained MPA and NEOS; two out of 11 samples were co-contaminated with T-2 and HT-2 toxins. The rate of mycotoxins in descending order was as follows: FB (with FB_1 prevailing) > DON and 15-AcDON > NEOS, MPA > T-2 and HT-2 toxins > ZEA, OTA, CIT, CTV.

FBs are the main mycotoxin of corn grain in Russia [30]. According to the FB levels, all the studied samples met the requirements of the regulation. In one sample from the Krasnodar Territory, OTA (13 μg/kg) exceeded the permissible level. The relatively high incidence of NEOS in corn is consistent with data from Spain [54], while no HEOS was detected in samples from Africa, Japan [55] and Brazil [56]. The main trichothecenes of group A, T-2 and HT-2 toxins, were detected in 18% of the samples. For comparison: in corn grain from Croatia (n = 71), the rate for these toxins was 27% (the total content varied from 15 to 332 μg/kg) [28]. Along with FB and DON, T-2 toxin contaminates domestic feed grain of corn [57, 58]. We did not detect Alternaria metabolites in corn, which may be due to the low susceptibility of corn kernels to infection because of a specific structure of the ear [36]. Several publications are in line with these data. For example, less than 7% of corn food grain samples from the southern Brazil contained AOH, AME, TE [56], the rate for AOH and TE in the samples from Serbia did not exceed 10%, while for AME and tenuazonic acid, it was 40 and 35%, respectively [59]. In corn fodder grain from the Central Federal District, as per Kononenko et al. [57], the rate for AOH was 13.3%.

Contamination of barley, oats, and rye grain. In these samples, mycotoxicological analysis also revealed the dependence of the detected grain contaminants on the region of crop growth (see Table 3). The number of studied samples in these species was less than for wheat and corn, therefore, we considered the conclusions about the prevalence of mycotoxins in barley, oats and rye from different regions as indicative. Nevertheless, the results obtained are in good agreement with each other. The main contaminants of grain in the Central, Volga, Urals and Siberian regions were T-2 and HT-2 toxins (20-50%) and secondary metabolites of Alternaria, primarily TE (33-100%). Among Alternaria toxins, ALT accumulation in a barley sample from the Altai Territory reached 15 μg/kg (the only case of this toxin in the studied grain samples). The predominant contamination of oat grain with T-2 and HT-2 toxins in combination with Alternaria mycotoxins corresponds to the incidence of fungal infections caused by the producers [60].

3. Rates and levels of mycotoxins in corn, barley, oats, and rye food grain from Russian regions (2018, HPLC/MS-MS)

Region	Contaminated samples/total number of samples (%)	Level (min-max; averaged), μg/kg (rate, %)														
		DON	15-AcDON	ZEA	T-2	HT-2	NEOS	OTA	CIT	FB_1	FB_2	AOH	AME	TE	CTV	MPA
Corn (n = 14)																
SFD + NCFD	11/11 (100 %)	50-950; 407 (55 %)	14-36; 25 (27 %)	34 (9 %)	25 and 67 (18 %)	41 and 194 (18 %)	1-21; 6 (27 %)	13 (9 %)	6 (9 %)	30-1560; 370 (91 %)	40-170; 97 (27 %)	–	–	–	57 (9 %)	10-95; 39 (27 %)
CFD + VFD	2/3 (66 %)	–	–	–	–	7 (33 %)	–	–	–	40 and 220 (66 %)	–	–	–	–	–	–
Barley (n = 17)																
CFD	6/6 (100 %)	–	–	–	2-7; 4 (50 %)	5-10; 8 (50 %)	–	–	–	–	–	< QL-135; 72 (67 %)	–	< QL -5; < ПКО (67 %)	–	–
VFD + UFD + SiFD	5/5 (100 %)	–	–	–	6 (20 %)	–	–	–	2 (20 %)	–	–	–	–	< QL -23; 11 (60 %)	–	–
SFD + NCFD	6/6 (100 %)	–	–	–	2 (17 %)	5-34; 15 (50 %)	–	11 (17 %)	–	< QL and 20 (33 %)	–	24 (17 %)	5 (17 %)	5 (17 %)	10 (17 %)	–
Rye (n = 8)																
CFD	2/3 (66 %)	–	–	–	2 (33 %)	6 (33 %)	–	–	–	–	–	5 (33 %)	< QL (33 %)	8 and 15 (66 %)	–	–
VFD + UFD + SiFD	1/3 (33 %)	–	–	–	–	–	–	5 (33 %)	–	–	–	–	–	20 (33 %)	–	–
SFD + NCFD	1/2 (50 %)	–	–	–	–	–	–	–	2.5 (50 %)	–	–	–	–	15 (50 %)	–	–
Oats (n = 8)																
CFD + VFD + SiFD	4/4 (100 %)	–	–	–	2 and 35 (50 %)	34 (25 %)	8 (25 %)	–	3 (25 %)	–	–	< QL (25 %)	< QL (25 %)	9-86; 27 (100 %)	–	–
SFD + NCFD	4/4 (100 %)	–	–	–	–	–	–	< QL (50 %)	–	–	–	6 (25 %)	–	2-38; 14 (75 %)	–	–

Note. DON — deoxynivalenol, 15-AcDON — 15-Acetyl-DON, ZEA — zearalenone, NEOS — neosolaniol, OTA — ochratoxin A, CIT — citrinin, FB_1, FB_2 — fumonisins B_1 и B_2, AOH — alternariol, AME — methyl ether of AOH, TE — tentoxin, CTV — citreoviridin, MPA — mycophenolic acid. See Table 1 for detection protocol. If the number of mycotoxin-contaminated samples was less than 3, the analyte content in the sample is indicated instead of the concentration range and average. DL — detection limit, QL — quantification limit; a dash means that mycotoxin was not detected (the content is below DL, < DL). CFD — Central Federal District, VFD — Volga Federal District, UFD — Ural Federal District, SFD — Siberian Federal District, FEFD — Far Eastern Federal District, SFD — Southern Federal District, NCFD — North Caucasus Federal District.

The samples from the Southern and North Caucasian regions differed in the mycotoxin profiles depending on the crop. FB_1 in two samples out of 6 studied was distinctive for barley. Cases of FB_1 in barley food grain were reported in Tunisia (46 μg/kg, one of 31 samples) [61] and in Poland (one of 8 samples) [62]. Monitoring of mycotoxins in the fodder grain of barley harvested in 2004-2014 from the Central, Southern and North Caucasian regions revealed the FB_1 rate of 19-79% [31]. Moreover, there are cases of FBs in wheat fodder grains that are atypical for food grains, namely 6% of samples harvested in 2017 contained FBs (from 75 to 1990 μg/kg) [63]; the rate of positive fodder wheat samples from 2004-2014 harvests in southern Russia reached 50% [31]. It is noteworthy that the studied samples of barley, oats, and rye did not contain DON even in trace amounts. These data are consistent with the results of long-term monitoring. From 2009 to 2016, we tested 50 samples of rye, 28 samples of oats from the Volga, Urals, and Siberian regions, and 12 samples of barley from the Central Federal District. DON was not found, while according to Gavrilova et al. [44], the rate for DON in barley from the Ural region was 20%, and we also detected NIV. One of the studied samples of barley from the Krasnodar Territory did not meet the safety requirements for OTA (11 μg/kg).

4. Examples of co-contamination with mycotoxins of food grain harvested in the Amur Province (Far Eastern Federal District,)

Crop, year	Level, μg/kgкг											
	DON	3-AcDON	15-AcDON	ZEA	β-ZEL	T-2	HT-2	OTA	CIT	STC	AOH	TE
Wheat, 2018	430	71	36	–	–	–	–	–	–	–	26	17
Wheat, 2018	530	14	–	–	366	5	13	–	–	–	–	90
Barley, 2018	2830[a]	65	54	–	–	–	–	–	–	–	40	–
Oat, 2016	650	5	–	180	-	2	12	9[a]	30	120	No data	

N o t e. DON — deoxynivalenol, ZEA — zearalenone, β-ZEL — β-zearalenol, OTA — ochratoxin A, CIT — citrinin, STC — sterigmatocystin, AOH — alternariol, TE — tentoxin. See Table 1 for detection protocol. A dash means that mycotoxin was not detected (the content is below the detection level DL, < DL). [a] — exceeding the maximum allowable level (MAL).

From the point of view of the diversity and the rate of mycotoxins, grain from the Far Eastern Federal District is of particular interest. Table 4 shows the profiles of three individual grain samples of food wheat and barley harvested in 2018, most clearly reflecting the multiple mycotoxin contamination observed in the region. In addition, there is one sample of oats harvested in 2016 which attracted attention during our long-term monitoring due to a variety of mycotoxins. All these samples were from the Amur Province. These data indicate that the regional conditions are favorable for grain infection with micromycetes and toxin production. For example, only the samples from this region, along with DON, contained both acetyl derivatives of DON (3- and 15-AcDON) and β-ZEL. Co-contamination with eight mycotoxins was shown for a sample of oats harvested in 2016, in particular, OTA + CIT and STC were detected.

Gagkaeva et al. [21] drew attention to the high infection rate of spring wheat and barley in the Amur Province in 2019. They found DON (912-13343 μg/kg), 3-AcDON (0-293 μg/kg), 15-AcDON (19-179 μg/kg, 3-glucoside DON (98-3803 μg/kg), ZEA (92-3670 μg/kg), and moniliformin (5-218 μg/kg). AOH and AME, T-2 and HT-2 toxins in grain were at the level of tens of micrograms per kilogram. APL, OTA, STC, CPC, MPA, NEOS, DAS, and FUSX were not detected. Abundant 3-AcDON in grain is peculiar to the Far Eastern Federal District. According to the available data [21], the level of the 3-AcDON-producing *F. graminearum* DNA in grain from the Amur Province was on average 1.1-1.3 times higher than that of the 15-AcDON-producing genotype.

In our survey, in several cases, STC, β-ZEL and CTV occurred in grain samples. It should be noted that we report the contamination of food grains with

citreoviridin in the Russian Federation for the first time. This mycotoxin was detected in samples from the Krasnodar Territory (56 μg/kg in wheat and corn and 10 μg/kg in one barley sample). The rate for CTV in cereals in the Krasnodar Territory was 12%. It is known that CTV producers are micromycetes of the genera *Aspergillus* and *Penicillium*, mainly *P. citreonigrum* traditionally found in rice. For example, the toxin level in rice from Brazil reached 97 μg/kg [64], which is comparable to the levels in the samples we studied. CTV accumulated in the body have a pathological effect on the central nervous system [65]. STC was found in two grain samples — in wheat from the Lipetsk region (1.3 μg/kg) and rye from the Saratov region (0.1 μg/kg). According to the reports, the rate of STC in wheat, rye and corn grain in countries having temperate climate reached 7%; STC was more abundant in barley (up to 44% of samples) and oats (up to 57% of samples) [38]. We detected β-ZEL at 366 μg/kg in one wheat sample from the Amur Province. Structural analogs of ZEA, the α-ZEL and β-ZEL were rarely detected in grain. There are no systematic data on these mycotoxins in special publications.

Among the studied 28 mycotoxins, we did not find NIV, FUSX, T-2 triol, α-ZEL, APL and CPA. According to data from Italy [66], Poland [62] and the Czech Republic [67], the incidence of these mycotoxins in wheat does not exceed 12%. NIV is more likely to contaminate oats and barley [68]. In the present study, we did not detect NIV and FUSX in any grain tested. Fusariotoxins T-2 triol and α-ZEL are also rarely detected, while APL is more abundant in grain from countries with subtropical and tropical climates, e.g., Syria [66] and African countries [69]. However, it should be borne in mind that the increase in average annual temperatures and frequent summer droughts in the countries of Southern Europe give grounds for unfavorable forecasts of *Aspergillus flavus* infection of maize and, therefore, APL accumulation [70]. It can be assumed that such a scenario is possible in the Southern Federal District and North Caucasian Federal District of the Russian Federation. CPA also refers to metabolites of *Penicillium* and *Aspergillus*, characteristic of the tropical and equatorial zones. The reports describe cases of co-detection of APL and CPA in food products from hot countries, for example, in corn [71]. For CPA, a low abundance in fodder corn, barley and wheat grain was show in the Russian Federation (3 out of 276 samples, or 1.1% at 50-80 μg/kg) [72].

Thus, in Russia in 2018, the main mycotoxins in food wheat grain were fusariotoxins deoxynivalenol (DON), zeralenone (ZEA), T-2 and HT-2 toxins and Alternaria metabolites tentoxin (TE), alternariol (AOH) and its methyl ester (AME). Ochratoxin A (OTA), citrinin (CIT) and mycophenolic acid (MPA) were also identified. In wheat from the Central, Volga, Ural and Siberian federal districts, alternariatoxins prevailed, primarily TE the rate of which varied from 62 to 91%. More than half of the samples from the Krasnodar Territory contained DON (in two of the 15 samples, it exceeded the MAL), the rate for TE was 13%. Among the regulated mycotoxins, along with DON, 7 out of 115 samples (6%) contained OTA, exceeding the MRL in two samples. Most of the wheat samples contaminated with OTA were from the Siberian Federal District. Mycotoxins were not detected in 61% of wheat samples from the North Caucasian Federal District and 87% of samples from the Rostov Province. In other regions, more than half of the samples were contaminated. DON exceeded the MAL in three samples (2.6%), OTA in two samples (1.7%). In corn grain from the Southern Federal District and North Caucasian Federal District, the main contaminants were fumonisins B_1 and B_2 (FB_1 or FB_1 + FB_2) and DON (DON or DON + 15-AcDON). A third of the samples, were MPA- and NEOS-positive. ZEA, T-2 and HT-2, OTA, CIT, and CTV were rarely found, Alternaria toxins were not detected. All the studied samples met the safety requirements, with the exception of sample from the Krasnodar

Territory with OTA exceeding the MAL. Among fusariotoxins, the barley grain from the southern regions of Russia contained T-2, HT-2, and FB_1. Alternaria toxins were more characteristic of the Central, Volga, Urals, and Siberian regions. In several cases, CIT, CTV and OTA were found, the latter exceeding the MAL. In rye and oat grain, regardless of the region, alternariatoxins, mainly TEs, prevailed compared to other mycotoxins. We also identified T-2 and HT-2 toxins, NEOS and CIT. In one rye sample, OTA did not meet the safety requirements. The barley, oats and rye grain was not contaminated with DON even in trace amounts. Our data on CTV contamination of food corn, barley and wheat in the Russian Federation nave been obtained for the first time. The survey traced the dependence of the mycotoxin profiles on the crop and the region of cultivation. The high rate of OTA-positive samples and OTA excess of the MAL in 45% of food grain are of concern.

REFERENCES

1. Pitt J.I., Miller J.D. A concise history of mycotoxin research. *Journal of Agricultural and Food Chemistry*, 2017, 65(33): 7021-7033 (doi: 10.1021/acs.jafc.6b04494).
2. *Tekhnicheskii reglament Tamozhennogo soyuza TR TS 015/2011 «O bezopasnosti zerna», 2011* [Technical regulations of the Customs Union TR CU 015/2011 «On grain safety», 2011]. Available: https://docs.cntd.ru/document/90232039). No date (in Russ.).
3. *Tekhnicheskii reglament Tamozhennogo soyuza TR TS 021/2011 «O bezopasnosti pishchevoi produktsii», 2011* [Technical regulations of the Customs Union TR CU 021/2011 «On food safety», 2011]. Available: https://docs.cntd.ru/document/902320560). No date (in Russ.).
4. Sulyok M., Berthiller F., Krska R., Schuhmacher R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrometry*, 2006, 20(18): 2649-59 (doi: 10.1002/rcm.2640).
5. Sulyok M., Krska R., Schuhmacher R. Application of a liquid chromatography-tandem mass spectrometric method to multi-mycotoxin determination in raw cereals and evaluation of matrix effects. *Food Additives and Contaminants*, 2007, 24(10): 1184-1195 (doi: 10.1080/02652030701510004).
6. Malachova A., Stranska M., Vaclavikova M., Elliott C. T., Black C., Meneely J., Hajslova J., Ezekiel C. N., Schuhmacher R., Krska R. Advanced LC-MS-based methods to study the co-occurrence and metabolization of multiple mycotoxins in cereals and cereal-based food. *Analytical and Bioanalytical Chemistry*, 2018, 410(3): 801-825 (doi: 10.1007/s00216-017-0750-7).
7. Steiner D., Sulyok M., Malachovб A., Mueller A., Krska R. Realizing the simultaneous liquid chromatography-tandem mass spectrometry based quantification of >1200 biotoxins, pesticides and veterinary drugs in complex feed. *Journal of Chromatography A*, 2020, 1629: 461502 (doi: 10.1016/j.chroma.2020.461502).
8. Amelin V., Korotkov A., Andoralov A. Identification and determination of 492 contaminants of different classes in food and feed by high-resolution mass spectrometry using the standard addition method. *Journal of AOAC International*, 2016, 99(6): 1600-1618 (doi: 10.5740/jaoacint.16-0069).
9. *Comission Regulation (EU) No 165/2010 amending Regulation No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. 2010.* Available: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:050:0008:0012:EN:PDF). No date.
10. Alexander J., Barregerd L., Bignami M., Brüschweiler B., Ceccatelli S., Cottrill B., Dinovi M., Edler L., Grasl-Kraupp B., Hogstrand Ch., Hoogenboom L., Knutsen H.K., Nebbia C.S., Oswald I.P., Petersen A., Rose M., Roudot A.-C., Schwerdtle T., Vleminckx Ch., Vollmer G., Wallace H. Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA Journal*, 2017, 15(9): 4718 (doi: 10.2903/j.efsa.2017.4718).
11. Alexander J., Barregerd L., Bignami M., Bruschweiler B., Ceccatelli S., Cottrill B., Dinovi M., Edler L., Grasl-Kraupp B., Hogstrand Ch., Hoogenboom L., Knutsen H.K., Nebbia C.S., Oswald I.P., Petersen A., Rogiers V.M., Rose M., Roudot A.-C., Schwerdtle T., Vleminckx Ch., Vollmer G., Wallace H. Appropriateness to set a group health based guidance value for T2 and HT2 toxin and its modified forms. *EFSA Journal*, 2017, 15(1): 04655 (doi: 10.2903/j.efsa.2017.4655).
12. Alexander J., Barregard L., Bignami M., Ceccatelli S., Cottrill B., Dinovi M., Edler L., Grasl-Kraupp B., Hogstrand Ch., Hoogenboom L., Knutsen H.K., Nebbia C.S., Oswald I.P., Petersen A., Rogiers V.M., Rose M., Roudot A.-C., Schwerdtle T., Vleminckx Ch., Vollmer G., Wallace H. Appropriateness to set a group health - based guidance value for zearalenone and its modified forms. *EFSA Journal*, 2016, 14(4): 4425 (doi: 10.2903/j.efsa.2016.4425).
13. Knutsen H-K., Barregerd L., Bignami M., Brüschweiler B., Ceccatelli S., Cottrill B., Dinovi M.,

Edler L., Grasl-Kraupp B., Hogstrand Ch., Hoogenboom L., Nebbia C. S., Petersen A., Rose M., Roudot A.-C., Schwerdtle T., Vleminckx Ch., Vollmer G., Wallace H., Dall'Asta Ch., Gutleb A.C, Humpf H.-U., Galli C., Metzler M., Oswald I.P., Parent-Massin D., Binaglia M., Steinkellner H., Alexander J. Appropriateness to set a group health-based guidance value for fumonisins and their modified forms. *EFSA Journal*, 2018, 16(2): 5172 (doi: 10.2903/j.efsa.2018.5172).

14. Gagkaeva T.Yu., Gannibal F.B., Gavrilova O.P. *Zashchita i karantin rastenii*, 2012, 1: 37-41 (in Russ.).
15. Khaneghah A.M., Martins L.M., von Hertwig A.M., Bertoldo R., Sant'Ana A.S. Deoxynivalenol and its masked forms: Characteristics, incidence, control and fate during wheat and wheat based products processing — a review. *Trends Food Science and Technol.*, 2018, 71: 13-24 (doi: 10.1016/j.tifs.2017.10.012).
16. Yli-Mattila T. Ecology and evolution of toxigenic *Fusarium* species in cereals in Nothern Europe and Asia. *Journal of Plant Pathology*, 2010, 92(1): 7-18 (doi: 10.4454/jpp.v92i1.10).
17. Gagkaeva T.Yu., Gavrilova O.P., Levitin M.M. *Biosfera*, 2014, 6(1): 36-45 (in Russ.).
18. Tutelyan V.A., Zaharova L.P., Sedova I.B., Perederyaev O.I., Aristarkhova T.V., Eller K.I. Fusariotoxins in Russian Federation 2005-2010 grain harvests. *Food Additives and Contaminants: Part B*, 2013, 6(2): 139-145 (doi: 10.1080/19393210.2013.767862).
19. Zakharova L.P., Sedova I.B., Aristarkhova T.V., Perederyaev O.I., Selifanov A.V., Eller K.I., Tutel'yan V.A. *Voprosy pitaniya*, 2009, 78(6): 26-31 (in Russ.).
20. Sedova I.B., Aksenov I.V., Zakharova L.P. *Voprosy pitaniya*, 2016, 85(S2): 35 (in Russ.).
21. Gagkaeva T.Yu., Gavrilova O.P., Orina A.S., Gogina N.N. *Zashchita i karantin rastenii*, 2020, 8: 19-21 (in Russ.).
22. Gagkaeva T.Yu., Gavrilova O.P. *Zashchita i karantin rastenii*, 2009, 12: 13-15 (in Russ.).
23. van der Lee T., Zhang H., van Diepeningen A., Waalwijk C. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. *Food Additives and Contaminants: Part A*, 2015, 32(4): 453-460 (doi: 10.1080/19440049.2014.984244).
24. Chen P., Xiang B., Shi H., Yu P., Song Y., Li Sh. Recent advances on type A trichothecenes in food and feed: analysis, prevalence, toxicity, and decontamination techniques. *Food Control*, 2020, 118: 107371 (doi: 10.1016/j.foodcont.2020.107371).
25. Gagkaeva T.Yu., Gavrilova O.P. *Zashchita i karantin rastenii*, 2014, 3: 30-32 (in Russ.).
26. Gavrilova O.P., Gagkaeva T.Yu. *Vestnik zashchity rastenii*, 2020, 103(3): 201-206 (doi: 10.31993/2308-6459-2020-103-3-13282) (in Russ.).
27. Gavrilova O.P., Orina A.S., Gogina N.N., Gagkaeva T.Yu. *Agrarnyi vestnik Urala*, 2020, 07(198): 29-40 (doi: 10.32417/1997-4868-2020-198-7-29-40) (in Russ.).
28. Kis M., Vulic A., Kudumija N., Sarkanj B., Jaki Tkalec V., Aladic K., Skrivanko M., Furmeg S., Pleadin J. A two-year occurrence of fusarium T-2 and HT-2 toxin in Croatian cereals relative of the regional weather. *Toxins*, 2021, 13(1): 39 (doi: 10.3390/toxins13010039).
29. Zinedine A., Soriano J. M., Molto J. C., Manes J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and Chemical Toxicology,* 2007. 45(1): 1-18 (doi: 10.1016/j.fct.2006.07.030).
30. Sedova I.B., Zakharova L.P., Kiseleva M.G., Chalyi Z.A., Tutel'yan V.A. *Nauchnye trudy SKFNTSSVV*, 2018, 21: 129-137 (in Russ.).
31. Drobin Yu.D., Soldatenko N.A., Sukhikh E.A., Kovalenko A.V. *Problemy veterinarnoi sanitarii, gigieny i ekologii*, 2015, 4 (16): 27-30 (in Russ.).
32. Aksenov I.V., Eller K.I., Tutel'yan V.A. *Voprosy pitaniya*, 2006, 75(1): 43-47 (in Russ.).
33. Palumbo R., Crisci A., Venancio A., Cortinas Abrahantes J., Dorne J. L., Battilani P., Toscano P. Occurrence and co-occurrence of mycotoxins in cereal-based feed and food. *Microorganisms*, 2020, 8(1): 74 (doi: 10.3390/microorganisms8010074).
34. Alexander J., Benford D., Boobis A., Ceccatelli S., Cottrill B., Cravedi J.-P., Di Domenico A., Doerge D., Dogliotti Eu., Edler L., Farmer P., Filipi M., Fink-Gremmels J., Fürst P., Guérin Th., Knutsen H.K., Machala M., Mutti A., Schlatter J., Rose M., van Leeuwen R. Scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal*, 2011, 9(10): 2407 (doi: 10.2903/j.efsa.2011.2407).
35. Gagkaeva T., Gavrilova O., Orina A., Burkin A., Khusaynov Kh. Microbiological quality of grain cultivated in the North Caucasus region in 2019. *BIO Web of Conferences*, 2020, 27: 00151 (doi: 10.1051/bioconf/20202700151).
36. Gannibal F.B. Factors affecting Alternaria appearance in grains in European Russia. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(3): 605-615 (doi: 10.15389/agrobiology.2018.3.605eng).
37. Orina A.S., Gavrilova O.P., Gagkaeva T.Yu., Gannibal F.B. *Mikologiya i fitopatologiya*, 2020, 54(5): 365-377 (doi: 10.31857/s0026364820050086) (in Russ.).
38. Sedova I.B., Kiseleva M.G., Zakharova L.P., Tutel'yan V.A. *Gigiena i sanitariya*, 2019, 98(1): 105-117 (doi: 10.18821/0016-9900-2019-98-1-105-117) (in Russ.).
39. Benford D., Ceccatelli S., Cottrill B., DiNovi M., Dogliotti E., Edler L., Farmer P., Fürst P.,

Hoogenboom L., Knutsen H.K., Haldorsen A-K.L., Metzler M., Nebbia C.S., O'Keeffe M., Rietjens I., Schrenk D., Silano V., van Loveren H., Vleminckx Ch., Wester P. Scientific opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. *EFSA Journal*, 2013, 11(6): 3254 (doi: 10.2903/j.efsa.2013.3254).
40. Zhao Y., Wang Q., Huang J., Ma L., Chen Z., Wang F. Aflatoxin B_1 and sterigmatocystin in wheat and wheat products from supermarkets in China. *Food Additives and Contaminants: Part B*, 2018, 11(1): 9-14 (doi: 10.1080/19393210.2017.1388295).
41. Kononenko G.P., Burkin A.A. Peculiarities of feed contamination with citrinin and ochratoxin A. *Agricultural Sciences*, 2013, 4(1): 34-38 (doi: 10.4236/as.2013.41006).
42. Gruber-Dorninger C., Novak B., Nagl V., Berthiller F. Emerging mycotoxins: beyond traditionally determined food contaminants. *Journal of Agricultural and Food Chemistry*, 2017, 65(33): 7052-7070 (doi: 10.1021/acs.jafc.6b03413).
43. Moldes-Anaya A.S., Asp T.N, Eriksen G.S., Skaar I., Rundberget T. Determination of cyclopiazonic acid in food and feeds by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 2009, 1216(18): 3812-3818 (doi: 10.1016/j.chroma.2009.02.061).
44. Gavrilova O.P., Orina A.S., Gogina N.N., Gagkaeva T.Yu. *Rossiiskaya sel'skokhozyaistvennaya nauka*. 2020, 6: 20-23 (doi: 10.31857/S2500262720060058) (in Russ.).
45. Burkin A.A., Kononenko G.P. Producers of mycophenolic acid in ensiled and grain feeds. *Applied Biochemistry and Microbiology*, 2010, 46(5): 545-550 (doi: 10.1134/S0003683810050145).
46. Burkin A.A., Ustyuzhanina M.I., Zotova E.V., Kononenko G.P. Reasons of contamination of production lots of sunflower (*Helianthus annuus* L.) seeds by mycotoxins. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(5): 969-976 (doi: 10.15389/agrobiology.2018.5.969eng).
47. Shaw L.M., Figurski M., Milone M.C., Trofe J., Bloom R.D. Therapeutic drug monitoring of mycophenolic acid. *Clinical Journal of the American Society of Nephrology*, 2007, 2(5): 1062-1072 (doi: 10.2215/CJN.03861106).
48. Kiseleva M.G., Chalyi Z.A., Sedova I.B., Minaeva L.P., Sheveleva S.A. *Analiz riska zdorov'yu*, 2020, 1: 38-51 (doi: 10.21668/health.risk/2020.1.04) (in Russ.).
49. Chalyi Z.A., Kiseleva M.G., Sedova I.B., Minaeva L.P., Sheveleva S.A., Tutel'yan V.A. *Voprosy pitaniya*, 2021, 90(1): 33-39 (doi: 10.33029/0042-8833-2021-90-1-33-39) (in Russ.).
50. Gagkaeva T.Yu., Gavrilova O.P., Orina A.S., Ablova I.B., Bespalova L.A. *Biotekhnologiya i selektsiya rastenii*, 2018, 1(1): 7-15 (doi: 10.30901/2658-6266-2018-1-7-15) (in Russ.).
51. Chen Ch., Frank K., Wang T., Wu F. Global wheat trade and Codex Alimentarius guidelines for deoxynivalenol: a mycotoxin common in wheat. *Global Food Security*, 2021, 29: 100538 (doi: 10.1016/j.gfs.2021.100538).
52. Juan Ch., Covarelli L., Beccari G., Colasante V., Maces J. Simultaneous analysis of twenty-six mycotoxins in durum wheat grain from Italy. *Food Control*, 2016, 62: 322-329 (doi: 10.1016/j.foodcont.2015.10.032).
53. Müller M.E.H., Korn U. *Alternaria* mycotoxins in wheat — a 10 years survey in the Northeast of Germany. *Food Control*, 2013, 34(1): 191-197 (doi: 10.1016/j.foodcont.2013.04.018).
54. Khaneghah A.M., Farhadi M., Nematollahi A., Vasseghian Y., Fakhri Y. A systematic review and meta-analysis to investigate the concentration and prevalence of trichothe-cenes in the cereal-based food. *Trends in Food Science & Technology*, 2020, 102: 193-202 (doi: 10.1016/j.tifs.2020.05.026).
55. Chen P., Xiang B., Shi H., Yu P., Song Y., Li Sh., Recent advances on type A trichothecenes in food and feed: analysis, prevalence, toxicity, and decontamination techniques. *Food Control*, 2020. 118: 107371 (doi: 10.1016/j.foodcont.2020.107371).
56. Oliveira M.S., Rocha A., Sulyok M., Krska R., Mallmann C.A. Natural mycotoxin contamination of maize (*Zea mays* L.) in the South region of Brazil. *Food Control*, 2017, 73: 127-132 (doi: 10.1016/j.foodcont.2016.07.033).
57. Kononenko G.P., Burkin A.A., Zotova E.V. *Veterinariya segodnya*, 2020, 2(33): 139-145 (doi: 10.29326/2304-196X-2020-2-33-139-145) (in Russ.).
58. Kononenko G.P., Burkin A.A., Zotova E.V., Smirnov A.M. *Rossiiskaya sel'skokhozyaistvennaya nauka*, 2019, 3: 28-31 (doi: 10.31857/S2500-26272019328-31) (in Russ.).
59. Hajnal E.J., Kos J., Malachova A., Steiner D., Stranska M., Krska R., Sulyok M. Mycotoxins in maize harvested in Serbia in the period 2012-2015. Part 2: non-regulated mycotoxins and other fungal metabolites. *Food Chemistry*, 2020, 317: 126409 (doi: 10.1016/j.foodchem.2020.126409).
60. Gavrilova O.P., Gannibal F.B., Gagkaeva T.Yu. *Fusarium* and *Alternaria* fungi in grain of oats grown in the North-Western Russia regarding cultivar specificity. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2016, 51(1): 111-118 (doi: 10.15389/agrobiology.2016.1.111eng).
61. Juan C., Berrada H., Manes J., Oueslati S. Multi-mycotoxin determination in barley and derived products from Tunisia and estimation of their dietary intake. *Food and Chemical Toxicology*, 2017, 103: 148-156 (doi: 10.1016/j.fct.2017.02.037).
62. Bryla M., Wa kiewicz A., Podolska G., Szymczyk K., Jedrzejczak R., Damaziak K., Sulek A.

Occurrence of 26 mycotoxins in the grain of cereals cultivated in Poland. *Toxins*, 2016, 8(6): 16 (doi: 10.3390/toxins8060160).
63. Kononenko G.P., Burkin A.A., Zotova E.V., Ustyuzhanina M.I., Smirnov A.M. *Rossiiskaya sel'skokhozyaistvennaya nauka*, 2018, 1: 17-21 (in Russ.).
64. Rosa C.A., Keller K.M., Oliveira A.A. Production of citreoviridin by *Penicillium citreonigrum* strains associated with rice consumption and beriberi cases in the Maranhro State, Brazil. *Food Additives and Contaminants: Part A*, 2010, 27(2): 241-248 (doi: 10.1080/19440040903289712).
65. Uchiyama Y., Takino M., Noguchi M., Shiratori N., Kobayashi N., Sugita-Konishi Y. The *in vivo* and *in vitro* toxicokinetics of citreoviridin extracted from *P. citreonigrum*. *Toxins*, 2019, 11(6): 360 (doi: 10.3390/toxins11060360).
66. Alkadri D., Rubert J., Prodi A., Pisi A., Manes J., Soler C. Natural co-occurrence of mycotoxins in wheat grains from Italy and Syria. *Food Chemistry*, 2014, 157: 111-118 (doi: 10.1016/j.foodchem.2014.01.052).
67. Chrpová J., Šíp V., Sumíková T., Salava J., Palicová J., Štočková L., Džuman Z., Hajšlová J. Occurrence of *Fusarium* species and mycotoxins in wheat grain collected in the Czech Republic. *World Mycotoxin Journal*, 2016, 9(2): 317-327 (doi: 10.3920/WMJ2015.1917).
68. Hietaniemi V., Rämö S., Yli-Mattila T., Jestoi M., Peltonen S., Kartio M., Sievilainen E., Koivisto T., Parikka P. Updated survey of *Fusarium* species and toxins in Finnish cereal grains. *Food Additives & Contaminants: Part A*, 2016, 33(5): 831-848 (doi: 10.1080/19440049.2016.1162112).
69. Gruber-Dorninger C., Jenkins T., Schatzmayr G. Multi-mycotoxin screening of feed and feed raw materials from Africa. *World Mycotoxin Journal*, 2018, 11(3): 369-383 (doi: 10.3920/WMJ2017.2292).
70. Moretti A., Pascale M., Logrieco A.F. Mycotoxin risks under a climate change scenario in Europe. *Trends in Food Science & Technology*, 2019, 84: 38-40 (doi: 10.1016/j.tifs.2018.03.008).
71. Hayashi Y., Yoshizawa T. Analysis of cyclopiazonic acid in corn and rice by a newly developed method. *Food Chemistry*, 2005, 93(2): 215-221 (doi: 10.1016/j.foodchem.2004.09.017).
72. Kononenko G.P., Burkin A.A. *Mikologiya i fitopatologiya*, 2008, 42(2): 178-184 (in Russ.).