



UDC: 639.37 (479.25)

## Risk Assessment of Tetracycline Residues in Ishkhan Fish Meat Cultured in Armenia

E.A. Ghrejyan, S.A. Stepanyan, L.A. Sireyan

Center for Ecological-Noosphere Studies of National Academy of Sciences, Republic of Armenia  
emma.ghrejyan@cens.am

### ARTICLE INFO

#### Keywords:

cultured fish,  
tetracyclines,  
estimated daily intake,  
health risk

### ABSTRACT

The aim of this study was to conduct dietary exposure assessment of tetracycline residues through consumption of cultured Ishkhan fish meat in Armenia. Tetracycline concentrations were detected by enzyme linked immunosorbent assay method (ELISA). The results indicated that the contents of tetracycline residues exceed the maximum allowable concentration (MAC) value in more than half of the samples. However, due to relatively low consumption of Ishkhan fish, the Hazard Quotient (HQ) is less than 1, indicating a low health risk.

### Introduction

Aquaculture is the fastest growing animal food producing sector that provides approximately 46% of total food fish supply for meeting the increasing protein needs. One of the most urgent problems of aquaculture, which can bring significant economic loss, is the fish mortality caused by infectious diseases that are often addressed with antibiotics (Agriculture Organization of the United Nations. Fisheries Department, 2000). Antibiotics are natural or semi-synthetic drugs, widely used among humans and animals for treating and preventing various diseases through their ability to kill or inhibit the growth of microorganisms (Bacanli, et al., 2019). To date, antibiotics, particularly tetracyclines are commonly used and often detected in high concentrations in aquatic production (Liu, et al., 2017). The presence of antibiotic residue in food products is a great concern according to international scientific authorities such as World Health Organization (WHO) and American Public Health Association, etc. (Graham et al., 2007). Antibiotic residues can cause various toxic effects including allergies, cancer, alterations in the intestinal flora, and can become a public health issue by contributing to the development of bacterial resistance (Mensah et al., 2014).

In Armenia, where fish farming is regulated by the EAEC Technical Regulation (Ministry of Foreign Affairs of the Republic

of Armenia, 2015), aquaculture is a developing field and the export of fish has a growing potential (Customs Service of the Republic of Armenia, 2017). As there are limited studies regarding tetracycline use in aquaculture, the present study aims to assess occurrence and dietary exposure of tetracycline residues and related human health risk through consumption of cultured Ishkhan fish meat in Armenia.

### Materials and methods

The sampling and analysis was performed in the framework of State Monitoring Program (Decision N 1142-N). In 2017, a total of 21 cultured Ishkhan fish samples were collected from different regions of Armenia and were analyzed for detecting the contents of tetracycline residues. The sampling was carried out during September-November, 2017 and the contents of tetracyclines were quantified via enzyme linked immunosorbent assay method (ELISA).

Afterwards Estimated Daily Intake (EDI) was calculated according to the following equation:

$$EDI = \frac{C_{\text{antibiotic}} \times C_{\text{fish}}}{BW}, \quad (1)$$

where  $C_{\text{antibiotic}}$  is the concentration of tetracyclines in  $\mu\text{g}/\text{kg}$ ,  $C_{\text{fish}}$  is the average daily consumption of Ishkhan fish, which is 2.94 g/day

for Armenia according to Statistical committee of the Republic of Armenia (List of Individual Consumption of Goods and Services, 2016). BW is the average body weight for the adult and is 70 kg. For those samples, where the concentration of tetracyclines residues was under the limit of detection (10 µg/kg), EDI values were not calculated.

For carrying out risk assessment the Hazard Quotients (HQ) were calculated as ratios between EDIs and the Acceptable Daily Intake (ADI, 0-3 µg/kg bw/day), recommended by WHO (JECFA, 1998):

$$HQ = \frac{EDI}{ADI} \quad (2)$$

HQ ≥ 1 is considered to indicate a high risk of an adverse health effect, while HQ < 1 indicates a low risk.

### Results and discussions

Concentrations of tetracyclines in Ishkhan fish samples are summarized in the Figure below. These concentrations were compared with a maximum allowable concentration (MAC= 10 µg/kg) established by the Eurasian Economic Commission (EAEC 021/2011).

Contents of tetracyclines exceeded the MAC value in 12 samples (F2, F3, F5, F6, F9, F10, F11, F12, F13, F14, F20 and F21) out of 21 (51,7%) and ranged between 11-28 µg/kg. It should be noted, that in other 9 investigated samples the contents of tetracyclines were below the limit of detection. The highest concentration levels were observed in the samples F5, F6 and F9 being 2.4 to 2.8 times higher than the corresponding MAC value. Likewise, in the recent study conducted in neighboring Iran, 63.1% of farmed rainbow trout samples contained the residues of tetracyclines, concentrations of which in positive samples ranged from 1.43 to 101.4 µg/kg. Nevertheless, these concentrations did not exceed the maximum residue level (200 µg/kg) recommended by the Institute of Standards and Industrial Research (Barani et al., 2015).

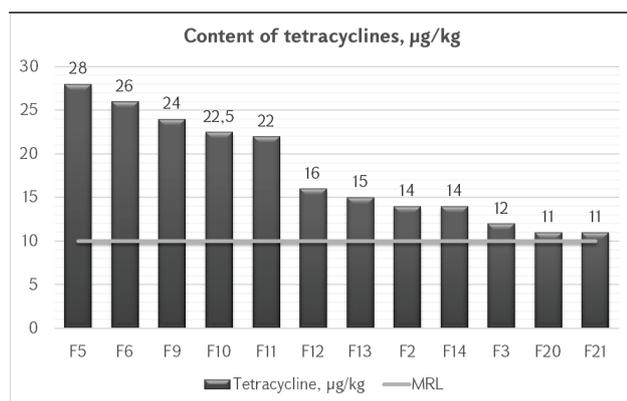


Figure . Concentrations of tetracyclines in Ishkhan fish samples.

Obtained EDI and HQ values are presented in the table below. The EDI's ranged from 4.62E-04 to 1.18E-03 µg/kg bw/day. However, the HQ for tetracyclines is much smaller than 1, which indicates no direct adverse effects from ingestion of these antibiotic-contaminated Ishkhan fish (Table).

Table. Concentrations, EDI and HQ values for tetracyclines in Ishkhan fish

Sample	Concentration, µg/kg	EDI, ng/kg bw/day	HQ
F1	n/d	-	-
F2	<b>14</b>	0.588	0.020
F3	<b>12</b>	0.504	0.017
F4	n/d	-	-
F5	<b>28</b>	1.176	0.039
F6	<b>26</b>	1.092	0.036
F7	n/d	-	-
F8	n/d	-	-
F9	<b>24</b>	1.008	0.034
F10	<b>22.5</b>	0.945	0.032
F11	<b>22</b>	0.924	0.031
F12	<b>16</b>	0.672	0.022
F13	<b>15</b>	0.630	0.021
F14	<b>14</b>	0.588	0.020
F15	n/d	-	-
F16	n/d	-	-
F17	n/d	-	-
F18	n/d	-	-
F19	n/d	-	-
F20	<b>11</b>	0.462	0.015
F21	<b>11</b>	0.462	0.015

Note: values below the limit of detection (10 µg/kg) are indicated as "not detected" (n/d), values that exceeded MRL are bolded. For samples where the content of tetracycline is below the limit of detection, EDI and HQ are not calculated.

In the comparative study, which included risk assessment of tetracyclines in 14 cultured fish species from a coastal city in the northern China (Liu et al., 2018), consumption of cultured fish from mentioned region was not associated with significant human health risks as well, whereas concentration of tetracycline residues exceeded the MRL (100 µg/kg) in two samples of fish species.

### Conclusion

Despite the fact, that in the majority of samples the concentrations of tetracycline residues exceeded the MAC value, they pose a low health risk as a result of relatively low consumption of Ishkhan fish. The obtained HQ values indicated that Ishkhan fish consumption does not have significant contribution in tetracycline exposure, so it is important to assess shares of its exposure due to consumption of other food items (i. g. meat and dairy products). Therefore, human health risk assessment of tetracyclines should be further investigated. Moreover, in this study concentrations of other antibiotics that might occur in cultured fish were not considered, which should be addressed in future studies.

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