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Review

CHEMICAL CONTAMINANTS IN FISH, SHELLFISH AND FISH PRODUCTS ON THE SERBIAN MARKET

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Abstract

Fish meat and fish products are usually considered as healthy foods, mostly because of their content of unsaturated fatty acids, which have a beneficial effect on human health. On the Serbian market, fish and shellfish are commonly present as fresh, frozen and canned, but not regulating and monitoring the existence of high levels of different chemical hazards can lead to human health problems. The aim of the present paper is to provide an overview of the contamination of fish and their products from the Serbian market with regard to the most significant chemical hazards. The following contaminants are considered: lead, mercury, cadmium, arsenic, copper, iron and zinc representing heavy metals and metalloids; endrin, aldrin, dieldrin, lindane, endosulfans, dichlorodiphenyltrichloroethane (DDT) and its derivatives as the most significant organochlorine pesticides and; benzo(a)pyrene, benz(a)anthracene, benzo(b) fluoranthene and chrysene as four priority polycyclic aromatic hydrocarbons (PAH4). Bearing in mind that there is no food, including fish and fish products, that does not contain harmful substances and various hazards, there is a need at national and lower

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levels to constantly monitor these hazards, establish maximum allowable level (MAL) and carry out risk assessments for each of them.

Key Words: aquaculture products, heavy metals, legislation, PAHs, pesticides

INTRODUCTION

With the increase of the human population on the planet, the production of fish and seafood from aquaculture is also increasing significantly, both annually and in the long term. The largest aquaculture production, with approximately 90% of the total world production, takes place in Asian countries, which are expected to double fish production by 2050 (FAO, 2018; 2020). As a continental country, Serbia produces freshwater fish species, with 77 registered common carp farms and 68 trout farms (Relić & Marković, 2021). The total production of freshwater fish in Serbia is about 15,000 tons. Common carp (11,000 tons), is followed by trout, bighead carp, grass carp, perch, European catfish and sturgeon (Ćirković et al., 2015). In addition to freshwater fish, there are also marine fish on the Serbian market, most often hake, tuna, sardines, sea bream and sea bass. Since Serbia does not produce shellfish, imports are from coastal countries. Mediterranean mussels (*Mytilus galloprovincialis*), due to their price, availability and consumer habits, are the most common and most widely consumed shellfish. With an average fish consumption per capita of 5-7 kg per year, Serbia belongs to the countries with very low fish consumption, and this needs to increase, especially among the health vulnerable categories of the population.

The nutritional quality of fish meat must be taken into account, since this food is a good source of protein, unsaturated fatty acids, minerals and vitamins of animal origin (Ljubojević et al., 2013). However, fish, like other foods, can sometimes endanger the health of consumers. Hazards in foods are defined as biological (bacteria, parasites, bio-toxins and viruses), chemical (heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)) or physical (metal fragments, glass, wood and plastic).

Lead, mercury, cadmium and arsenic, as the most toxic heavy metals, and the less toxic elements (zinc, copper, nickel and tin) have a particularly important place among pollutants (Olmedo et al., 2013; Zaza et al., 2015). Due to their high toxicity and ability to accumulate in various organisms, organochlorine pesticides can pose a significant risk to the health of people as the end consumers in the food chain (Kartalović et al., 2016). In addition to heavy metals, PAHs are very significant pollutants that drastically impair the quality of the environment. When placing fish and fish products, shellfish and other seafood on the market, care must be taken that no product contains hazards exceeding the maximum allowable level. In particular, chemical contaminants included for monitoring in the national legislation framework through two specific regulations (Serbia, 2019; 2020), have a special significance. The goal of this review is to highlight the most toxic chemical contaminants (heavy metals, organochlorine pesticides, PAH,

PCBs and dioxins) in fish, shellfish and fish products, as well as to present the Serbian and European Union legislation related to this important issue.

HEAVY METALS

Heavy metals are widely distributed hazards in the environment, so they easily enter food chains. Lead, mercury, cadmium and arsenic are among the most toxic metals that are not biogenic elements. They are toxic even in low concentrations to most living beings, leading to various health problems in humans that include carcinogenic, renal and endocrine effects (ATSDR, 2003, 2007, 2012, 2019). Some biogenic elements, like copper, iron and zinc, are necessary for living organisms and can also be toxic, but only in higher concentrations (Yi et al., 2017). Fish and other aquatic organisms are contaminated with heavy metals mostly through poorly or inadequately treated industrial waste waters that end up in aquatic systems, but sewage is also a significant source of contamination (Andayesh et al., 2015). Contamination of water is of particular importance for shellfish, which, due to their filtration ability, can accumulate 100 to 1000 times higher levels of heavy metals than are present in the water in which they are reared (Lehel et al., 2018). In canned fish, some metals can occur as a consequence of the canning process itself, when they pass into the fish meat from the can (Mol, 2011). Heavy metals are the most significant contaminants associated with fish as food, especially shellfish and long-lived species such as tuna (Guérin et al., 2011).

Lead is a widespread and highly toxic metal that exhibits harmful effects in various cells and tissues. One of the confirmed mechanisms of lead action is enzyme inhibition (*Debnath* et al., 2019). Chronic intoxication leads to various clinical manifestations, of which the most common are connected to kidney damage, allergies, mental disorders, and haematologic and cardiovascular toxicity (Wani et al., 2015). In the national legislation (Serbia, 2019), the MAL for lead in fish meat and cephalopods is 0.3 mg kg^{-1}, while in the meat of crustaceans it is 0.5 mg kg^{-1}. The maximum allowable level in the meat of bivalve molluscs is 1.5 mg kg^{-1}. Legislation in the European Union (EC, 2015) prescribes the same values.

Cadmium is also a very toxic metal that accumulates in various tissues and organs for a long time (Jaishankar et al., 2014). It causes significant health problems and damage in the liver, lungs, bones, testicles and other tissues (Genchi et al., 2020). Humans ingest cadmium most often through various foods, including fish and fish products. According to the national (Serbia, 2019) and European Union regulation (EC, 2014a), the MAL for cadmium in fish meat of most species is 0.05 mg kg^{-1}. However, some species have different maximum allowable levels of cadmium, which are separately specified in the regulations. Therefore, the MAL for cadmium in sardine (*Sardina pilchardus*) meat is 0.25 mg kg^{-1}, and in crustaceans it is 0.50 mg kg^{-1}, while the highest value of 1.0 mg $kg^{-1}$is allowed in bivalve mollusc and cephalopod meat.

Mercury and the poisonous form of mercury, methylmercury (MeHg), are very toxic, often associated with being introduced into the human body through the consumption of fish and shellfish. In particular, consumption of large quantities of fish and shellfish is not recommended for pregnant women, primarily due to the ability of this hazard to pass through the placenta to the foetus, where it can consequently cause different forms of damage (Alina et al., 2016). The maximum allowable level for mercury in fish meat and fishery products of species that are not specified separately in the regulation has been set at 0.5 mg kg^{-1}, according to European (EC, 2006) and Serbian (Serbia, 2019) legislation. However, the MAL for tuna, sea bream, swordfish and other indicated species is 1.0 mg kg^{-1}.

Arsenic is a metalloid, occurs in high levels in groundwater and is also widely distributed in the Earth's crust. This metalloid can be found in both its organic and inorganic forms in food. On chemical analysis, it is often shown as total arsenic, from which it is difficult to determine how much is organic and how much is inorganic. Organic arsenic is less toxic, while inorganic arsenic is more toxic. In fish meat and fish products, arsenic is present predominately in its organic form (Francesconi, 2010; Julshamn et al., 2012), which is why it is considered as a less significant contaminant than lead, mercury and cadmium. This is one of the main reasons that arsenic is no longer mandatorily monitored in fish and fish products, shellfish and other seafood in the European Union or in Serbia. In Serbia, this element was mandatory for monitoring until 2019, within the regulation (Serbia, 2014) that allowed maximum allowable level for total arsenic of 2 mg kg^{-1} in fish. The MAL for marine fish products was 3 mg kg^{-1}, while the same limit for products of tuna species was set at 12 mg kg^{-1}.

Iron, copper and zinc are biogenic metals and are necessary for numerous body functions because they participate in various biological processes. They are toxic to humans and animals only if they are found in high concentrations. As such, they are difficult to find in fish and fish products, so they no longer require monitoring according to European Union and Serbian legislation. The previous national regulation (Serbia, 2014) specified obligatory control only of canned fish products, in which the determined MAL for iron and copper was 30 mg kg^{-1}, while zinc's MAL was 100 mg kg^{-1}.

Several studies investigated the content of heavy metals in different fish species from Serbian natural waters and aquaculture, as well as from fish and shellfish present on the Serbian market but imported from other countries. Jovanović (2016) analysed the content of heavy metals and metalloids in various tissues from fish caught in the Danube River and other natural waters in Serbia. The same group (Jovanović et al., 2017) investigated the presence of heavy metals in six fish species originating from the Danube River: barbell, pike perch, common and Prussian carp, bream and catfish. They analysed mercury, lead and arsenic levels. All obtained values reported (Jovanović et al., 2016; 2017) were below the MALs set by national and European regulations. Milanov et al. (2016) studied levels of cadmium, lead, mercury and arsenic in bighead carp, European catfish and common carp. They also did not find elevated values of the

investigated metals, concluding that these fish species are safe for human consumption. Lenhardt et al. (2012) investigated concentrations of cadmium, lead, arsenic, copper, zinc and iron in fish from the Danube River: *Abramis brama, Hypophthalmichthys molitrix*, *Blicca bjoerkna*, *Silurus glanis* and *Cyprinus carpio*. They found concentrations at acceptable levels, not exceeding the MAL for each analysed element. On the other hand, Subotić et al. (2013) found the concentrations of mercury and zinc in predators (catfish, pikeperch) from the Belgrade section of the Danube were higher than the MALs. They connected their results to high pollution levels in the investigated area and pointed out the importance of monitoring. Novakov et al. (2017) conducted a study of heavy metal concentrations in canned fish collected from supermarkets in Serbia. They investigated levels of heavy metals and biogenic elements (zinc, iron and copper) in canned tuna, smoked sprats and sardines. They found some of the samples contained cadmium, arsenic and copper above the MAL set by the national legislation, reaching maximum levels of 0.12 mg kg^{-1}, 3.73 mg kg^{-1} and 52.2 mg kg^{-1}, respectively. Novakov et al. (2021) studied heavy metal concentration (mercury, cadmium, lead and arsenic) in imported mussels collected in Serbia. The ranges of these elements (mg kg^{-1}) were 0.01-0.74 for lead, 0.01-0.38 for cadmium, 0.01-0.15 for mercury and 1.12-5.87 for total arsenic. Concentrations of all analysed metals were under the maximal allowable limits.

ORGANOCHLORINE PESTICIDES

Organochlorine pesticides (OCPs), due to their long-term use in agriculture, can still be found in food, including fish. Long-term retention in the environment and in different food chains is made possible thanks to OCPs' properties of slow degradation and deposition in fatty tissues of living organisms (Williams et al., 2008). Due to the long-term consumption of products containing OCPs or the consumption of food with high levels, health problems in humans, connected mostly to the carcinogenic potential of OCPs, can occur (Gilden et al., 2010). The most abundant OCPs in fish and fish products are endrin, aldrin, lindane, dieldrin, endosulfans, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE). The European Union has not controlled the content of OCPs in fish and seafood for many years. On the other hand, Serbia controlled the content of some OCPs in fish for a long time, allowing MAL of 50 μg kg^{-1} for lindane, 20 μg kg^{-1} for the sum of aldrin and dieldrin, 10 μg kg^{-1} for endrin, 100 μg kg^{-1} each for DDT, DDE and DDD, and 50 μg kg^{-1} for endosulfans. Other OCPs were not subject to obligate monitoring in fish (Serbia, 2014). In the law harmonization process with the European Union, Serbia cancelled the obligation to control OCPs (Serbia, 2019) in fish and derived products.

There are scarce data on the presence of OCPs in fish from Serbia. Kartalović et al. (2016) conducted a study to determine concentrations of nineteen OCPs in canned tuna and sardines from the Serbian market. They found 93.2% of all analysed samples

contained OCP residues, while some OCPs were present at levels above the MAL. The highest dieldrin level was 39.7 μg kg^{-1} in one sample of canned tuna, and the average endosulfans level in canned sardines was 85.0 μg kg^{-1}, which in both cases, were above the respective MAL.

POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are compounds containing only carbon and hydrogen, which are arranged in the form of aromatic rings. They are derived from incomplete combustion of organic matter, both in natural conditions during a fire or in industrial conditions in stoves and engines. PAHs are widely distributed in nature and can be found in soil, water and air. Since most PAHs are not soluble in water, in aquatic systems they can be deposited in sediment. Although some PAHs enter foodstuffs through the environment, most of them end up in foods that are thermally processed, including smoking, roasting, grilling and frying (Ledesma et al., 2015). The highest content of PAH in fish is found in smoked products and smoked fish. That is why the technology itself, which can be industrial or traditional, the temperature of wood burning and the duration of exposure of the product to the smoke are very important (Babić et al., 2018; Mastanjević et al., 2019; 2020). Although more than a hundred different PAHs have been isolated so far, and the US Environmental Protection Agency (US EPA, 2008) has singled out 16 priority ones that have the most harmful effects on human health, primarily due to their carcinogenic potentials. The 16 priority PAHs are: acenaphthene (Ane), acenaphthylene (Anl), anthracene (Ant), benz(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(g,h,i)perylene (BghiP), chrysene (Chry), dibenz(a,h)anthracene (DahA), indeno(1,2,3-cd)pyrene (InP), fluorene (Flu), fluoranthene (Flt), phenanthrene (Phen) and naphthalene (Nap). Benzo(a)pyrene is the most toxic and most often investigated PAH. Its metabolites are mutagenic and highly carcinogenic. BaP is classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogen. It is especially dangerous due to the disruption of cell membranes, enzyme system dysfunction and interactions with DNA (Bogdanović et al., 2019). Due to its properties, toxicity and fact that it is the most common carcinogenic compound in smoke, the quantity of BaP in foods is limited by European (EC, 2011; EC, 2014b; EC, 2020) and Serbian (Serbia, 2019) legislation.

Serbian national regulation (Serbia, 2019) set MAL for BaP at 5.0 μg kg^{-1}, and for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene (together termed PAH4) at 30.0 μg kg^{-1} for some smoked fish and canned smoked fish, including fresh and frozen shellfish. The regulation (Serbia, 2019) specifies the fish by species and includes the length for some of the species. The MAL for BaP in smoked shellfish is 6.0 μg kg^{-1}, and for the sum of PAH4 it is 35.0 μg kg^{-1}. MAL of BaP and PAH4 for meat of other smoked fish, including smoked crustaceans, crabs and crab-like crustaceans, are 2.0 and 12.0 μg kg^{-1}, respectively. MAL of BaP and PAH4 are the

same for the European Union (EC, 2011) and Serbia. However, the European Union in 2020 revised (EC, 2020) the maximum levels of PAHs in traditionally smoked fish and its products. Now, some Member States (Latvia, Finland and Sweden) can authorize placing on the market smoked fish and smoked fish products, manufactured in the traditional way and placed only in their territories, which have MALs higher than 2.0 and 12.0 μg kg^{-1} BaP and PAH4, respectively, but the levels must be under 5.0 μg kg^{-1} (BaP) and 30.0 μg kg^{-1} (PAH4).

There are few investigations regarding PAH levels in fish marketed in Serbia. Novakov et al. (2017) found total PAH levels of 17.67 μg kg^{-1} in canned tuna and 15.12 μg kg^{-1} in canned sardines; these products were not smoked, but only canned. Smoked sprats contained total PAHs at a level of 57.19 μg kg^{-1}. Some individual samples of smoked sprats had high concentrations, which was above the MAL. Babić et al. (2018) investigated levels of PAHs in carp meat that was traditionally smoked, but where different filters were used absorb these toxic substances from the smoke. They reported the analysed fish meat was safe for consumption, both in the group where filters were used and in the control group. Also, the zeolite filters proved to be the most effective for traditionally smoked carp (Babić et al., 2018). Novakov et al. (2021) found PAH levels in all analysed samples of fresh and frozen mussels from the Serbian market were under the MAL set by European and Serbian legislation, and so they considered the shellfish to be safe for human consumption.

POLYCHLORINATED BIPHENYL AND DIOXINS

Polychlorinated biphenyls (PCBs) are chemical compounds that cannot be found naturally in the environment, but are the result of artificial synthesis. They are very stable, do not dissolve in water, but are soluble in oil and fats. PCBs are insensitive to light and are very resistant to heat, disintegrating only at temperatures above 1000°C. PCBs' properties make them widely used in numerous industries. Due to their very high toxicity and carcinogenic potential, the US EPA issued in 1997 the Toxic Substances Control Act, which restricts their use and control their import and elimination (Yang et al., 2006; Lauby-Secretan et al., 2013). Depending on the positions of the chlorine atoms, PCBs are classified into two groups: dioxin-like PCBs and PCBs that are not similar to dioxins. Foods that contain the highest amounts of PCBs are most often milk, meat with a higher fat content, fatty fish and shellfish (Li et al., 2008; Kartalović et al., 2020).

Dioxins (i.e. polychlorinated dibenzo-p-dioxins (PCDDs)) are highly toxic and widely distributed compounds. Most often, they are created as by-products of various industrial processes in the production of some chemicals and waste burning. Dioxins are found as mixtures of numerous congeners and dioxin-like PCBs (Sweetman et al., 2000). Serbian national regulations (Serbia, 2019; 2020), which are harmonized with EU law, and which sometimes specify World Health Organisation (WHO) toxic equivalents (TEQ), prescribe MALs for WHO-PCDD/F-TEQ, the sum of dioxins

and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ) and the sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB180 (ICES-6) of 3.5 pg g^{-1}, 6.5 pg g^{-1} and 75 ng g^{-1} of wet mass, respectively. These levels are prescribed for fish meat and fishery products except: caught eels, hunted *Squalus acanthias*, caught freshwater fish, excluding diadromous fish species from freshwater farming, fish liver, fish liver products and oils of marine aquatic organisms. The MAL for shellfish refers to meat from the abdomen and appendages. In the case of crabs and crayfish and similar shellfish, the MAL refers to meat in the appendages. Fish liver and fish liver products both have maximum allowable limits of 20 pg g^{-1} and 200 ng g^{-1} for the sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ) and the sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB180 (ICES-6), respectively.

CONCLUSION

Although fish is a healthy foodstuff due to its favourable composition of micro and macro elements, it can contain harmful substances, like any other food. For this reason, fish and fish products must be regularly monitored, and chemical hazards determined in accordance with the current legislation. Internationally recognized standard methods are recommended for determination of chemical contaminants level in food. Although in most studies conducted in Serbia, the concentrations of chemical contaminants in fish and fish products were not above the MALs, systems to control and supervise chemical hazards in these products require constant development.

Risk assessments must be continually updated and new, threatening hazards should be taken into consideration.

Authors' contributions

NN is responsible for data synthesis, writing and correspondence. BK wrote the section regarding pesticides. JM wrote the part related to heavy metals. JV wrote the section related to PAHs. BĐ and DL analysed and wrote about the national and international legislation. MP and DLJP wrote the section related to PCBs and dioxins.

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the manuscript.

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HEMIJSKI KONTAMINANTI U RIBI, ŠKOLJKAMA I PROIZVODIMA OD RIBE NA TRŽIŠTU SRBIJE

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Kratak sadržaj

Meso riba i proizvoda od riba spada u zdrave namirnice, prvenstveno zbog sadržaja nezasićenih masnih kiselina, koje povoljno deluju na zdravlje ljudi. Riba i školjke se na tržištu Srbije mogu naći najčešće kao sveže, zamrznute i konzervirane, pri čemu se vodi računa o prisustvu i količini hemijskih zagađivača, koji mogu izazvati značajne zdravstvene probleme kod potrošača ukoliko su iznad dozvoljenih vrednosti. Osnovni cilj ovog rada je da prikaže pregled kontaminacije ribe i njenih proizvoda sa tržišta Srbije sa najznačajnijim hemijskim kontaminantima. Treba uzeti u obzir sledeće zagađivače: olovo, živu, kadmijum, arsen, bakar, gvožđe i cink kao predstavnike teških metala i metaloida; endrin, aldrin, dildrin, lindan, endosulfane, dihlorodifeniltrikloretan (DDT) i njegove derivate kao najznačajnije organohlorne pesticide i benzo(a)pirene, benz(a)anthracene, benzo(b)fluoranthene i hrizene kao četiri prioritetna policiklična aromatična ugljovodonika (PAH4). Imajući u vidu da nema hrane, uključujući ribu i proizvode od ribe, koja ne sadrži štetne materije i različite hazarde, na državnom nivou i nižim nivoima je neophodno neprestano sprovoditi monitoring, utvrđivati maksimalne dozvoljene koncentracije i raditi ocene rizika za svaki od njih.

Ključne reči: proizvodi akvakulture, teški metali, legislativa, PAH, pesticidi