International Journal of Food Science + Technology

Institute of Food Science + Technology ifst

International Journal of Food Science and Technology 2021, **56**, 6685–6696

Original article

Toxic elements in eggs and egg-based products: occurrence, exposure assessment and risk characterisation for the Serbian population

Marija Mitrovic,[1*] Igor Tomasevic,[1] Srđan Stefanovic,[2] Vesna Djordjevic[2] & Ilija Djekic[1]

1 Faculty of Agriculture, University of Belgrade, Belgrade 11080, Serbia
2 Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade 11040, Serbia

(Received 5 August 2021; Accepted in revised form 21 September 2021)

Summary This study aimed to assess the exposure of the Serbian adult population to As, Cd, Pb and Hg through the consumption of eggs and egg-based products and to assess health risks. Coccidiostat residues were also examined, and their contribution to acceptable daily intake was calculated. Egg samples were collected from 2018 to 2020. The consumption survey was conducted during 2020. A Monte Carlo simulation was performed to estimate the intake of toxic elements based on one-day and seven-day recalls of egg consumption. The results obtained showed that the adult population in Serbia is not exposed to health risks from the studied toxic elements when consuming eggs and egg-based products. The presence of anticoccidial agents was detected in 22% of egg samples at concentrations above the limits prescribed by current regulations in Serbia (which do not allow detectable levels of coccidiostats in eggs), with the main risk of exposure being to coccidiostat residues associated with maduramycin.

Keywords Arsenic, cadmium, coccidiostats, health risk assessment, lead, mercury, toxic elements.

Introduction

Hen eggs are a food of animal origin and are consumed globally. According to reports from the Food and Agriculture Organization (FAO), egg consumption, as well as total food production of animal origin, has increased sharply (by about 50%) in the period from 2000 to 2018 (FAO, 2020). Demand for hen eggs and their products are expected to continue to grow due to their widespread consumer acceptance, increasing consumption at individual level and population growth (FAO, 2018).

Based on their multifunctional properties and simple preparation methods, fresh eggs are used as a main source of protein in the human diet around the world (Domingo, 2014; Lesnierowski & Stangierski, 2018). They contain an optimal ratio of essential amino acids, fatty acids, liposoluble vitamins (A, D, E, K) and B vitamins (B1, B2, B5, B6, B7, B9, B12, choline). In addition, they contain minerals such as calcium, iron, magnesium, phosphorus, selenium, sodium and zinc and antioxidants that reduce free radicals caused by cellular metabolism (Rodríguez-Gonzalo *et al.*, 2017). Owing to their known nutritional characteristics, availability to the population around the world and affordable prices (Domingo, 2014), eggs are very often combined with other foods in human nutrition (FAO, 2018).

In order to provide sufficient quantities of food of animal origin to the world market, there has been intensive development of agricultural production in recent decades. Modern, intensive agricultural production requires increased use of protective substances and growth stimulants, and it has also produced uncontrolled waste disposal. These activities significantly contribute to concentrations of toxic elements being above the permitted values in eggs (Kabir & Simul, 2019), which leads to the manifestation of negative effects in the environment (pollution), food chain and human health (Kacholi & Sahu, 2018). Along with the rapid growth of the hen egg industry, there is rising concern about the exposure of food of animal origin to contamination by toxic elements from various sources throughout the food chain (Filazi *et al.*, 2017).

Contaminants such as toxic metals, banned chemicals and veterinary drug residues are often found in food of animal origins such as meat, fish, dairy products and eggs (EFSA, 2016). However, there are relatively few peer-reviewed studies that have included assessment of

---

*Correspondent: E-mail: marijamitrovic66@gmail.com

doi:10.1111/ijfs.15366

consumer exposure to toxic elements and health risk assessment for eggs and egg-based products (EEBP) (Fu *et al.*, 2014). Research on toxic metals (As, Cd, Hg, Pb) in hen eggs have been conducted by authors mainly from Asia, (Fu *et al.*, 2014; Shaheen *et al.*, 2016; Hashemi *et al.*, 2018; Giri & Singh, 2019), which is expected given that Asian countries account for 60% of total world egg production. Regarding Europe, research was conducted in the European Union (EU), in Spain (Rubio *et al.*, 2018) and Italy (Esposito *et al.*, 2016) and in Bosnia and Herzegovina, a non-EU country (Vehab *et al.*, 2019). Studies of coccidiostats in food of animal origin were mainly conducted within the EU (Dorne *et al.*, 2013; Roila *et al.*, 2019).

The most common causes of veterinary drug residues in products of animal origin are their uncontrolled use, incorrect dosing, non-compliance with the waiting period and cross-contamination during the production, storage and transport of animal feed (Roila *et al.*, 2019). Most coccidiostats approved by European regulations are classified as feed additives but are prohibited for laying hens due to their excretion through the egg and high risk for consumers (Daeseleire *et al.*, 2017; Wang *et al.*, 2020). Examination of coccidiostats in eggs included the following: diclazuril (DICL), robenidine (ROBN), narasin (NAR), nicarbazin (DNC), monensin (MON), salinomycin (SAL), maduramicin (MAD), lasalocid (LAS) and toltrazuril (TOL), as defined in EU Commission Regulation (124/2009) and (610/2012) (EC, 2009, 2012).

Coccidiostats and heavy metals exhibit various toxic effects on human health, especially due to long-term exposure to low concentrations after consuming contaminated food of animal origin (Roila *et al.*, 2019). In order to assess the exposure of the local population to toxic compounds/elements (veterinary drug residues and toxic metals) from hen EEBP, it is necessary to determine the concentration of toxic elements present in these products in relation to their mean daily consumption (Anadón & Martínez-Larrañaga, 2014).

Consumption of foods containing some of these substances poses a major health risk to consumers worldwide, due to the substances' persistence, toxicity (Kabir & Simul, 2019) and negative impact on cellular metabolism (Dorne *et al.*, 2013). In order to protect consumers, legal obligations for monitoring and control of residues in products of animal origin have been defined at EU level, by establishing maximum residue limits (MRLs) for those foods in which residues can be tolerated. Systematic control of residues is further carried out through national surveillance programmes, which define the conditions and measures for monitoring residues in food of animal origin, control methods and methods for response when legal limits are exceeded (Sundlof, 2014).

Despite strict regulations for the monitoring of chemical residue, the possible contamination of food of animal origin through industrial and agricultural activities remains a major problem worldwide (Okamoto & Motomura, 2017). Health risks will depend on the eating habits of each individual, the level of contamination, different types of products used in the diet and the portion size. Therefore, monitoring and assessing exposure to toxic elements and veterinary drugs residues in EEBP is of great importance if we consider that these foods are economically affordable and often consumed (Basha *et al.*, 2013; Khan *et al.*, 2016). Regarding eggs and EEBP, there are papers that analysed microbial hazards mainly pathogens, such as in the papers of Upadhyaya *et al.* (2017), Lin *et al.* (2021), and Cardoso *et al.* (2021). However, our focus was on chemical hazards. A large number of authors have addressed the health risks associated with chemical residues in meat, fish, fruits, vegetables, grains and their products. However, concerning eggs and egg-based products, focus of this type of research was mainly only on eggs with limited studies that have examined the exposures and risks associated with consuming EEBP (Fu *et al.*, 2014). Therefore, this was identified as a research gap by the authors of this paper.

The aim of this study was to provide an assessment of exposure to toxic elements (heavy metals and veterinary drug residues) through the consumption of hen EEBP in Serbia and to assess health risks. This goal was met by analysing the content of toxic elements in eggs over a period of three years (2018–2020) and using data from a survey on food consumption conducted during 2020.

Materials and methods

Analyses of eggs

Analysis of eggs was performed in a laboratory accredited according to the reference standard ISO/IEC 17025:2017. Test methods were validated according to the relevant EU regulations and are used in routine work for testing eggs.

Sampling and preparation

A total of 255 samples were analysed during the period of 2018-2020. Eggs were collected from various commercial layer farms throughout Serbia. Sampling included all large, all medium and some small producers within the part of the national residue monitoring programme.

Analysis of toxic elements

As, Cd, Pb and Hg were determined using inductively coupled plasma mass spectrometry (ICP-MS). Each previously homogenised sample was subjected to oxidation with nitric acid and hydrogen peroxide followed by microwave digestion. After dilution, digestions were analysed using an iCAP q instrument (Thermo Scientific, Bremen, Germany) operating in the kinetic energy discrimination (KED) in order to remove isobaric interferences. Four-point calibration was obtained before and after each sample batch, in the range 0.2–2 µg L^{-1}.

Heavy metal concentrations below limit of quantification (LOQ) were substituted with a constant value of LOQ/2 in further analysis since this is recommended as a method for dealing with left-censored values (Beal, 2001). The quality control of the analytical procedure was achieved by the analysis of blank samples fortified with analytes of interest (0.5 µg L^{-1} for Cd, Hg, Pb and As). The recovery was always in the range of 92–104%. Additional quality control was performed analysing two certified reference materials at the beginning and end of each series of samples (Mussel tissue – ERM-CE278k, JRC Geel, Belgium and TET010RM – milk powder, Fera Science Ltd, Sand Hutton, UK). For deviations of ±10% (including uncertainty), proportional corrections of results were applied, and for deviations >10%, the analysis was repeated (Nardi *et al.*, 2009; Millour *et al.*, 2011).

Analysis of coccidiostats

Nine compounds registered in Serbia as therapeutic drugs or feed additives were analysed during the study: diclazuril (DICL), robenidin (ROBN), narasin (NAR), nicarbazin (DNC), monensin (MON), salinomicin (SAL), maduramycin (MAD), lasalocid (LAS) and toltrazuril (TOL). Liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used. Simple sample preparation was applied, consisting of extraction with acetonitrile and direct injection of crude extract after filtration. Quantification was performed using a five-point matrix-matched calibration procedure.

Samples were analysed by a Shimadzu LC-MS system (Kyoto, Japan) with a triple quadrupole mass spectrometer LCMS-8040. An analytical column Kinetex 100 × 2.1 mm 2.6 µm C18 100A with UltraGuard cartridge (Phenomenex, Thorens, CA, USA) was used. Chromatographic separation was achieved in gradient mode using water acidified with 0.1% formic acid (mobile phase A) and acetonitrile acidified with 0.1% formic acid (mobile phase B) at a flow rate of 0.4 mL min^{-1}. Electrospray ionisation (ESI) was used in both positive and negative modes, with the following parameters: interface voltage 4 kV, heat block temperature 400 °C, desolvation line temperature 250 °C, spray and drying gas flow 3 and 15 L min^{-1}, respectively. Argon was used as a collision gas (Olejnik *et al.*, 2010; Cronly *et al.*, 2011).

Consumption of eggs and egg-based products

For the purpose of the survey on consumption of EEBP, 1000 residents were interviewed face-to-face during 2020. All interviewees confirmed consumption of EEBP in the last seven days, while for the one-day recall, forty-one had not consumed EEBP (Table 1). Regarding age and location, the sample was predetermined in terms of interviewing adult population older than 18 years of age and living in large cities as places of residence (Djekic *et al.*, 2019). A brief introduction to the survey was provided to the interviewees, informing them about basic principles of anonymity, confidentiality and data protection (Udovicki *et al.*, 2019) enshrined in the study. Before proceeding, all interviewees gave their verbal consent.

The questionnaire was developed using guidance outlined by the European Food Safety Authority (EFSA), to enable analysis of frequencies and amounts of food consumed (EFSA, 2009). The questionnaire had the following sections: (i) demographic data – gender, age, height and body weight (bw). The last two enabled calculation of the body mass index – BMI = -weight [kg]/height^2 [m^2] (Brouwer-Brolsma *et al.*, 2018); (ii) consumption patterns of EEBP, giving the respondents an opportunity to specify frequency of consumption and; (iii) questions designed to determine consumption of ten different types of EEBP based on two periods: ’yesterday’ (one-day recall) and ’in the last seven days’ (seven-day recall). In order to capture habitual intake, the use of two recall periods in combination is preferable (Udovicki *et al.*, 2019), since some authors promote a 1-day recall period as the most common, as opposed to EFSA advising that additional

Table 1 Demographic profile of the sample population (*n* = 1000)

Demographic factor	Sub-group	Total
Gender	Male	345 (34.5%)
	Female	655 (65.5%)
Age	<34 years	439 (43.9%)
	35–49 years	354 (35.4%)
	Over 50 years	207 (20.7%)
Weight	Below 70 kg	520 (52.0%)
	Above 70 kg	480 (48.0%)
BMI	14.5 ≤ BMI ≤ 24.9	662 (66.1%)
	BMI ≥ 24.9	338 (33.8%)
Average body weight (kg)		73.36 ± 14.59
Average intake of eggs and egg-based products – 1-day recall (kg)		0.147 ± 0.115
Average intake of eggs and egg-based products – 7-day recall (kg)		0.790 ± 0.577

n is the number of respondents; (%) is the percentage in the sub-group for each demographic factor.

recall periods are sometimes more effective (EFSA, 2009). After analysis of scientific literature, ten EEBP were pre-selected for the consumption survey as follows: eggs, pastry products, pasta, meat products with eggs, fried vegetables, other fried food, fish and seafood, cakes, pancakes and mayonnaise. Prior to the consumption survey, authors prepared photographs of these products with predetermined mass/volume of portions, which were provided to the interviewees as visual aids.

Calculation of the amount of egg in egg-based products

To calculate the content of egg in the egg-based products, 100 eggs in four different classes XL, L, M, S according to the Serbian Regulation (Serbian Regulation, 2019a) were purchased from retail. Their mass was measured on an analytic balance SARTORIUS CP2202S. The average egg weight of 52.62 ± 7.26 g was used for all further calculations. For each of the egg-based product groups (Table 2), the average egg content per 100 g of final product was estimated. This value for each group was calculated based on several common recipes (4–10) for each of the product types. The recipes are commonly used in Serbia and were captured from suitable, available literature (Pejovic, 2014; Ilic, 2018; Serbian Regulation, 2018).

Exposure assessment

The exposure to toxic elements through consumption of EEBP was calculated based on data from the consumption survey, concentration of a toxic element in eggs and body weight (from the consumption survey). For the two recall periods, the following equations apply (Djekic *et al.*, 2019; Udovicki *et al.*, 2019):

$$\mathrm{EDI} = \frac{\sum_{i=1}^{n} D_i}{\mathrm{bw}} * C_t \qquad 1 - \text{day recall} \qquad (1)$$

Table 2 Content of egg in egg-based products included in the study

Egg-based product	Content of egg (g) in 100g of final product
Pastry products	11.42
Pasta	17.12
Meat products with eggs	11.05
Fried vegetables	19.15
Other fried food	19.07
Fish and seafood	20.12
Cakes	31.36
Pancakes	24.21
Mayonnaise	19.47

$$\mathrm{EDI} = \frac{\sum_{i=1}^{n} w_i}{7} * \frac{1}{\mathrm{bw}} * C_t \qquad 7 - \text{day recall} \qquad (2)$$

EDI is the estimated daily intake of toxic elements [g kg bw^{-1} day^{-1}]. D_i is the quantity of eggs consumed based on the '1-day' recall [kg]; 'n' is the number of consumers. W_i is the quantity of eggs consumed based on the '7-day' recall [kg]; 'n' is the number of consumers. Body weight (bw) is expressed in [kg]. C_t is the concentration of a toxic element in eggs [μg kg^{-1}].

Non-carcinogenic and carcinogenic risks

The potential for non-carcinogenic effects was estimated as the ratio of exposure to the mean food intake of the toxic substance and the corresponding reference dose (eqn 3) (USEPA, 1989; Fu *et al.*, 2014):

$$\mathrm{THQ}_{i,x,y} = \frac{E_{i,x,y}}{\mathrm{RfD}} \qquad (3)$$

$\mathrm{THQ}_{i,x,y}$ is the target risk factor for non-carcinogenic effects for a single toxic substance; $E_{i,x,y}$ is exposure to the mean intake of a toxic substance as a function of time, for a representative individual [mg kg^{-1} day^{-1}]; RfD is the level of exposure to a toxic agent at which there will be no negative effects on human health [mg kg^{-1} day^{-1}]; the oral RfD values for As, Cd and Hg were taken from the database established by USEPA (IRIS, 2020), while the value for Pb was captured according to the provisional tolerable daily intake (PTDI) proposed by FAO/WHO (JECFA) (Fu *et al.*, 2014).

The non-carcinogenic toxic effect of exposure to the combined action of different toxic substances was estimated as the total hazard factor (hazard index), as presented in eqn 4 (USEPA, 2003; Hashemi *et al.*, 2018):

$$\text{Total HI} = \sum_{x=1}^{n} \mathrm{THQ}_{i,x,y} \qquad (4)$$

The potential for carcinogenic effects was assessed as the product of exposure and the carcinogenicity coefficient, eqn 5 (USEPA, 2005; Atamaleki *et al.*, 2020):

$$\mathrm{CR}_{i,x,y} = E_{i,x,y} * \mathrm{SF}_x \qquad (5)$$

$\mathrm{CR}_{i,x,y}$ is the carcinogenic risk for a representative individual due to exposure to a toxic substance in the environment; SF_x is the carcinogenicity coefficient of a toxic substance [mg kg^{-1} day^{-1}]; the oral SF values for As, Pb and Cd were taken from a database established by USEPA (IRIS, 2020), and studies conducted in Iran, (Hashemi *et al.*, 2018) and Thailand (Aendo *et al.*, 2019), respectively.

Statistical methods

The Chi squared test was used to analyse possible relationships between egg consumption patterns and demographic data such as gender, age, BMI and bw. The level of statistical significance was set at 0.05. Statistical processing was performed using Microsoft Excel 365 and SPSS Statistics 17.0.

For calculating EDI, authors used Monte Carlo simulations consisting of 100 000 iterations to estimate the daily intake of selected toxic elements based on the consumption of EEBP. These simulations were used as probabilistic models for calculating dietary exposure assessment, as recommended by WHO (FAO/WHO, 2009) and EFSA (EFSA, 2011). Minitab was used for distribution fitting of bw, daily and weekly intake of EEBP and Monte Carlo simulation. The fitting analysis showed normal distribution for bw and positively skewed distributions of daily/weekly intake of EEBP. Based on the distribution results, all data were randomised 100 000 times to perform Monte Carlo simulation, and EDIs were calculated (Djekic *et al.*, 2020). Exposure calculation was based on the mean value of all toxic elements (Table 4) since, over time, it is expected that an individual will be exposed to this concentration in EEBP (FAO/WHO, 2009). As proposed by Sun & Wu (2016), the uncertainty of Monte Carlo simulation was obtained from the confidence intervals (95% CIs) of the mean values.

Results and discussion

Consumption of eggs and egg-based products

The results in Table 1 depict the demographic profile of the interviewees and show that the survey was completed by more female (65.5%) than male respondents (34.5%). Over 40% of interviewees were younger than 34 years of age, followed by interviewees between 35 and 49 (35%) and those older than 50 years of age (20%). The average bw of the interviewees was 73.36 kg, which is in line with the EFSA recommendation that the average bw of an adult European citizen is around 70 kg (EFSA, 2012a). One-third of the interviewees were overweight with BMI >24.9, which coincides with the results of a study on the consumption of dairy products in Serbia during 2020 (Djekic *et al.*, 2020). Data from a national survey indicated that half of the population (54.5%) in Serbia during 2006 had a BMI above 24.9 (out of a total of 19 796 participants) (Grujic *et al.*, 2010). Also, research by the World Health Organization indicates that the number of overweight people is growing in recent years and that Europe is in second place in terms of obesity (EUROSTAT, 2014). Our consumption survey data indicate a decrease in obesity among adults in Serbia could have occurred since 2006, but it should be kept in mind that the number of interviewees in this consumption survey (1000) differs from the number in the 2006 survey on BMI (Grujic *et al.*, 2010). The average weight of EEBP consumed on a daily basis was 0.147 kg, while an average weight of 0.790 kg of EEBP was consumed weekly.

The average consumption of eggs per capita in the world is growing from year to year and reached the value of 9.68 kg in 2018. Kuwait ranks highest in terms of consumption of 21.9 kg, followed by Mexico (19.9 kg), Japan (19.8 kg) and China (19.8 kg). Egg consumption in most EU member states has been stable in recent years and amounts to about 210 eggs per year, with a significant increase in the use of egg products. Some countries, such as Spain, Germany and Hungary, have higher egg consumption levels of around 270 eggs per year, while the United Kingdom has around 200 eggs per capita. Serbia ranks 58th in the group of 156 countries in the world, which our results confirm somewhere (FAOSTAT, 2021).

The consumption survey showed the population of Serbia most often consumes fresh eggs (60.5%). Eggs are mostly consumed for breakfast (69.8%), prepared as fried eggs (36.3%), omelettes (19.7%) and boiled eggs (15.2%). Since these three culinary methods of egg preparation predominated, the relationship between their consumption patterns and demographic characteristics was analysed (Table 3).

There was a statistically significant difference between BMI and age of interviewees with respect to their consumption patterns ($P < 0.05$). The younger population (52.85%) under the age of 34 and those aged 34–49 (53.38%) consume fried eggs and omelettes for breakfast several times a week. Interviewees over the age of 50 consume only fried eggs several times a month. Interviewees with BMI $\leq$ 24.9 and weighing up to 70 kg consume fried eggs (32.11%) several times a month, while those with BMI $\geq$ 24.9 and weighing over 70 kg consume fried eggs (40.84%) several times a week. Females are more likely to consume fried eggs (58.92%), while males are more likely to consume omelettes (65.79%).

Toxic elements in eggs

From 2018 to 2020, low levels of toxic elements were detected in eggs. The levels were within the safety limits outlined in the current regulations in Serbia (Serbian Regulation, 2019b). The results of the analysis of toxic elements in eggs are shown in Table 4. The mean levels of As, Cd, Pb and Hg in hen eggs were 2.78, 0.92, 2.51 and 0.84 μg kg^{-1}, respectively (Table 4).

Our results showed a lower As content compared with data from Italy, 0.007 mg kg^{-1} (Esposito *et al.*, 2016); from Bangladesh, 0.3 mg kg^{-1} (Shaheen *et al.*, 2016); and Iran, 0.029, 0.056 mg kg^{-1} (Hashemi *et al.*, 2018). From 2000 to 2017, the mean As level in eggs from Belgrade, Serbia, was below the limit of detection (Mitrovic *et al.*, 2019). The mean Cd level was lower than that observed in home-produced eggs (0.30 mg kg^{-1}) and in eggs from local farms

Table 3 Consumption pattern of fresh hen eggs in Serbia (n = 1000)

Country	Daily	Several times a week	Several times a month	Rarely	Total
Gender					
Male	31 (8.98%)	182 (52.75%)	108 (31.30%)	24 (6.97%)	345 (100%)
Female	51 (7.78%)	325 (49.62%)	243 (37.10%)	36 (5.55%)	655 (100%)
X^2 = 3.8; $P > 0.05$					
Age					
Less than 34	51 (11.62%)	232 (52.85%)	128 (29.15%)	28 (6.38%)	439 (100%)
35–49 years	56 (15.82%)	189 (53.38%)	104 (29.38%)	5 (1.41%)	354 (100%)
Over 50 years	9 (4.35%)	86 (41.55%)	97 (46.86%)	15 (7.24%)	207 (100%)
X^2 = 47.93; $P < 0.05$					
Weight					
Below 70 kg	41 (7.88%)	269 (51.73%)	180 (34.61%)	30 (5.77%)	520 (100%)
Above 70 kg	41 (8.54%)	251 (52.29%)	135 (28.12%)	30 (6.25%)	480 (100%)
X^2 = 3.00; $P > 0.05$					
BMI					
BMI ≤ 24.9	55 (8.31%)	336 (50.76%)	231 (34.89%)	40 (6.04%)	662 (100%)
BMI ≥ 24.9	27 (7.99%)	171 (50.59%)	137 (40.53%)	3 (0.88%)	338 (100%)
X^2 = 15.79; $P < 0.05$					

n – the number of respondents; (%) is the percentage in the entire population sample.

Table 4 Concentration of toxic elements in eggs (2018–2020)

	Arsenic (μg kg^{-1})	Cadmium (μg kg^{-1})	Lead (μg kg^{-1})	Mercury (μg kg^{-1})
Average	2.78 ± 1.09	0.92 ± 0.39	2.51 ± 1.68	0.84 ± 0.22
Permittable value[a]	100	300	1000	100
PTDI (μg kg bw^{-1} day^{-1})	2.143[b]	0.357	3.57[b]	0.571

PTDI, provisional tolerable daily intake.

[a] Values obtained for current legislation.

[b] New values to be adopted by EFSA.

(0.82 mg kg^{-1}) from Bosnia and Herzegovina (Vehab *et al.*, 2019). However, in contrast to our current results, Cd levels in eggs above the detection limit were not detected in Spain (Rubio *et al.*, 2018). The mean Pb level in the eggs was much lower than reported on free-range farms in Italy, 0.03 mg kg^{-1} (Esposito *et al.*, 2016), in Spain, 0.025 mg kg^{-1} (Rubio *et al.*, 2018) and from different regions in neighbouring Bosnia and Herzegovina – 3.43 mg kg^{-1} (Vehab *et al.*, 2019). Similarly, the mean Hg level in eggs in this study was significantly lower than reported in Iran (0.01 mg kg^{-1}) (Hashemi *et al.*, 2018). Authors from Italy reported Hg levels in eggs were below the limit of detection (Esposito *et al.*, 2016).

Coccidiostats in eggs

From 2018 to 2020, a total of 56 (19%) egg samples were non-compliant with current Serbian regulation (Serbian Regulation, 2011) on the presence of coccidiostats (Table 5). Individually, MAD (14.2%) was most often detected, followed by LAS (6.27%), SAL (3.53%), ROBN (1.96%) and DNC (1.17%). Eight egg samples were contaminated with two substances (most often MAD and SAL, then; MAD and ROBN; MAD and DNC; LAS and DNC).

Regulatory requirements regarding the monitoring of coccidiostats in the EU and Serbia are not harmonised (Table 5). EU Commission Regulation (124/2009) defines maximum values (MRL) for the following coccidiostats in eggs: NAR, SAL, MON and ROB, while the values for LAS, MAD, DNC and DICL are defined by EU Commission Regulation (610/2012). Values for TOL in eggs are not prescribed by regulation, because its use is prohibited for poultry that will be used for human consumption (Roila *et al.*, 2019). In Serbia, products of animal origin must not contain veterinary drugs in quantities that can be proven by recognised methods (Serbian Regulation, 2011).

Research conducted in recent years indicates the variability of data regarding coccidiostat residues in products of animal origin, especially in eggs (Roila *et al.*, 2019). The latest EFSA report on the monitoring of veterinary drugs in products of animal origin analyses the data for 2018, and states thirty-three egg samples (0.65%) were non-compliant for anticoccidial agents out of 5098 tested egg samples in twenty-eight EU member states. The most common non-compliant coccidiostat was LAS, reported in Germany, Poland, the United Kingdom and Malta (EFSA, 2020).

Given the differences in regulatory requirements, the scope of control and the method of rearing laying hens

Table 5 Results of determination of coccidiostats in eggs in Serbia (2018–2020)

Analyte	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Tested (positive samples)	Incidence (%)	Min-Max (mg kg^{-1})	Regulation Serbia (mg kg^{-1})	Regulation EU (mg kg^{-1})	ADI EFSA (mg kg^{-1} bw)
Diclazuril	0.002	0.003	255 (0)	–	<LOD	Absence	0.002	0.029
Robenidine	0.0005	0.0015	255 (5)	1.96	0.013–0.017	Absence	0.025	0.0375
Narasin	0.001	0.002	255 (0)	–	<LOD	Absence	0.002	0.005
Nicarbazin	0.001	0.002	255 (3)	1.17	0.007–0.041	Absence	0.005	0.77
Monensin	0.0005	0.002	255 (0)	–	<LOD	Absence	0.002	0.003
Salinomycin	0.0005	0.002	255 (9)	3.53	0.002–10	Absence	0.003	0.005
Maduramicin	0.0005	0.002	255 (36)	14.12	0.002–17.50	Absence	0.012	0.001
Lasalocid	0.002	0.005	255 (16)	6.27	0.006–0.083	Absence	0.150	0.005
Toltrazuril	0.005	0.01	255 (0)	–	<LOD	Absence	Forbidden	Not applicable

ADI, acceptable daily intake; LOD, limit of detection; LOQ, limit of quantification.

Table 6 Estimated daily intake of toxic elements from egg consumption by adults in Serbia

	Arsenic ng kg bw^{-1} day^{-1}		Cadmium ng kg bw^{-1} day^{-1}	
Toxic metal intake	**1-day recall**	**7-day recall**	**1-day recall**	**7-day recall**
Mean	5.856	4.465	1.920	1.462
Minimum	0.070	0.068	0.023	0.022
1st quartile	2.443	2.632	0.801	0.863
3rd quartile	7.683	5.233	2.519	1.716
Maximum	63.998	61.842	22.711	21.783
95% confidence interval of mean	5.823–5.888	4.436–4.495	1.909–1.931	1.455–1.474

	Mercury ng kg bw^{-1} day^{-1}		Lead ng kg bw^{-1} day^{-1}	
Toxic metal intake	**1-day recall**	**7-day recall**	**1-day recall**	**7-day recall**
Mean	1.759	1.341	5.278	4.024
Minimum	0.021	0.020	0.063	0.061
1st quartile	0.734	0.791	2.202	2.372
3rd quartile	2.308	1.572	6.925	4.716
Maximum	20.548	18.169	60.644	59.509
95% confidence interval of mean	1.749–1.769	1.333–1.350	5.248–5.307	3.998–4.051

All values are calculated based on the Monte Carlo simulation.

within the EU and non-member countries, it is difficult to compare the results for coccidiostats from this study and results available in the literature. However, based on analytical data on the level of tested coccidiostats in eggs, their contributions to ADIs were calculated and compared with the results of other studies. Our results show that among the coccidiostats examined, DNC, ROBN and LAS contributed the lowest amounts (<1%, <1% and 1%, respectively) towards their individual respective ADIs, which coincides with results reported in similar studies conducted in Spain and Italy (Dorne *et al.*, 2013; Roila *et al.*, 2019). The contribution of SAL was significantly higher (56%), while the contribution of MAD exceeded the recommended ADI for MAD (130%).

Exposure assessment

The mean exposures of the Serbian population to toxic elements through egg consumption were As = 0.0058 µg kg bw^{-1} day^{-1}; Cd = 0.0019 µg kg bw^{-1} day^{-1}; Pb = 0.0052 µg kg bw^{-1} day^{-1}; Hg = 0.0017 µg kg bw^{-1} day^{-1}; (Table 6). Figure 1 displays estimated total daily intake of all four toxic elements after a Monte Carlo simulation for each of the two recall periods.

The EDIs were compared with the mean EDIs in the EU for each metal individually, and with the current values of PTDI for Cd and Hg, while for As and Pb they were compared with the last withdrawn values. The mean As exposure through diet for the European adult population (EFSA, 2014) was 110–170 µg kg

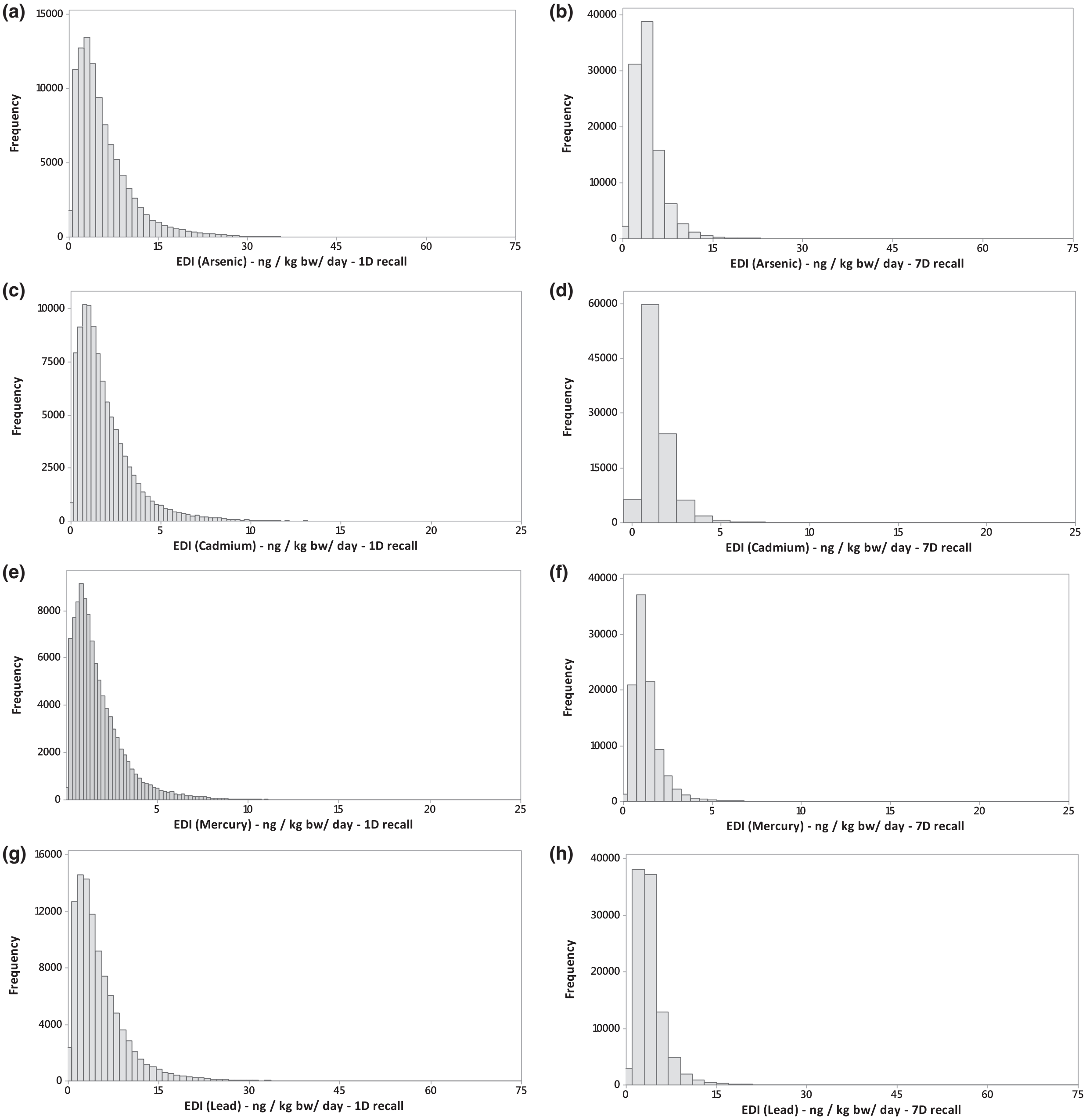

Figure 1 Comparison of estimated total daily intake of toxic elements after Monte Carlo simulation of 100 000 iterations. (a) Arsenic 1-day recall; (b) Arsenic 7-day recall; (c) Cadmium 1-day recall; (d) Cadmium 7-day recall; (e) Mercury 1-day recall; (f) Mercury 7-day recall; (g) Lead 1-day recall; (h) Lead 7-day recall.

bw^{-1} day^{-1} (min lower bound (LB) and upper bound (UB) approach) and 240–380 µg kg bw^{-1} day^{-1} (min LB-max UB). The EDI calculated for As through the intake of EEBP by consumers in Serbia was significantly below (i.e. was <1% of) the stated EU EDI and was below the previous assumed PTDI of 2.143 µg kg bw^{-1} day^{-1}.

The mean Cd exposure through diet for the European adult population was 2.04 to 3.66 µg kg bw^{-1} week^{-1} (95th percentile over a lifetime). The relative contribution to this exposure through EEBP consumption in nineteen EU member states was about 0.1% (EFSA, 2012a), which is the same as the contribution from EEBP for consumers in Serbia in the current research (0.09%).

The mean Pb exposure through diet for the European adult population was 0.40–59 μg kg bw^{-1} day^{-1}. The relative contribution to this exposure through EEBP consumption in EU member states was 0.4%, which is less than the contribution from EEBP for consumers in Serbia (0.42–1.45%). The FAO/WHO Joint Expert Committee on Additives and Foods (JECFA) withdrew the PTDI for Pb of 25 μg kg bw^{-1} week^{-1} (or 3.57 μg kg bw^{-1} day^{-1}) as inappropriate and concluded that it was not possible to establish a new value to protect consumer health. However, the EDI calculated for Pb through the intake of EEBP by consumers in Serbia was far below (i.e. was <1% of) the now withdrawn PTDI of 3.57 μg kg bw^{-1} day^{-1} (EFSA, 2012b).

The mean inorganic Hg exposure through diet for the European adult population was 0.13 μg kg bw^{-1} week^{-1} (lowest minimum LB) and 0.25 μg kg bw^{-1} week^{-1} (lowest minimum LB 95th percentile). The relative contribution to this exposure through EEBP consumption in nineteen EU member states was 0-0.5%, while our calculated contribution for EEBP consumers in Serbia was 0.68-1.3%. Consequently, the EDI for Hg is also far below (<1%) the established PTDI of 0.571 μg kg bw^{-1} day^{-1} (EFSA, 2012c).

Health risk assessment

The level of risk for carcinogenic and non-carcinogenic diseases associated with the intake of toxic elements from EEBP is given in Table 7. The risk assessment criteria for the development of chronic health diseases were determined based on the value of the target risk factor (THK) as follows: low risk THQ = 0.1 or <1; mean risk level THQ = 1; high risk level THQ > 1. Our results for As, Cd, Pb and Hg individually do not indicate a health risk, because the THQ values are far below 1. Also, the value for the summary hazard index was below 0.1. When assessing non-carcinogenic risks, a toxic agent on human health is expected to impact on human health when the estimated dose of exposure exceeds the value for RfD (Fowle & Dearfield, 2000), which did not occur in our study for any of the toxic elements.

The International Agency for Research on Cancer classifies As as a carcinogen for humans (Group 1), and Cd (Group B1) and Pb (Group B2) as likely carcinogens for humans (IARC, 2016), so the potential for carcinogenic risk (CR) was calculated from the exposure levels for these elements. The probability of occurrence of risk was determined based on the following criteria: CR < 10^{-6} negligible risk; CR = 10^{-4} to 10^{-6} acceptable risk; CR > 10^{-4} unacceptable risk (Atamaleki *et al.*, 2020). Our results (Table 7) show the probability of carcinogenic effects for each element through the intake of EEBP by consumers in Serbia is far below the minimum acceptable CR, <10^{-6}.

Table 7 Non-carcinogenic (HI) and carcinogenic (CR) risk of toxic elements from egg consumption by adults in Serbia

(*n* = 255)	Arsenic	Cadmium	Lead	Mercury
RfD	0.0003	0.0010	0.0035	0.0001
SF	1.5	8.5E-03	3.8E-01	5E-04
THQ	0.0193	0.0019	0.0015	0.0176
HI (THQ)	0.04			
CR	8.70E-6	7.20E-7	1.00E-7	Not applicable

CR, carcinogenic risk; HI (THQ), hazard index (total target hazard quotient); RfD, oral reference dose; SF, slope factor (carcinogenicity coefficient); THQ, target hazard quotient.

Conclusions

From the consumption survey, it can be concluded that fresh eggs are widely consumed in Serbia. The entire sampled sub-population confirmed consumption of eggs at least on a weekly basis, with a large number of interviewees (95.9%) consuming eggs every other day. Eggs were indirectly consumed through egg-based products in almost the same amount as fresh eggs.

Regardless of the large consumption of EEBP, the adult population in Serbia is not exposed to the intake of toxic elements As, Cd, Pb and Hg beyond tolerable values (daily and weekly) resulting from EEBP consumption. Consequently, there are no health risks, and hen eggs are a safe product regarding exposure to As, Cd, Pb and Hg. However, the presence of illegal, detectable levels of coccidiostats in eggs in Serbia indicates the need for promoting good veterinary practices by all stakeholders in the hen egg chain.

Since contamination of eggs could be associated with environmental contaminants, further research should assess the types of environmental impacts on the safety of hen table eggs and the health risks associated with them. This is pronounced in developing countries like Serbia, even though conditions for table egg production are currently being harmonised with European requirements.

The main limitations of this study included, firstly, the egg consumption survey being conducted only in large cities, so any future survey should encompass a population-based sample of residents. Secondly, the overall hazard index for the occurrence of non-cancerous effects, in addition to the examined toxic elements, should include other known toxic elements. Therefore, their concentrations in hen eggs should be also measured and taken into account for individual and summary health risks to the Serbian population.

Another limitation of this study is the lack of data for children.

Conflict of interest

All authors of this manuscript declare that they do not have any conflict of interest.

Author contribution

Marija Mitrovic: Conceptualization (equal); Data curation (equal); Investigation (equal); Visualization (equal); Writing-original draft (equal). **Igor Tomasevic:** Supervision (equal); Validation (equal); Writing-review & editing (equal). **Srđan Stefanovic:** Data curation (equal); Formal analysis (equal); Investigation (equal). **Vesna Djordjevic:** Data curation (equal); Investigation (equal); Resources (equal). **Ilija Djekic:** Formal analysis (equal); Methodology (equal); Supervision (equal); Visualization (equal); Writing-review & editing (equal).

Ethical approval

This study was performed according to the Codex of professional ethics of the University of Belgrade (Kodeks profesionalne etike Univerziteta u Beogradu 193/2016).

Informed consent

All participants involved in the field survey gave consent before beginning this study.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15366.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aendo, P., Thongyuan, S., Songserm, T. & Tulayakul, P. (2019). Carcinogenic and non-carcinogenic risk assessment of heavy metals contamination in duck eggs and meat as a warning scenario in Thailand. *Science of the Total Environment*, **689**, 215–222.

Anadón, A. & Martínez-Larrañaga, M. (2014). Veterinary drugs residues: coccidiostats. In: *Encyclopedia of Food Safety* (edited by Y. Motarjemi). Pp. 63–75. Orlando, USA: Academic Press. https://doi.org/10.1016/B978-0-12-378612-8.00246-8

Atamaleki, A., Sadani, M., Raoofi, A. *et al.* (2020). The concentration of potentially toxic elements (PTEs) in eggs: a global systematic review, meta-analysis and probabilistic health risk assessment. *Trends in Food Science & Technology*, **95**, 1–9.

Basha, A.M., Yasovardhan, N., Satyanarayana, S.V., Reddy, G.V.S. & Kumar, A.V. (2013). Assessment of heavy metal content of hen eggs in the surroundings of uranium mining area, India. *Annals. Food Science and Technology*, **14**, 6.

Beal, S.L. (2001). Ways to fit a PK model with some data below the quantification limit. *Journal of Pharmacokinetics and Pharmacodynamics*, **28**, 481–504.

Brouwer-Brolsma, E.M., van Lee, L., Streppel, M.T. *et al.* (2018). Nutrition Questionnaires plus (NQplus) study, a prospective study on dietary determinants and cardiometabolic health in Dutch adults. *British Medical Journal Open*, **8**, e020228.

Cardoso, M.J., Nicolau, A.I., Borda, D. *et al.* (2021). *Salmonella* in eggs: from shopping to consumption—a review providing an evidence-based analysis of risk factors. *Comprehensive Reviews in Food Science and Food Safety*, **20**, 2716–2741.

Cronly, M., Behan, P., Foley, B., Malone, E., Shearan, P. & Regan, L. (2011). Determination of eleven coccidiostats in animal feed by liquid chromatography–tandem mass spectrometry at cross contamination levels. *Analytica Chimica Acta*, **700**, 26–33.

Daeseleire, E., Van Pamel, E., Van Poucke, C. & Croubels, S. (2017). Veterinary drug residues in foods. In: *Chemical Contaminants and Residues in Food* (edited by D. Schrenk & A. Cartus). Pp. 117–153. Cambridge, UK: Woodhead Publishing Limited. https://doi.org/10.1016/B978-0-08-100674-0.00006-0

Djekic, I., Petrovic, J., Jovetic, M. *et al.* (2020). Aflatoxins in milk and dairy products: occurrence and exposure assessment for the Serbian population. *Applied Sciences*, **10**, 7420.

Annotated references: This paper provided a methodological framework for the Monte Carlo simulation. Also, the study was helpful in terms of consumption patterns.

Djekic, I., Udovicki, B., Kljusurić, J.G. *et al.* (2019). Exposure assessment of adult consumers in Serbia, Greece and Croatia to deoxynivalenol and zearalenone through consumption of major wheat-based products. *World Mycotoxin Journal*, **12**, 431–442.

Domingo, J.L. (2014). Health risks of human exposure to chemical contaminants through egg consumption: a review. *Food Research International*, **56**, 159–165.

Dorne, J.L.C.M., Fernández-Cruz, M.L., Bertelsen, U. *et al.* (2013). Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. *Toxicology and Applied Pharmacology*, **270**, 196–208.

EC. (2009). Commission Regulation (EU) 124/2009 of 10 February 2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed. *Official Journal of the European Union L*, **40**, 7.

EC. (2012). Commission Regulation (EU) No 610/2012 of 9 July 2012 amending Regulation (EC) No 124/2009 of 10 February 2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed. *EFSA Journal*, **2011**, 1952.

EFSA. (2009). General principles for the collection of national food consumption data in the view of a pan-European dietary survey. *EFSA Journal*, **7**, 1–51.

EFSA. (2011). Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. *EFSA Journal*, **9**, 1–33.

EFSA. (2012a). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, **10**, 2579. https://doi.org/10.2903/j.efsa.2012.2579

EFSA. (2012b). Lead dietary exposure in the European population. *EFSA Journal*, **10**, 2831. https://doi.org/10.2903/j.efsa.2012.2831

EFSA. (2012c). Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*, **10**, 2985. https://doi.org/10.2903/j.efsa.2012.2985

EFSA. (2014). Dietary exposure to inorganic arsenic in the European population. *EFSA Journal*, **12**, 3597. https://doi.org/10.2903/j.efsa.2014.3597

EFSA. (2016). Report for 2014 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. *EFSA Supporting Publications*, **13**, EN-923. https://doi.org/10.2903/sp.efsa.2016.EN-923

EFSA. (2020). Report for 2018 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. *EFSA Supporting Publications*, **17**, EN-1775. https://doi.org/10.2903/sp.efsa.2020.EN-1775

Esposito, M., Cavallo, S., Chiaravalle, E. *et al.* (2016). Trace elements in free-range hen eggs in the Campania region (Italy) analyzed by inductively coupled plasma mass spectrometry (ICP-MS). *Environmental Monitoring and Assessment*, **188**, 326.

EUROSTAT. (2014). Statistics Explained – European Commission. Overweight and obesity – BMI statistics. Retrieved from https://ec.europa.eu/eurostat/statistics-explained/index.php/Overweightandobesity-BMIstatistics/. Accessed March 5, 2021.

FAO. (2018). Eggs: Harnessing their power for the fight against hunger and malnutrition [Report of activity No154]. FAO Global Forum on Food Security and Nutrition (FSN Forum).

FAO. (2020). World Food and Agriculture – Statistical Yearbook 2020. Rome. https://doi.org/10.4060/cb1329en

FAO/WHO. (2009). Principles and methods for the risk assessment of chemicals in food. In: *Environmental Health, Criteria 240. Food and Agriculture Organization of the United Nations & World Health Organization: Geneva, Switzerland.*

FAOSTAT. (2021). Food and Agriculture Organization of the United Nations Statistics Division. Food and Agriculture Organization of the United Nations. Retrieved from http://faostat3.fao.org/home/E. Accessed September 10, 2021.

Filazi, A., Yurdakok-Dikmen, B., Kuzukiran, O. & Sireli, U.T. (2017). Chemical contaminants in poultry meat and products. In: *Poultry Science* (edited by M. Manafi). Pp. 172–190. London, UK: InTechOpen. https://doi.org/10.5772/64893

Fowle, J.R. & Dearfield, K.L. (2000). *Risk Characterization: Handbook: (519222012-001)*. Science Policy Council. Washington, DC: U.S. Environmental Protection Agency. https://doi.org/10.1037/e519222012-001

Fu, Q., Liu, Y., Li, L. & Achal, V. (2014). A survey on the heavy metal contents in Chinese traditional egg products and their potential health risk assessment. *Food Additives & Contaminants: Part B*, **7**, 99–105.

Giri, S. & Singh, A.K. (2019). Heavy metals in eggs and chicken and the associated human health risk assessment in the mining areas of Singhbhum copper belt, India. *Archives of Environmental & Occupational Health*, **74**, 161–170.

Grujic, V., Dragnic, N., Radic, I. & Harhaji, S. (2010). Overweight and obesity among adults in Serbia: results from the National Health Survey. *Eating and Weight Disorders*, **15**, 9.

Hashemi, M., Sadeghi, A., Dankob, M. *et al.* (2018). The impact of strain and feed intake on egg toxic trace elements deposition in laying hens and its health risk assessment. *Environmental Monitoring and Assessment*, **190**, 540.

Annotated references: This study helped in the methodological framework for health risk assessment. We applied the assessment to eggs and egg-based products that are most commonly consumed in Serbia.

IARC. (2016). Agents Classified by the IARC Monographs (edited by CANCER, I. A. F. R. O.). Pp. 1–116.

Ilic, M. (2018). *Traditional Serbian food and recipes/Tradicionalni recepti domaće srpske kuhinje*. National Library of Serbia/Narodna biblioteka Srbije. COBISS.SR-ID 261177612.

IRIS. (2020). Integrated Risk Information System US environmental protection Agency. Cincinnati. Retrieved from https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables/. Accessed February 22, 2021.

Kabir, A. & Simul, B. (2019). Heavy metals in egg contents of hens (*Gallus gallus domesticus*) and Ducks (*Anas platyrhynchos*) from Chittagong Region, Bangladesh. *Journal of Pollution Effects & Control*, **7**, 6.

Kacholi, D.S. & Sahu, M. (2018). Levels and health risk assessment of heavy metals in soil, water, and vegetables of Dar es Salaam, Tanzania. *Journal of Chemistry*, **2018**, 1–9.

Khan, Z., Sultan, A., Khan, R., Khan, S. & Farid, K. (2016). Concentrations of heavy metals and minerals in poultry eggs and meat produced in Khyber Pakhtunkhwa, Pakistan. *Meat Sciences and Veterinary Public Health*, **1**, 4–10.

Lesnierowski, G. & Stangierski, J. (2018). What's new in chicken egg research and technology for human health promotion? – A review. *Trends in Food Science & Technology*, **71**, 46–51.

Lin, Q., Chousalkar, K.K., McWhorter, A.R. & Khan, S. (2021). Salmonella Hessarek: an emerging food borne pathogen and its role in egg safety. *Food Control*, **125**, 107996.

Millour, S., Noël, L., Kadar, A., Chekri, R., Vastel, C. & Guérin, T. (2011). Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: method validation. *Journal of Food Composition and Analysis*, **24**, 111–120.

Mitrovic, B., Vranjes, B., Kostic, O., Perovic, V., Mitrovic, M. & Pavlovic, P. (2019). Presence of radionuclides and toxic elements in feedstuffs and food of animal origin. *Veterinarski Glasnik*, **73**, 30–39.

Nardi, E.P., Evangelista, F.S., Tormen, L. *et al.* (2009). The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. *Food Chemistry*, **112**, 727–732.

Okamoto, Y. & Motomura, N. (2017). Anxiety and depression in cardiovascular surgery. In: *Depression* (edited by D. Breznoščáková). Pp. 39–54. London, UK: InTechOpen. https://doi.org/10.5772/67064

Olejnik, M., Szprengier-Juszkiewicz, T. & Jedziniak, P. (2010). Confirmatory method for determination of coccidiostats in eggs. 8. *Bulletin of the Veterinary Institute in Pulawy*, **54**, 327–333.

Pejovic, D. (2014). *The national illustrated cookbook/Ilustrovani narodni kuvar*. Serbia: Akia Mali Princ.

Rodríguez-Gonzalo, E., Mateos-Vivas, M., Domínguez-Álvarez, J., García-Gómez, D. & Carabias-Martínez, R. (2017). Anthelmintic benzimidazoles in eggs. In: *Egg Innovations and Strategies for Improvements* (edited by P.Y. Hester). Pp. 465–474. San Diego, California, USA: Nikki Levy. https://doi.org/10.1016/B978-0-12-800879-9.00044-5

Roila, R., Branciari, R., Pecorelli, I. *et al.* (2019). Occurrence and residue concentration of coccidiostats in feed and food of animal origin; human exposure assessment. *Foods*, **8**, 477.

Annotated references: This study provided a review of the literature on coccidiostats in eggs and helped calculate their contribution to the recommended acceptable daily intake and discussion of results.

Rubio, C., Ojeda, I., Gutierrez, A.J., Paz, S., González-Weller, D. & Hardisson, A. (2018). Exposure assessment of trace elements in fresh eggs from free-range and home-grown hens analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). *Journal of Food Composition and Analysis*, **69**, 45–52.

Serbian Regulation. (2011). Ordinance on the quantities of pesticides, metals and metalloids and other toxic substances, chemotherapeutics, anabolic and other substances that may be found in foods. In (Vol. No 28/2011). Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Serbian Regulation. (2018). Regulation on the quality of grain, mill and bakery products and pasta. In (Vol. No 56/2018). Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Serbian Regulation. (2019a). Regulation on egg quality. Official Gazette of the Republic of Serbia. In (Vol. No 7/2019). Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Serbian Regulation. (2019b). Regulation on the maximum permitted quantities of residues plant protection products in food and feed and on food and feed for which maximum permitted quantities of residues of plant protection products. In (Vol. No 76/2019). Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Shaheen, N., Ahmed, MdK, Islam, MdS *et al.* (2016). Health risk assessment of trace elements via dietary intake of 'non-piscine protein source' foodstuffs (meat, milk and egg) in Bangladesh. *Environmental Science and Pollution Research*, **23**, 7794–7806.

Sun, J. & Wu, Y. (2016). Evaluation of dietary exposure to deoxynivalenol (DON) and its derivatives from cereals in China. *Food Control*, **69**, 90–99.

Sundlof, S. (2014). Veterinary drugs residues: veterinary drugs – general. In: *Encyclopedia of Food Safety* (edited by Y. Motarjemi). Pp. 35–38. San Diego, California, USA: Elsevier Science & Technology. https://doi.org/10.1016/B978-0-12-378612-8.00248-1

Udovicki, B., Djekic, I., Kalogianni, E. & Rajkovic, A. (2019). Exposure assessment and risk characterization of aflatoxin M1 intake through consumption of milk and yoghurt by student population in Serbia and Greece. *Toxins*, **11**, 205.

Annotated references: This paper provides a methodological framework for exposure and provides and consumer survey. For our research, it gave the idea to include egg-based products in the consumption research.

Upadhyaya, I., Yin, H.-B., Nair, M.S. & Venkitanarayanan, K. (2017). Natural approaches for improving postharvest safety of egg and egg products. In: *Producing Safe Eggs* (edited by S.C. Ricke & R.K. Gast). Pp. 391–420. Orlando, USA: Academic Press. https://doi.org/10.1016/B978-0-12-802582-6.00019-7

USEPA. (1989). Risk assessment guidance for superfund. Human Health Evaluation Manual Part A. Interim Final, 1, 289. United States Environmental Protection Agency Washington, DC. EPA/540/1-89/002.

USEPA. (2003). Framework for Cumulative Risk Assessment. Risk Assessment Forum U.S. Environmental Protection Agency Washington, DC. EPA/630/P-02/001F.

USEPA. (2005). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum U.S. Environmental Protection Agency Washington, DC. EPA/630/P-03/001F.

Vehab, A., Huremović, J., Sabina, Ž. & Šabanović, E. (2019). Essential and toxic metals content in hen eggs at different geographic area in Bosnia and Herzegovina. *Annals. Food Science and Technology*, **20**, 97–104.

Wang, B., Liu, J., Zhao, X. *et al.* (2020). Determination of eight coccidiostats in eggs by liquid-liquid extraction–solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Molecules*, **25**, 987.