

Journal of the Hellenic Veterinary Medical Society

Vol 71, No 1 (2020)



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doi: [10.12681/jhvms.22941](https://doi.org/10.12681/jhvms.22941)

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To cite this article:

PELIĆ, M., KARTALOVIĆ, B., ŽIVKOV BALOŠ, M., MIRILOVIĆ, M., ĐORĐEVIĆ, M., TEODOROVIĆ, V., ĆIRKOVIĆ, M., & LJUBOJEVIĆ PELIĆ, D. (2020). Health Risks associated with residual pesticide levels in fish reared in purified wastewater from slaughterhouse. *Journal of the Hellenic Veterinary Medical Society*, 71(1), 1991–1996. <https://doi.org/10.12681/jhvms.22941>

Health Risks associated with residual pesticide levels in fish reared in purified wastewater from slaughterhouse

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ABSTRACT. The main objective of the present research was to determine the concentrations of the selected pesticides in muscle, liver and skin of common carp. Fish were sampled in two different seasons from fish pond which received previously treated slaughterhouse wastewater. Pesticides including etridiazole, chloroneb, trifluralin, propachlor, chlorothalonil, hexa-chlorocyclopentadiene, atrazine, simazine, alachlor, metribuzin, metolachlor, DCPA, cyanazine, chlorobenzilate, endrin aldehyde, cis permethrin and trans permethrin were determined by using a GS-MS method. Many of pesticides were not determined or determined in low concentrations. Propachlor was found in muscle, skin and liver. The recommended acceptable daily intake was higher in comparison with the estimated daily intake for examined pesticides via fish reared in treated slaughterhouse wastewater. It is very important to maintain the safety of the fresh fish produced in wastewater in order to ensure food safety and avoid health problems in humans.

Keywords: common carp; food safety; environmental protection; integrated aquaculture system, propachlor, risk assessment

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Date of initial submission: 25-11-2019

Date of revised submission: 13-12-2019

Date of acceptance: 25-12-2019

INTRODUCTION

Fish meat is highly acceptable protein source worldwide. Moreover, fish represents an excellent source of fatty acids in healthy nutrition (Ljubojević et al., 2015). From another point of view, consumers are more and more oriented to the safety issues due to the potential presence of different environmental contaminants in fish meat. Consumption of fish contaminated with pesticides may lead to poisoning of consumers (Aamir et al., 2016). Pesticides are widely used in agriculture (Hladik et al., 2016). It is highly important to understand the nature, bioaccumulation and distribution of pesticides in aquatic environment and also the routes of contamination in order to control and prevent outbreaks of poisoning associated with fish consumption. Pesticides are present in aquatic environment and can be transferred into fish and enter into the food chain (Guo et al., 2008). Due to the fact that pesticides are highly soluble in fats, they accumulate in fish tissue (El-Shahawi et al., 2010). According to Cao et al. (2007) pesticides can remain in soil and water for years. There is increasing trend in the use of wastewater in fish production, particularly in developing countries. The fish rearing in waste water may represent a significant risk for public health (Kim and Aga, 2007). Fish may represent a bioindicator of environmental contamination and the health risk arising from fish produced in wastewater filled fish ponds is questionable. Thus, the safety of fish reared in wastewater filled ponds could be a serious concern for public health. There are several studies regarding the level of pesticides in common carp, mainly organochlorine pesticides in different parts of the world (Svobodová et al., 2003; Hu et al., 2010; Ondarza et al., 2010; Pelić et al., 2019), but no available data exists about the level of other pesticides in fish produced in treated slaughterhouse wastewater in scientific literature. It should be highlighted that such systems may be long-term solution for aquaculture but also for slaughterhouses worldwide. The main objective of the present research was to determine the concentrations of the selected pesticides in muscle, liver and skin of common carp reared in fish pond which received previously treated slaughterhouse wastewater. For that purpose, common carp meat, liver and skin were examined using GS-MS, for 17 pesticides (etridiazole, chloroneb, trifluralin, propachlor, chlorothalonil, hexa-chlorocyclopentadiene, atrazine, simazine, alachlor, metribuzin, metolachlor, DCPA, cyanazine, chlorobenzilate, endrin aldehyde, cis permethrin and trans permethrin) in order

to evaluate the slaughterhouse wastewater treatment systems for their efficiency as well as the safety of produced fish meat.

MATERIAL AND METHODS

An earthen pond with an area of 1 ha and average depth of 1.3 m was built in the Pećinci village (N 44°54'19", E 19°57'35"), Srem District, the Republic of Serbia. Purified water from slaughterhouse was used for filling the pond. Continuous aeration was provided. Two year old fingerlings of common carp were stocked at density 2500 individuals/ha. Industrially produced completed feed mixture and locally available ingredients were used as fish feed and feed was provided manually twice per day. Seven individuals of common carp were harvested from the pond in spring (April) and in autumn (October). All samples were dissected, dorsal muscles without skin, liver and skin were homogenised and prepared for analyses. All chemicals and reagents used in this experiment were of analytical grade with high purity. Preparation of sample which was based on extraction with acetonitrile (CAN), (Sigma-Aldrich, St. Louis, USA) in the presence of anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium acetate (CH_3COONa) Merck, Darmstadt, Germany) was performed as described by Kartalović et al. (2016). The gas-mass chromatography was Agilent 7890B/5977A MSD and operating conditions were as follow: fused silica column [30 m x 0.25 μ m film of HP-5M - thickness]; injection temperature was set at 280°C using the splitless mode and the volume injected was 4 μ L. The column temperature was programmed as follows; held at 50°C for 0.4 min; 50-195 °C at 25 °C per min, held 1.5 min; 195-265 at 8 °C per min and maintained at 315 °C for 1.25 minutes on 20 °C per min, MSD temperature was 280 °C. Calculation of precision, accuracy, linearity, LOD and LOQ (Table 1) was performed by MassHunter Software (Agilent Technologies, Santa Clara, CA, USA). The quantification was based on external calibrations curves prepared from the standard solution of each of the pesticides.

Data analysis was performed using Excel (Microsoft Excel, 2007) to determine the descriptive statistic parameters.

Table 1. Method performance data obtained in blank common carp samples, spiked with 50 µg/kg (n=20)

Pesticides	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Linearity (r ²)	Recovery (%)	RSD (%)
Etridiazole	1.5	4.9	4.8	1.00	97.4	6.2
Chloroneb	0.9	2.1	7.8	1.00	98.1	7.9
Trifluralin	0.4	1.3	0.8	1.00	99.3	9.2
Propachlor	0.3	1	9	1.00	99.6	8.3
Chlorothalonil	0.4	1.2	5.4	1.00	98.1	12.6
Hexa-chlorocyclopentadiene	1.2	4.1	3.4	1.00	97.3	3.8
Atrazine	0.4	1.2	4.4	1.00	90.4	8.1
Simazine	0.5	1.6	3.2	1.00	94.2	4.2
Alachlor	1.1	3.6	4.5	1.00	91.8	8.6
Metribuzin	0.8	2.6	8.3	1.00	89.3	8.9
Metolachlor	1.2	4.4	3.1	1.00	96.6	3.7
DCPA	1.3	4.8	3.8	1.00	95.4	3.9
Cyanazine	0.8	2.9	8.1	1.00	93.4	11.2
Chlorobenzilate	1.1	3.7	4.7	1.00	91.6	10.3
Endrin aldehyde	1.4	4.8	3.5	1.00	95.2	8.3
Cis permethrin	1.2	4.2	3.8	1.00	96.4	3.4
Trans permethrin	1.4	4.7	11.7	1.00	112.3	12.3

LOD, Limits of detection; LOQ, Limits of quantification; RSD, relative standard deviation

Table 2. Concentrations (µg/kg) of pesticides in meat, skin and liver of common carp (n = 7) in spring

Pesticide	Muscle tissue			Skin			Liver		
	Frequency (%)	Range	X±SD	Frequency (%)	Range	X±SD	Frequency (%)	Range	X±SD
Propachlor	100	11.4-11.5	11.4±0.05	100	20.40- 21.32	20.83±0.38	100	11.3-11.5	11.4±0.1
Atrazine	14.29	<lod-26.88	/	0	<lod	/	0	<lod	/
Endrin aldehyde	71.43	<lod-38.25	37.30±0.53	0	<lod	/	0	<lod	/

* All other analysed pesticides were below LOD.

Table 3. Concentrations (µg/kg) of pesticides in meat, skin and liver of common carp (n = 7) in autumn

OC Pesticide	Muscle tissue			Skin			Liver		
	Frequency (%)	Range	X±SD	Frequency (%)	Range	X±SD	Frequency (%)	Range	X±SD
Chloroneb	0	<lod	/	28.57	<lod-22.31	13.40±12.61	0	<lod	/
Propachlor	100	20.46-20.59	20.53±0.06	100	20.46-34.15	24.49±6.59	100	20.47-20.74	20.58±0.12
Atrazine	0	<lod	/	28.57	<lod-115.83	102.51±18.84	0	<lod	/
Simazine	14.29	<lod-45.28	/	28.57	<lod-91.99	85.27±9.51	0	<lod	/
Alachlor	0	<lod	/	42.86	<lod -90.78	78.53±21.13	0	<lod	/
Metribuzin	0	<lod	/	28.57	<lod-126.74	126.67±0.1	0	<lod	/
Metolachlor	0	<lod	/	28.57	<lod-44.09	44.11±0.2	0	<lod	/
DCPA	0	<lod	/	14.29	<lod-28.47	/	0	<lod	/
Cyanazine	0	<lod	/	28.57	<lod-404.85	385.8±26.92	0	<lod	/
Chlorobenzilate	0	<lod	/	14.29	<lod-138.31	/	0	<lod	/
Endrin aldehyde	0	<lod	/	14.29	<lod-111.13	/	0	<lod	/

* All other analysed pesticides were below LOD.

RESULTS

Table 1 shows method performance data for 17 selected pesticides. The results of the study are shown in Table 2 (spring) and Table 3 (autumn). Generally, the concentrations of pesticides were low in all analysed samples in both seasons. A number of pesticides were not detected neither in muscle tissue, and neither in liver and skin samples. It includes the following: etridiazole, trifluralin, chlorothalonil, hexachlorocyclopentadiene, cis permethrin and trans permethrin. In autumn concentrations of propachlor were two fold higher compared to concentrations obtained in spring and were $20.53 \pm 0.06 \mu\text{g/kg}$ in muscle tissue, in skin concentrations were in range 20.46 - 34.15 $\mu\text{g/kg}$ and in liver was $20.58 \pm 0.12 \mu\text{g/kg}$. Atrazine was above the limit of detection in two samples of skin and simazine in one sample of muscle tissue and two samples of skin. Alachlor was found in three samples of skin and metribuzin in two. Metachlor, DCPA and cyanazine were found only in skin samples. Chlorobenzilate was found only in one sample of skin, while endrin aldehyde was present only in one sample of skin in amount of 111.13 $\mu\text{g/kg}$.

DISCUSSION

Concentrations of pesticides were generally comparable or lower in comparison with previous results reported in studies of common carp and *Cyprinidae* species worldwide (Svobodová et al., 2003; Darko et al., 2008). Etridiazole, chlorothalonil, atrazine, simazine, metribuzin, metolachlor, DCPA and chlorobenzilate are registered for use in Serbia. All of those pesticides except etridiazole and chlorothalonil were detected in low levels in skin of common carp. Pesticides such as atrazine, simazine, trifluralin, alachlor are listed as priority substances of the Directive 2008/105/EC (EEC, 2008) and the Directive 2013/39/EC (EEC, 2013) and three of those pesticides were detected in the present study. A number of pesticides not registered (chloroneb) or banned in Serbia were also found (mainly propachlor, alachlor and endrin aldehyde) and their presence could be attributed to illegal use. The pesticides reported in this study are not often examined so the data regarding their content in fish including common carp are scarce. Papadakis et al. (2015) reported the detection of etridiazole, metolachlor, atrazine, and alachlor (maximum concentrations of 0.026, 0.688, 1.465 and 1.098 $\mu\text{g/L}$, respectively, in the surface waters of Lake Vistonis Basin, Greece. Kong et al. (2008) highlighted that pesticides were used widely in China, and that pesticides like at-

razine, simazine, and alachlor have a harmful effects on endocrine system. All the mentioned pesticides were detected but only in skin and in relatively low concentrations in common carp in our study. Inadequate regulation and poor agriculture management are the main reasons for the fact that banned pesticides are still in illegal usage. Among pesticides determined in the common carp samples of muscle, liver and skin residual concentration of propachlor was dominant in all samples in both season. The determined concentrations were the lowest in liver, then in muscle, while the highest levels were measured in skin. Ondarza et al. (2010) also reported that the pesticide levels were higher in muscle than in liver. Since the skin is a kind of protective barrier, it may be the reason for the highest concentrations of pesticides detected in it. Thomas et al. (2012) examined levels of pesticides in muscles of farmed common carp in France and also found that the levels of pesticides were low. Also, Padula et al. (2008) didn't detect any pesticide residues in wild and farmed southern bluefin tuna (*Thunnus maccoyii*). Kong et al. (2008) evaluated the municipal sewage treatment system in China for their pollutant removal efficiency. They found that concentrations of alachlor, acetochlor, atrazine were 0.074 - 0.021 $\mu\text{g/l}$, 0.160 - 0.096 $\mu\text{g/l}$ and 0.238 - 0.184 $\mu\text{g/l}$, respectively, and the total removal efficiency of atrazine was poorest through the sewage treatment systems. Our results showed that the wastewater treatment systems in slaughterhouse were efficient in removing pesticides, and consequently that the discharged effluent is not harmful for the aquatic environment. Furthermore, Khalil and Hussein (1997) reported that the primary and secondary treated waste effluents were successfully used to growth the Nile tilapia. The presented results showed that common carp could be successfully reared in treated slaughterhouse wastewater with regard to pesticides contamination. Janković et al. (2012) reported that estimated weekly intake of common carp in Serbia is 29.4 g. The risk related to pesticides via consumption of examined fish for human of body weight of 70 kg is compared with acceptable daily intake (ADI) of selected pesticides recommended by various organization (Table 4). The estimated daily intake (EDI) was significantly lower than ADI for each pesticide, even in the worst case scenario. Having that in mind, it can be concluded that this intake would not pose a health hazard in human populations.

Table 4. Estimated daily intakes (EDI) of pesticides through common carp muscle by human (average body wt 70 kg) in Serbia

Pesticide	Average concentration ($\mu\text{g/g}$ wet wt)	EDI ($\mu\text{g/kg}$ body wt/day)	ADI ($\mu\text{g/kg}$ body wt/day)	Reference for ADI
Propachlor	0.02	0.0012	0.013 (mg/kg bw/day)	US EPA (1998)
Atrazine	0.03	0.0018	5 $\mu\text{g/kg}$	WHO (1990)
Simazine	0.05	0.003	5 mg/kg bw per day	US EPA (1987)
Endrin aldehyde	0.038	0.0023	0.2	US EPA (1989)

EDI, Estimated daily intake; ADI, acceptable daily intake

CONCLUSIONS

Pesticide levels in all tissues of examined fish were below the permitted levels. The pesticides included in the present study are not usually included in the monitoring studies in Serbia but also in different states worldwide. However, the obtained results revealed that several of those pesticides could pose a significant human health hazard and also an environmental hazard. This should be taken into account by the authority so that those pesticides should be included in some new monitoring schemes. The knowledge and proper management of pesticides associated with the fish consumption are of considerable medical, economic and environmental importance. Continuous monitoring, including appropriate testing of fish meat in order to determine the presence of pesticides is necessary. It is important due to the fact that fish is an important food source but also is an important indicator of environmental pollution. The obtained results provide important data for the risk assessment of pesticides from fish reared in integrated systems.

However, the further research are necessary for developing risk management tools for integrated aquaculture production systems.

ACKNOWLEDGEMENTS

This research is a part of a project (grant number: TR 31011) of the Ministry of Science and Technological Development, Republic of Serbia, entitled "The influence of the quality of the components of food for cyprinid fish species on the quality of meat, losses and the profitability of production". We would like to thank Milana Bomeštar, technical assistant for her encouragement and assistance during the study. For us, she had a considerable contribution. Also, we would like to thank Dragica Sofinkić, Kristina Stanišić and Jelena Menićanin for technical assistance.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

REFERENCES

- Aamir M, Khan S, Nawab J, Qamar Z, Khan A (2016) Tissue distribution of HCH and DDT congeners and human health risk associated with consumption of fish collected from Kabul River, Pakistan. *Ecotoxicol Environ Saf* 125: 128-134. <https://doi.org/10.1016/j.ecoenv.2015.12.005>
- Cao H Y, Liang T, Tao S, Zhang CS (2007) Simulating the temporal changes of OCP pollution in Hangzhou, China. *Chemosphere* 67:1335-1345. <https://doi.org/10.1016/j.chemosphere.2006.10.082>
- Darko G, Akoto O, Oppong C (2008) Persistent organochlorine pesticide residues in fish, sediments and water from Lake Bosomtwi, Ghana. *Chemosphere* 72:21-24. <https://doi.org/10.1016/j.chemosphere.2008.02.052>
- EU Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. *Official Journal of the European Union*, 348:84-97.
- EU Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance. *Official Journal of the European Union*, 226:1-17.
- El-Shahawi MS, Hamza A, Bashammakh AS, Al-Saggaf WT (2010) An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta* 80:1587-1597. <https://doi.org/10.1016/j.talanta.2009.09.055>
- Guo L, Qiu Y, Zhang G, Zheng GJ, Lam PK, Li X (2008) Levels and bioaccumulation of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) in fishes from the Pearl River estuary and Daya Bay, South China. *Environ Pollut* 152:604-611. <https://doi.org/10.1016/j.envpol.2007.06.067>
- Hladik ML, Vandever M, Smalling KL (2016) Exposure of native bees foraging in an agricultural landscape to current-use pesticides. *Sci Total Environ* 542: 469-477. <https://doi.org/10.1016/j.scitotenv.2015.10.077>
- Hu G, Dai J, Mai B, Luo X, Cao H, Wang J, Li F, Xu M (2010) Concentrations and accumulation features of organochlorine pesticides in the Baiyangdian Lake freshwater food web of North China. *Arch Environ Con Tox* 58:700-710. <https://doi.org/10.1007/s00244-009-9400-1>
- Janković S, Antonijević B, Čurčić M, Radičević T, Stefanović S, Nikolić D, Čupić V (2012) Assessment of mercury intake associated with fish consumption in Serbia. *Tehnol mesa* 53:56-61.
- Kim S, Aga DS (2007) Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. *J Toxicol Environ Health B Crit Rev* 10:559-573. <https://doi.org/10.1080/15287390600975137>
- Kartalović B, Novakov N, Mihaljev Ž, Petrović J, Prica N, Babić J, Čirković MA (2016) Organochlorine pesticides in canned tuna and sardines on the Serbian market. *Food Addit Contam B* 9:299-304. <https://doi.org/10.1080/19393210.2016.1234004>
- Khalil MT, Hussein HA (1997) Use of waste water for aquaculture: an experimental field study at a sewage-treatment plant. *Aquac Res* 28:859-865. <https://doi.org/10.1046/j.1365-2109.1997.00910.x>
- Kong XJ, Li D, Cao LQ, Zhang XM, Zhao Y, Lv Y, Zhang J (2008) Evaluation of municipal sewage treatment systems for pollutant removal efficiency by measuring levels of micropollutants. *Chemosphere* 72:59-66. <https://doi.org/10.1016/j.chemosphere.2008.02.005>
- Ljubojević D, Radosavljević V, Puvača N, Živkov Baloš M, Đorđević V, Jovanović R, Čirković M (2015) Interactive effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio* L.). *J Food Compos Anal* 37:44-50. <https://doi.org/10.1016/j.jfca.2014.09.005>
- Ondarza PM, Miglioranza KSB, Gonzalez M, Shimabukuro VM, Aizpún JE, Moreno VJ (2010) Organochlorine compounds in common carp (*Cyprinus carpio*) from Patagonia Argentina. *J Braz Soc Ecotoxicol* 5:41-47.
- Padula DJ, Daughtry BJ, Nowak BF (2008) Dioxins, PCBs, metals, metalloids, pesticides and antimicrobial residues in wild and farmed Australian southern bluefin tuna (*Thunnus maccoyii*). *Chemosphere* 72:34-44. <https://doi.org/10.1016/j.chemosphere.2008.02.029>
- Papadakis EN, Tsaboula A, Kotopoulou A, Kintzikoglou K, Vryzas Z, Papadopoulou-Mourkidou E (2015) Pesticides in the surface waters of Lake Vistonis Basin, Greece: Occurrence and environmental risk assessment. *Sci Total Environ* 536:793-802. <https://doi.org/10.1016/j.scitotenv.2015.07.099>
- Pelić M, Kartalović B, Đorđević V, Puvača N, Teodorović V, Čirković M, Ljubojević Pelić D (2019) Occurrence and dietary exposure of organochlorine pesticides in common carp obtained from integrated production systems. *Food Addit Contam Part B Surveill* 12: 303-309. <https://doi.org/10.1080/19393210.2019.1663372>
- Svobodova Z, Žlábek V, Randak T, Machova J, Kolářová J, Hajšlová J, Suchan P (2003) Profiles of persistent organochlorine pollutants (POPs) in tissues of marketable common carp and in bottom sediments of selected ponds of South and West Bohemia. *Acta Vet Brno* 72:295-309.
- Thomas M, Lazartigues A, Banas D, Brun-Bellut J, Feidt C (2012) Organochlorine pesticides and polychlorinated biphenyls in sediments and fish from freshwater cultured fish ponds in different agricultural contexts in north-eastern France. *Ecotoxicol Environ Saf* 77:35-44. <https://doi.org/10.1016/j.ecoenv.2011.10.018>
- USEPA (1998) Health and environmental effects profile for propachlor. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/X-84/335 (NTIS PB88162177).
- USEPA (1987) Health advisory - simazine. Office of Drinking Water. Washington, DC.
- USEPA (1989) Exposure assessment handbook. United States Environmental Protection Agency. Office of Health and Environmental Assessment, Washington, DC.
- WHO (1989) Aldrin and dieldrin: health and safety guide. Health and Safety Guide No. 21., Geneva. pp 1-48.
- WHO (1990) Atrazine health and safety guide. Health and Safety Guide No. 47. Geneva. pp 1-22.