Pesticide residues in cow’s milk

DOI: 10.15567/mljekarstvo.2021.0302

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Received: 17.03.2021. / Accepted: 14.06.2021.

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Abstract

Quality of milk plays a key role in its wide consumption but also of its involvement in a large series of derived products, such as yogurt, cheese, butter, and ice cream. An effective LC-MS/MS method has been validated for the determination of 81 different pesticide residues in milk samples. The analyses comprised 44 milk samples collected during 2019. From all inspected pesticides, only metalaxyl (present in 11.36 % samples), bifenthrin and metolachlor (9.09 %), dimethoate (4.55 %), prochloraz and thiacloprid were detected in 2.27 % of analysed samples. The detections of trifloxystrobin and bifenthrin were above the maximum residue levels (MRLs). The Commission Regulation (EU) 2017/1135 standardizes the MRLs for dimethoate and omethoate in certain products, but a MRL for dimethoate residues in milk has not been established jet. All the other pesticide detections were below the MRLs.

Key words: milk; milk safety; pesticides; cow milk
**Introduction**

Milk and milk products are considered to be the essential components of a balanced human diet as they have tremendous nutritional values regarding a good proportion of proteins, fats, and important minerals (Vuković et al., 2020; Puvacba et al., 2020). According to Puvacba et al. (2020), significant health benefits of milk and its products are related to the proteins, especially their nutritive value and biological properties. Furthermore, milk is a nutrient-dense food with important nutritional value as it contains a good proportion of calcium, potassium, phosphorus, cholecalciferol, cobalamin, retinol, riboflavin, and proteins. Milk is an important source of niacin equivalents as it has a sufficient content of the tryptophan amino acid, a niacin precursor (Puvacba et al., 2020). Free of contaminants, liquid milk is an essential alimentative and healthful food for infants and elderly population (Battu et al., 2004). Consequently, cow milk is the most widely manufactured and consumed product in the world, and the European countries produce 24% of the total world milk production (Witczak and Mituniewicz-Malek, 2019).

Milk contamination with pesticide residues is a source of serious concern (Battu et al., 2004). Nowadays, in the majority of plant origin food productions the application of pesticides became imperative and essential. The production in all agricultural fields has been increased due to the possible chemical control of weeds, insects, microorganisms and other pests. Nevertheless, in this way obtained economic benefits are not without direct or indirect risks to human and environmental health (Ignjatijevic et al., 2020). Pesticides and their metabolites enter the human body through contaminated food and water. The European Union makes great effort to achieve sustainable use of these compounds in order to avoid the pesticide level augmentation in the environment and food (Bursic et al., 2021). Many pesticides and their residues that enter the human body through the food chains are recognized as stimulants, causative agents or primarily contributory factors in several serious human diseases, such as disruption of the endocrine system, neurological and immune system disorders, sterility, different cancer types (breast, lung, cervix, and prostate), heart diseases, Alzheimers’s and Parkinsonism (Nath et al., 2013; Bedi et al., 2015).

The pesticide toxicity to the target and non-target organisms commonly depends on the quantity in the environment, the available proportion and finally, on the amount actually encountered and absorbed by the organism (Ibigbami et al., 2019). The animal digestion and absorption is often not studied for toxic and unwanted substances such as dioxins, mycotoxins, heavy metals, pesticides, veterinary drugs and polycyclic aromatic hydrocarbons, while these contaminants are often analysed in feed, food and animal products (Vukovic et al., 2019). According to Kan and Meijer (2007), sometimes researchers presume complete absorption of these harmful substances as a worst-case scenario in order to predict residues in animal products from those detected in food and feed. Consequently, the physiological processes occurring during intestine transit and after absorption into the general circulation, as well as the intermediary metabolism, are ignored. This methodology does not improve the existing knowledge to identify or implement possible control points for harmful residue levels reduction in animal products. In addition to the great importance for human diet, milk has been studied as a bio-concentration process indicator of the environmentally persistent organic pollutants, such as pesticides.

Reddy and Reddy (2015) highlighted that pesticide residues are often retained in the animal milk and fat either by the direct contact or by indirect contamination of soil, water, feed and fodder. For example, domestic animals may be exposed to the pesticides during the cattle barn treatments for parasite control procedures and consequently, avoidance of the vector-borne diseases. In time of spraying, the water and feed should be well protected, as the accidental spills may occur, which may result in feed and water contamination and the further pesticide ingestion by the animals (Goulart et al., 2008). Furthermore, feed and fodder for cattle are frequently contaminated with pesticide residues. After feeding and ingestion, these residues inevitably assimilate into the animal body systems (Ittkhar et al., 2014). Pesticide bio-concentration and bioaccumulation in animal tissues are possible to reach the toxic levels even if the exposure was low. According to Tsiplakou et al. (2010), once ingested, lipophilic pesticides are absorbed from the intestines into the systematic circulation. High lipid solubility pesticides and their metabolites tend to accumulate in high fat content tissues, such as adipose tissue, brain, liver, kidney, and subsequently translocated and excreted through milk or other excrements (Rothwell et al., 2001). Occurrence of pesticide residues in milk is not so rare, especially for cow’s milk (EFSA, 2019). Additionally, an increasing amount of pesticides and their residues is entering into our environment, which is undoubtedly hazardous to human, animals and ecosystems health.

Therefore, the aim of this study was to apply liquid chromatography coupled with tandem quadrupole mass spectrometry (LC-MS/MS) method, for the analyses of 81 pesticide residues in 44 cow milk samples. The pesticide residues were extracted using a modified QuEChERS sample preparation procedure followed by validated LC-MS/MS method according to SANTE/12682/2019.

**Materials and methods**

**Chemicals and reagents.** All pesticide reference standards were obtained from Dr. Ehrenstorfer. Standard solution of each pesticide was prepared at 1 ng/mL in methanol (or acetone). Working standard mixture solutions were prepared at 10 µg/mL and 1 µg/mL in acetonitrile and stored in the dark at -20 °C. Carbofuran-D3 (10 µg/mL) was used as internal standard. Acetonitrile (MeCN) and methanol (MeOH) were purchased from J.T. Baker. Both
organic solvents were HPLC Ultra Gradient grade. Acetone was purchased from Sigma-Aldrich. Formic acid was analytical grade (Fisher Scientific UK). The Hillium QuECHERS extraction pouch 550 mL (P/N QEHLL0510P) and Hillium QuECHERS dispersive kit 15 mL (P/N QDHLL15032) were used for extraction and clean-up.

Validation parameters. The analytical data of the methods validated for all 81 pesticides in milk are presented in Table 1. The limits of detection (LOD) were calculated from a signal-to-noise ratio of 5.0, using the chromatogram of the sample spiking at the lowest concentration level. Limit of quantification (LOQ) was set at 0.01 mg/kg. Blank milk extracts were used for the estimation of the background noise of the chromatographic analysis. Linearity of the methods was checked in the range from 10 to 100 µg/kg using internal standard calibration. For the linearity the correlation coefficients ($R^2$) of all investigated pesticides were >0.99, indicating a good linearity. The precision of the methods, expressed as repeatability (%RSDr), was evaluated by analyzing milk samples spiked at 10 and 100 µg/kg.

The fragmentation of the $H^+$ molecular ion obtained by LC-MS/MS in the positive electrospray ionization (ESI+) of the 81 pesticides is given in Table 1, as well as $R^2$ and average recoveries. The selected reaction monitoring mode (SRM) was carried out to obtain the maximum sensitivity for each pesticide detection, while the pesticide confirmation, two SRM transitions were used taking into account the matching of the retention time (Rt). The fragmentation energy of all investigated pesticides was set at 166 V.

Instrumentation. HPLC Agilent 1290 Infinity II chromatograph equipped with a quaternary pump, multisampler, and column compartment thermostat was used for the detection of 81 pesticides. The HPLC system was coupled to an Agilent 6495 LC/TQ triple quadrupole mass spectrometer with AJS ESI (Jet Stream Technology Ion Source). A Zorbax Eclipse Plus C18 column Rapid Resolution HD (50×2.1mm, 1.8 µm particle size) was used for the chromatographic separation. The flow rate (0.25 mL/min) of the mobile phase was used and the injection volume for the LC system was 2 µL. Also, the column temperature was held on at 35 ºC. The chromatographic separation of pesticides was carried out with mobile phase consisted of water (A) and methanol (B) both contained formic acid (0.1%, v/v) in a gradient mode. A gradient elution started at 5 % of B and held 1 min. This composition was increased to 40 % B at 7 min, 90% B at 8 min and held for 2 min.

Table 1. MRM transitions, collision energies, Rt, recovery and $R^2$

| Pesticide   | Precursor ion (m/z) | Product ions (m/z) | CE (V) | Rt (min) | Recovery (%) ± RSD | $R^2$   |
|-------------|---------------------|--------------------|--------|----------|-------------------|---------|
| Acetamiprid | 223.1               | 126 90             | 27     | 45       | 89.6±12.71        | 0.9987  |
| Azoxystrobin| 404.1               | 372.1 344.1        | 8      | 24       | 91.8±10.43        | 0.9990  |
| Bendiocarb  | 224.1               | 167.1 109.1        | 12     | 25       | 90.9±9.22         | 0.9917  |
| Benalaxyl   | 326.2               | 148.1 102.3        | 20     | 15       | 78.3±11.58        | 0.9991  |
| Bitertanol  | 338.2               | 296.2 99.1         | 5      | 10       | 94.7±10.02        | 0.9928  |
| Bifenazate  | 301.1               | 198.2 170.1        | 20     | 16       | 90.8±9.65         | 0.9991  |
| Benoximate  | 364.0               | 105.0 99.1         | 15     | 15       | 82.9±8.43         | 0.9954  |
| Boscalid    | 343.0               | 377.1 271.2        | 18     | 12       | 93.9±7.88         | 0.9992  |
| Buprofezin  | 306.2               | 201.1 116.1        | 5      | 10       | 87.9±6.89         | 0.9921  |
| Butafenacil | 492.1               | 348.8 330.8        | 10     | 23       | 84.1±10.27        | 0.9908  |
| Carbaryl    | 202.1               | 145.1 127.1        | 4      | 28       | 94.7±13.02        | 0.9989  |
| Carbendazim | 192.1               | 160.1 132.1        | 16     | 32       | 79.9±8.24         | 0.9971  |
| Carbofuran  | 222.1               | 165.1 123.1        | 20     | 30       | 99.2±14.71        | 0.9990  |
| Chlorpyrifos| 349.93              | 198.0 97.0         | 20     | 41       | 98.9±8.11         | 0.9976  |
| Clothianidin| 250.0               | 169.0 131.9        | 8      | 8        | 96.2±11.06        | 0.9989  |
| Pesticide            | Precursor ion (m/z) | Product ions (m/z) | CE (V) | Rt (min) | Recovery (%) ± RSD | R²   |
|----------------------|---------------------|--------------------|--------|----------|------------------|------|
| Cyproconazole        | 292.1               | 125.0 70.0         | 32 16  | 8.45     | 73.4±7.55        | 0.9954 |
| Cymoxanil            | 199.1               | 128.0 111.1        | 4 16   | 5.07     | 87.8±14.68       | 0.9997 |
| Cyprodinil           | 226.1               | 76.0 65.1          | 50 56  | 7.45     | 79.2±6.99        | 0.9964 |
| Carfentrazone-ethyl  | 412.1               | 365.9 345.9        | 12 20  | 10.48    | 91.6±13.48       | 0.9965 |
| Chlorantraniliprole  | 483.9               | 452.9 285.9        | 16 8   | 7.93     | 94.8±12.50       | 0.9976 |
| Cyazofamid           | 325.0               | 261.0 108.0        | 4 8    | 10.47    | 83.78±8.92       | 0.9983 |
| Diclobutrazol        | 328.1               | 159.0 70.0         | 32 20  | 9.38     | 101.7±9.34       | 0.9997 |
| Diethofencarb        | 268.2               | 226.1 120.0        | 0 30   | 8.70     | 94.2±10.88       | 0.9981 |
| Difenconazole        | 406.1               | 337.0 251.0        | 10 20  | 10.51    | 75.7±8.94        | 0.9926 |
| Dimethoate           | 230.0               | 198.8 125.0        | 16 8   | 4.73     | 79.2±18.0        | 0.9902 |
| Hexaconazole         | 314.1               | 159.0 70.1         | 30 20  | 9.47     | 79.2±8.99        | 0.9908 |
| Hexythiazox          | 353.1               | 227.9 168.1        | 16 8   | 12.79    | 80.1±10.17       | 0.9875 |
| Fenhexamide          | 302.1               | 97.1 55.2          | 28 56  | 9.30     | 90.8±14.43       | 0.9987 |
| Fenoxycarb           | 302.1               | 116.0 88.0         | 17 29  | 9.82     | 71.4±10.03       | 0.9913 |
| Fenpropimorph        | 304.3               | 147.1 120.0        | 54 25  | 9.55     | 76.9±9.47        | 0.9964 |
| Flusilazole          | 316.1               | 165.0 120.1        | 16 32  | 3.17     | 86.4±7.26        | 0.9919 |
| Flutriafol           | 302.1               | 95.0 70.1          | 56 16  | 6.71     | 78.4±4.23        | 0.9899 |
| Furalaxyl            | 302.1               | 242.1 95.0         | 10 27  | 8.53     | 80.9±6.45        | 0.9904 |
| Furathiocarb         | 383.2               | 195.0 162.0        | 16 36  | 12.56    | 92.0±11.11       | 0.9987 |
| Kresoxim-methyl      | 314.1               | 267.0 222.1        | 0 10   | 10.49    | 73.9±9.44        | 0.9997 |
| Imazalil             | 297.1               | 159.0 123.1        | 20 20  | 8.26     | 80.8±17.14       | 0.9916 |
| Imidacloprid         | 256.0               | 208.9 175.0        | 12 12  | 4.66     | 91.8±9.17        | 0.9942 |
| Indoxacarbe          | 528.1               | 203.0 126.1        | 24 15  | 6.77     | 84.6±9.27        | 0.9985 |
| Isoprocarb           | 194.1               | 95.0 70.1          | 20 40  | 7.21     | 76.4±12.87       | 0.9953 |
| Metolachlor          | 284.2               | 252.1 176.1        | 8 24   | 9.67     | 90.8±10.49       | 0.9936 |
| Mepronil             | 270.1               | 119.0 91.1         | 20 40  | 9.74     | 80.3±10.77       | 0.9912 |
| Mepanipyrim          | 224.1               | 77.0 56.1          | 24 10  | 11.28    | 86.4±12.55       | 0.9979 |
| Metalaxyl            | 280.2               | 220.1 160.1        | 10 20  | 6.89     | 96.3±8.64        | 0.9954 |
| Metconazole          | 320.1               | 125.0 70.1         | 48 24  | 9.68     | 79.8±15.61       | 0.9989 |
| Methomyl             | 163.1               | 106.0 88.0         | 5 5    | 3.75     | 102.6±9.21       | 0.9847 |
| Methiocarb           | 226.1               | 169.0 121.1        | 4 12   | 8.37     | 79.9±10.24       | 0.9954 |
| Myclobutanil         | 289.1               | 125.1 70.1         | 32 16  | 8.81     | 85.6±14.81       | 0.9926 |
| Omethoate            | 214.0               | 125.0 109.0        | 16 24  | 1.84     | 92.6±11.78       | 0.9932 |
| Oxadixyl             | 279.1               | 219.2 132.3        | 5 32   | 5.70     | 76.3±9.44        | 0.9899 |
| Pesticide         | Precursor ion (m/z) | Product ions (m/z) | CE (V) | Rt (min) | Recovery (%) ± RSD | R²      |
|------------------|---------------------|--------------------|--------|----------|---------------------|---------|
| Oxamyl           | 237.1               | 90.0 72.0          | 5 12   | 3.66     | 94.5±11.12          | 0.9903  |
| Pencycuron       | 329.1               | 125.1 89.1         | 24 60  | 11.27    | 90.3±7.21           | 0.9905  |
| Paclobutrazol    | 294.1               | 125.0 70.1         | 48 20  | 8.21     | 103.4±8.66          | 0.9924  |
| Phoxim           | 299.0               | 129.1 77.1         | 4 24   | 11.4     | 79.9±8.21           | 0.9921  |
| Phosmet          | 317.99              | 160.0 133.0        | 8 36   | 8.96     | 100.5±9.48          | 0.9992  |
| Pirimiphos-methyl| 306.2               | 164.4 108.1        | 20 20  | 10.77    | 77.9±9.46           | 0.9912  |
| Penconazole      | 284.1               | 158.9 70.1         | 30 15  | 9.47     | 79.5±8.43           | 0.9902  |
| Picoxystrobin    | 368.1               | 265.2 90.0         | 4 20   | 10.67    | 83.4±12.3           | 0.9917  |
| Promecarb        | 208.1               | 151.0 109.1        | 0 10   | 8.77     | 104.2±8.21          | 0.9917  |
| Prochloraz       | 376.0               | 308.0 265.9        | 4 12   | 8.17     | 84.2±94.12          | 0.9964  |
| Propiconazole    | 342.1               | 159.0 69.1         | 32 16  | 9.86     | 103.0±9.21          | 0.9896  |
| Proxynur         | 210.1               | 168.1 111.0        | 0 10   | 6.23     | 90.8±4.24           | 0.9978  |
| Proquinazid      | 372.9               | 331.0 289.0        | 5 20   | 13.55    | 92.6±7.99           | 0.9983  |
| Pyriproxyfen     | 322.2               | 185.0 96.0         | 20 12  | 12.75    | 109.2±7.24          | 0.9982  |
| Pyraclostrobin   | 388.11              | 193.8 163.1        | 8 20   | 11.09    | 82.6±9.71           | 0.9914  |
| Pyrimethanil     | 200.1               | 106.9 82.0         | 20 25  | 6.20     | 69.7±9.49           | 0.9897  |
| Pyridaben        | 365.1               | 309.1 147.2        | 4 20   | 13.83    | 70.4±11.28          | 0.9901  |
| Quinoxynfen      | 308.0               | 196.9 161.9        | 36 45  | 11.59    | 82.4±7.01           | 0.9912  |
| Spiroxamine      | 298.28              | 144.1 100.1        | 18 32  | 6.87     | 70.0±15.68          | 0.9941  |
| Tebufenozide     | 353.2               | 297.2 133.1        | 5 15   | 10.26    | 73.8±10.26          | 0.9877  |
| Tetraconazole    | 372.0               | 159.0 70.0         | 36 20  | 9.27     | 80.9±9.47           | 0.9912  |
| Thiabendazole    | 202.0               | 175.0 131.0        | 24 36  | 3.75     | 119.4±15.99         | 0.9833  |
| Thiacloprid      | 253.0               | 186.0 126.0        | 10 20  | 5.31     | 96.7±18.12          | 0.9914  |
| Thiamethoxam     | 292.0               | 211.1 181.1        | 8 20   | 4.25     | 99.7±14.05          | 0.9997  |
| Thiophenate-methyl| 343.0              | 151.0 123.1        | 50 24  | 8.19     | 90.6±7.78           | 0.9928  |
| Tricyclazole     | 190.0               | 163.1 136.1        | 24 28  | 4.89     | 108.6±17.0          | 0.9922  |
| Tebuconazole     | 308.1               | 124.9 70.0         | 47 40  | 9.26     | 98.1±14.23          | 0.9958  |
| Thiodicarb       | 355.1               | 108.1 88.1         | 8 8    | 6.22     | 93.5±4.81           | 0.9916  |
| Triflunoxydorbin | 409.1               | 186.0 145.0        | 12 22  | 11.84    | 77.6±16.75          | 0.9915  |
| Trichlorazine    | 318.1               | 125.2 70.2         | 41 33  | 8.35     | 80.3±12.46          | 0.9898  |
| Zoxamide         | 336.0               | 187.0 159.0        | 16 44  | 11.07    | 73.8±10.44          | 0.9977  |
| Carbofuran-D3    | 225.1               | 165 123            | 10 22  | 6.25     |                     |         |

*MRM - multiple reaction monitoring mode; Rt - retention time, R² - correlation coefficient; CE - collision energy; RSD - relative standard deviation
The composition of the mobile phase returned to the initial conditions in 1 min and the system was equilibrated during 2 min. The total running time was 11 min. The ESI source was used with the following settings: drying gas (nitrogen) temperature 200 °C, drying gas flow rate 16 L/min, nebulizer pressure 30 psi, sheath gas temperature 300 °C, sheath gas flow 12 L/min, and capillary voltage 3000 V. The detection was performed using the dynamic multiple reactions monitoring mode (dMRM). The Agilent MassHunter software (version B.10.0 SR1 Agilent Technologies, 2006-2019) was used for the optimization and quantification.

**Sample collection.** The analyses comprised 44 milk samples collecting during 2019 (10 samples of pasteurized milk with 3.2 % milk fat, 9 samples of pasteurized milk with 2.8 % milk fat, 7 samples of homogenized pasteurized milk with 2.8 % milk fat, 10 samples of pasteurized partially skimmed milk with 2.8 % milk fat and 8 samples of pasteurized partially skimmed milk with 1.6 % milk fat.

The sampling was performed in accordance with SANTE/12682/2019 and stored at -10 °C to the time of the analysis.

**Sample preparation.** Pesticides were extracted from milk samples using the QuEChERS method described in Figure 1.

**Results and discussion**

The used validated LC-MS/MS method in accordance with SANTE/12682/2019 Document obtained a good response correlation coefficient regarding the linearity in the range of 10 to 100 µg/kg for all pesticides, with the $R^2>0.99$ for 90.12 % of investigated pesticides. The matrix effect was estimated on matrix and solvent calibration graph slopes and it indicated that milk has a strong influence on 73 pesticides. The matrix effects (ME) was compensated with matrix match calibration. The chromatogram of spiking milk samples on the level of 20 µg/kg is given in Figure 2.
The LOQ as the lowest quantified value was set for each pesticide on 0.01 mg/kg and it was confirmed experimentally. The recovery studies were appraised at two levels (20 and 100 µg/kg), spiking blank milk samples. Only one pesticide i.e. pyrimethanil showed the average recovery of 69.7 %, while all the others recovery values were in interval from 70.0 (spiroxamine) to 119.4 % (thiabendazole). The repeatability, expressed as a relative standard deviation (%RSD), was between 4.81 and 18.12 %.

The analyses of 44 milk samples were done with this highly sensitive and selective LC-MS/MS method. The total ion chromatogram (TIC) chromatograms of eight milk samples were given in Figure 3.

From all covered pesticides (81), only seven were detected at the trace levels: metalaxyl (present in 5 samples), bifenthrin (4), metolachlor (4), dimethoate (2), and trifloxystrobin (2) while the thiacloprid was detected in one of the analyzed samples.

Only one detection of trifloxystrobin in the concentration of 0.031 mg/kg was above the MRL of 0.02 mg/kg (EC Reg. 396/2005, Official Gazette 81/19), but taking in the account measurement uncertainty this detection could be considered compliant. The presence of pesticides was determined in 11 samples (25 %) out of a total of 44 analyzed samples. Only 5 samples (11.4 %) were contained pesticide residues above LOQ (bifenthrin: 0.023 and 0.019 mg/kg, thiacloprid 0.018 mg/kg and trifloxystrobin 0.031 and 0.015 mg/kg) (Figure 4).

According to the Commission Regulation (EU) 2017/1135 of 23 June 2017 amending Annexes II and III to Regulation (EC) No 396/2005 the MRLs for dimethoate in milk is not established. Various types of milk with different content of fat did not affect the appearance of pesticides. One of the reasons is probably the consequence of no analysed pesticides that are well soluble in fats.

According to the last available data from 2019, published by the Serbian National Household Budget Survey (2020), the average milk consumption in the Republic of Serbia is 100 mL/person/day, while based on a 7-day recall data, Đekić et al. (2020) emphasized that the average milk consumption is 153.6 mL/person/day. Considering the low concentrations of pesticide residue detections, it could be concluded that average milk consumption should not represent a threat and will not have a negative exposure assessment for human health.

The obtained results are in correlation with those published by Fagani et al. (2011) who also analysed pesticide residues in milk. In their study, the most frequently detected pesticide was fenthion, in more than a third of the samples (33.33 %). Dimethoate and malathion were present in 25 and 8.33 % of the examined samples, respectively. From the group of carbamates, carbofuran was detected in 25 %, aldicarb in 16.67 % and carbaryl in 8.33 % of the analysed milk samples. The results of our study emphasize that pesticide residues were present in 25 % of the examined samples, while multiple pesticide detections were recorded in 13.6 % of milk samples. Regarding the time window of the ten years between the obtained results published by Fagani et al. (2011) and this study, it is evident that the continuous monitoring of the cow milk and dairy products health safety is more than necessary.

Plant protection products (PPP) are a large group of different chemical compounds used to control insects, weeds, parasitic fungi, rodents, and other potentially harmful organisms. On the one hand, their long-term application has contributed to the productivity growth of many agricultural crops, but on the other hand, it has made a noteworthy danger to the environment and human health. Long-term studies have shown that the pesticide persistence and accumulation in the environment, their biodegradation resistance, as well as the potential degradation in more toxic metabolites have important human and animal health implications and are a crucial factor in considering the extent of their use (Witczak and Mituniewicz-Malek, 2019). The majority of previously published studies are based on the reports of organochlorine (OCPs) and organophosphorus (OPPs) pesticide residues in milk. OCPs are banned for more than 50 years regarding their toxicity and adverse harmful effects to the human,
animal and environmental health. Although, organophosphorus pesticides (OPPs) have relatively low stability, they still can accumulate in the food chains and consequently, they have been frequently detected in milk. The OPPs capacity to covalently link proteins can lead to their persistence in the milk, which is supported by the fact that casein, mainly with its seryl and phosphoryl residues, can surely bind with OPPs. Furthermore, the lipophilic nature of most OPPs favours their accumulation in a fatty matrix such as milk. For that reason, milk can be one of the human dietary sources of OPPs accumulation. The pesticide residues continuous monitoring in milk and dairy products is an imperative for correct evaluation of the overall consumers’ risk, emphasizing the particular attention to the infants and children safety.

**Conclusion**

The pesticide application is indispensable to enhance the overall agricultural production and control parasites responsible for transmission and maintenance of the vector-borne human and animal diseases. Nevertheless, the inappropriate use of pesticides may lead to the occurrence of their residues in concentrate feeds and fodder supplied to the cattle, particularly if the withdrawal period is not respected.

Milk and dairy products contamination with pesticide residues is a matter of a serious public health concern, since they are widely consumed by infants, children and elderly population. This is crucial since young children may be highly exposed to the pesticide residues and may be at a higher risk of adverse health effects due to their physiology and metabolism.

Regarding the fact that of 81 analysed, three pesticides were detected above LOQ in this study, in 11.4 % of analysed milk samples, the imperative is to continuously monitor animal feedstuffs and milk for pesticide residues in order to identify the main sources of milk contamination and consequently apply strict and well-designed prevention measures and risk assessment protocols.

**Ostaci pesticida u kravljem mlijeku**

**Sažetak**

Kvaliteta mlijeka igra ključnu ulogu u širokoj potrošnji, ali i u sudjelovanju u velikom nizu derivata, kao što su jogurt, sir, maslac i sladoled. Utvrđena je učinkovita LC-MS/MS metoda za određivanje 81 različitog ostatka pesticida u uzorcima mlijeka. Analize su obuhvatile 44 uzorka mlijeka prikupljenih tijekom 2019. godine. Od svih pregledanih pesticida u 2,27 % analiziranih uzoraka otkriveni su samo metalaksil (prisutan u 11,36 % uzoraka), bifentrin i metolaklor (9,09 %), dimetoat (4,55 %), prokloraz i tiakloprid. Detekcije trifloksistrobina i bifentrina bile su iznad maksimalnih razina ostataka (MRL). Uredba Evropske komisije (EU) 2017/1135 standardizira MRL za dimetoat i ometoat u određenim proizvodima, ali nije utvrđena MRL za ostatke dimetoata u mlijeku. Svi ostali pesticida bila su ispod granica MRL-a.

**Ključne riječi:** mlijeko, sigurnost mlijeka, pesticidi, kravlje mlijeko

**Acknowledgments**

This research was supported by the Ministry for Education, Science and Technological Development of the Republic of Serbia.
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