DIOXINS AND DIOXIN-LIKE PCBs IN CHICKEN EGGS AND FISH FROM ALAVERDI, ARMENIA

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Introduction

Dioxins are a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) congeners and 135 polychlorinated dibenzofuran (PCDF) congeners, of which 17 are of toxicological concern. Polychlorinated biphenyls (PCBs) are a group of 209 different congeners which can be divided into two groups according to their toxicological properties: 12 congeners exhibit toxicological properties similar to dioxins and are therefore often referred to as 'dioxin-like PCBs' (dl PCBs)[1]. Levels of PCDD/Fs and dl PCBs are expressed in total WHO-TEQ calculated according to toxic equivalency factors (TEFs) set by a WHO expert panel in 2005[2].

The PCDD/Fs and dl PCBs are products of incomplete combustion that are unintentionally produced during thermal processes. As such they are listed under Annex C of the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) which requires, under Article 5, reduction of *"total releases derived from anthropogenic sources of each of the chemicals …, with the goal of their continuing minimization and, where feasible, ultimate elimination"*[3].Major industrial source categories which have *"the potential for comparatively high formation and release of these chemicals to the environment"* are also specified in Annex C to the SC[3].

In this study, we present the results of the analyses of free-range hens' eggs and fish from the Tumanyan District in the north of Armenia, in order to evaluate potential contamination of food chain with PCDD/Fs and dl PCBs. Copper smelter has been operated in Alaverdi since the end of 19th century[4]. Metallurgy belongs to the industries listed in Annex C to the SC[3], and copper production is a proven source of PCDD/Fs and dl PCBs[5-7]. Free range chickens and their eggs are considered ideal "active samplers" and indicator species for POPs contamination. Eggs have been found to be sensitive indicators of PCDD/F and PCB contamination in soils and are an important exposure pathway from soil pollution to humans. Eggs from contaminated areas can readily lead to exposures which exceed thresholds for the protection of human health[8-10]. There are a range of studies on PCDD/Fs and PCBs in free range chicken eggs[11-14]. Fish meat can be also an important source of the PCDD/Fs and other POPs in diet. POPs accumulate mainly in lipids, so fatty fish species can be an important source of exposure to POPs[15-17]. The amount of bioaccumulative contaminants increases towards to the higher trophic levels of the food chain.

Materials and methods

In 2018-2020 one individual and five pooled samples of free-range chicken eggs and 1 pooled fish sample of chub were collected at three localities in Tumanyan district, Lori Province (Marz) located on the northern borders of Armenia. One pooled egg sample was taken from a supermarket in the city of Yerevan, considered as a background reference sample for Armenia, following precedents from other studies[18]. The localities that were chosen were expected to be influenced by pollution from a copper smelter as they are within the range of its common plume area. One sample was taken in Mets Ayrum, a locality affected by mining of metals and tailing ponds, not metallurgic industries. The samples of freshwater fish were collected form the river Debed downstream from the locations affected by mining as well as by the copper smelter and its wastes in Alaverdi.

The sampled eggs were collected into typical plastic egg packaging and were boiled for approximately 7 minutes immediately after sampling. The fish and eggs samples were kept in cold conditions during their storage and transport to the laboratory. The homogenates from the edible parts of the eggs and fish muscles were used for the analyses in the laboratories. The samples for analysis were mixed from 3-4 eggs from the same location, if available. All samples were analyzed for their content of individual PCDD/Fs and dl PCBs by GC/HRMS in an ISO 17025 accredited laboratory at the State Veterinary Institute in Prague, Czech Republic, with a resolution >10,000 using ^{13}C isotope labelled standards. PCDD/F and dl-PCB analysis followed the methods of analysis for the control of levels of PCDD/Fs and dl-PCBs in foodstuffs according the EU regulation[19]. First results from 2018 were partly published in a previous report[20].

The results are presented in pg WHO TEQ g^{-1} of fat in eggs and in wet weight (ww) in fish samples if not specified otherwise. TEFs defined in 2005[2] were used to evaluate dioxin toxicity in samples.

Results and discussion

Levels of PCDD/Fs and dl PCBs measured in 6 free range chicken eggs from Alaverdi and neighboring localities are summarized in Table 1. It also shows the results of measurements for reference sample from the supermarket in Yerevan and for the pooled fish sample from Debed river. The map at Figure 1 shows the location of the samples with results of measured PCDD/Fs/dl PCBs in the eggs and copper smelter location. Prevailing winds are in the diagram attached to that map.

Table 1. Summarized results of analyses for PCDD/Fs and dl PCBs in egg and fish samples from Armenia.

Year	Matrix	Name of the site	Eggs/fish in pooled sample	Fat	PCDD/Fs	dl PCBs	PCDD/Fs + dl PCBs
			number	%	pg WHO TEQ g^{-1} fat		
2018	Eggs	Alaverdi 1	3	14.4	7.5	19.3	26.8
2019		Alaverdi 2	4	12.0	14.6	25.5	40.1
2018		Alaverdi 3	4	13.1	4.4	9.1	13.5
2020		Alaverdi 4	3	12.3	1.7	3.3	5
2020		Sanahin	2	11.2	2.0	21.5	23.5
2020		Mets Ayrum	1	12.1	0.41	1.57	1.99
2018		Yerevan-Supermarket	4	8.7	0.20	0.14	0.34
	Fish (chub				pg WHO TEQ g^{-1} ww		
2020		Alaverdi F	6	4.8	0.10	1.25	1.35
					pg WHO TEQ g^{-1} fat		
2020		Alaverdi F	6	4.8	2.0	26.1	28.1

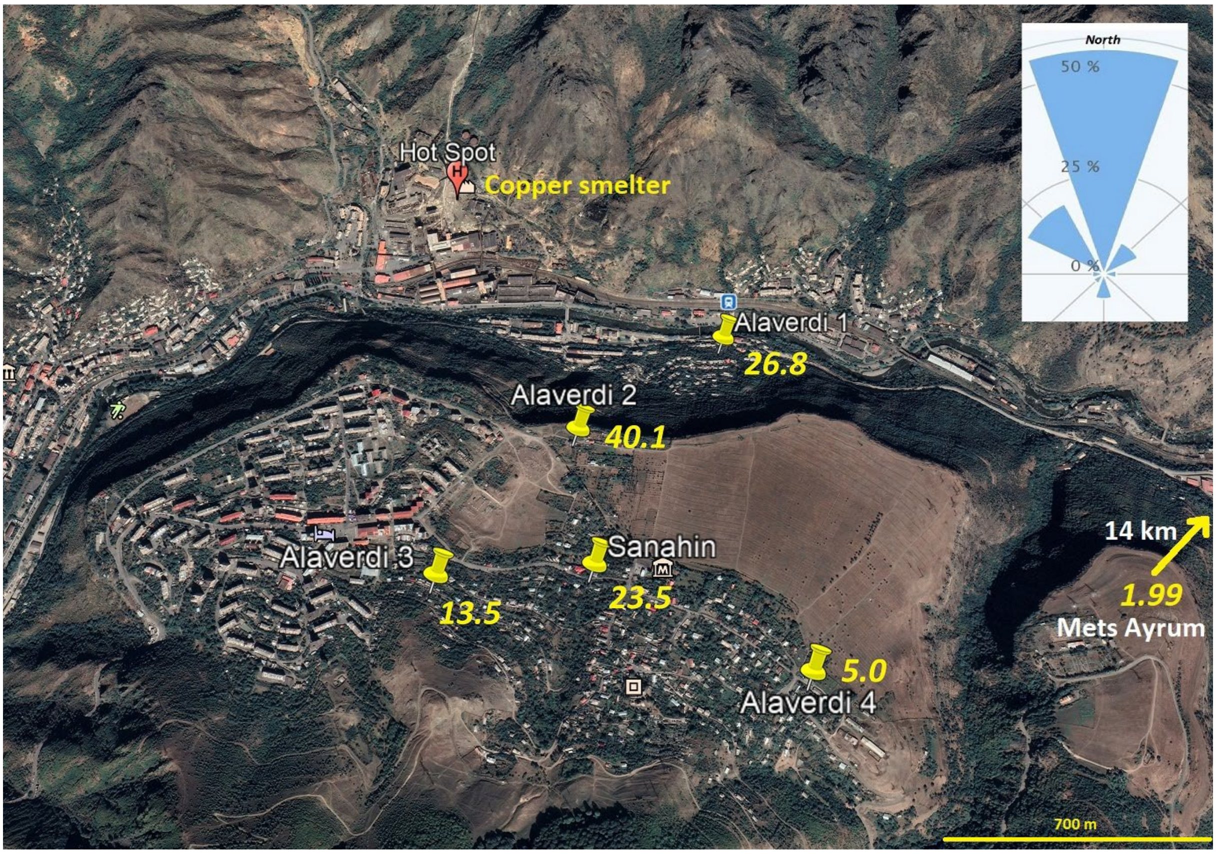

Figure 1: Map of Alaverdi with marked sampling sites and measured levels of PCDD/Fs/dl PCBs. Prevailing wind directions are in diagram in the upper right corner of the map. Mets Ayrum is 14 km to north-east.

Three samples, specifically Alaverdi 1, 2 and 3 out of a total of six pooled free range egg samples from Alaverdi and adjacent areas exceeded the Armenian and Russian limit for sum of PCDD/Fs (3.0 pg WHO TEQ g^{-1} fat)[21,22], as well as the EU ML for PCDD/Fs (2.5 pg WHO TEQ g^{-1} fat) and for PCDD/Fs and dl PCBs (5.0 pg WHO TEQ g^{-1} fat) respectively[23]. The levels for PCDD/Fs and dl PCBs measured in hens' eggs from a supermarket in Yerevan were 0.20 and 0.14 pg WHO-TEQ g^{-1} fat, respectively and they can be accepted as background levels for PCDD/Fs/dl PCBs in eggs from Armenia. All collected samples of free-range hens' eggs from Alaverdi and neighboring locations, exceeded levels of PCDD/Fs and dl PCBs measured in the reference sample of eggs from Yerevan by 2 – 73 and 11 - 182 fold respectively. The highest level of 40.1 pg WHO TEQ

g^{-1} fat of PCDD/Fs/dl PCBs was measured in a pooled egg sample from site Alaverdi 2 located on the south of the copper smelter, right in the direction of prevailing winds (see map at Figure 1). On the map it can be observed that levels of PCDD/Fs/dl PCBs in eggs are decreasing with the distance from the copper smelter and that they are also quite clearly in agreement with prevailed wind directions carrying the pollution from the copper smelter. The lowest level of PCDD/Fs/dl PCBs (1.99 pg WHO-TEQ g^{-1} fat) was measured in the eggs from Mets Ayrum, 14 km distant from Alaverdi and affected rather by mining and tailing ponds which are not sources of PCDD/Fs and dl PCBs. The copper smelter was shut down in 2018 however pollution is most likely remaining in the soil and dust.

Arnika and AWHHE had already sampled and analysed free range hens' eggs for POPs in 2010 at different locations, including Alaverdi[24]. The eggs were analysed for total dioxin activity by DR CALUX analysis expressed in bioanalytical toxicity equivalents (BEQs) [25], the results of which can be compared with the results of the GC-HRMS analyses[26-27] in this report. The level of 11.9 pg BEQ g^{-1} fat was measured in eggs from Alaverdi in 2010, which is lower than the levels of PCDD/Fs and dl PCBs in most of samples from 2018-2020 except Alaverdi 4. The sample was taken 1 km further to the south-east of the smelter, further than Alaverdi 1 presented in this study, but it was still located in the valley influenced by smelter emissions. The other locations sampled in 2010 were around obsolete pesticide storage areas, e.g. in Griboedov or Nubarashen[24], and measured levels were in the range of 0.8 and 37 pg BEQ g^{-1}. Quite high levels of dioxin activity at 508 and 1120 pg BEQ g^{-1} dry matter were measured in samples of waste from the copper smelter stored near Lernahank village sampled in 2010[24].

Levels of 5 – 40.1 pg WHO-TEQ g^{-1} in the eggs from the vicinity of the copper smelter in Alaverdi are comparable to those measured in the neighborhoods of other metallurgical plants, e.g. in Beihai, China (24 – 37 pg BEQ g^{-1} fat) [28] or near a copper smelter in Balkhash, Kazakhstan (12.7 – 30.1 pg WHO-TEQ g^{-1} fat) [29].

PCDD/Fs and dl PCBs levels of 0.1 and 1.25 pg WHO-TEQ g^{-1} in chub fish from Debed river, sampled in the surroundings of Alaverdi town, were below the EU ML for PCDD/Fs and PCDD/Fs/dl PCBs of 3.5 and 6.5 pg WHO TEQ g^{-1} ww respectively set for fish meat[23]. They are also lower than levels of PCDD/Fs and dl PCBs of 0.48 – 0.88 and 3.9 – 4.4 pg WHO-TEQ g^{-1} ww respectively measured in chub samples from the Elbe (Labe) river in 2005[30]. Levels of PCDD/Fs and dl PCBs of 0.04 - 0.09 and 0.53 – 1.4 pg WHO-TEQ g^{-1} ww respectively which were found also in chub samples caught in upper part of Sava river, Croatia in 2015[31] are almost the same as those observed in pooled sample of chub from Debed river. Chub is also considered to be a nomadic species[30] which means that the levels found in chub from Alaverdi do not necessarily show just localised contamination.

Dietary intake evaluation: The share of eggs in total food consumption in Armenia in 2007 was close to 0.8% of the total food basket per day, according to the World Atlas – Food Security data[32], and changes in its share have not increased greatly since 2002. If we count 50 g per hen's egg as the average weight, it will mean the consumption of one third of an egg per person per day as the general consumption pattern for the current Armenian population. The consumption of eggs within families who raise hens at the sampled sites is in agreement with this assumption, as they confirmed during our sampling. In this study the dietary intake was calculated for PCDD/Fs and dl PCBs contaminants per day for all pooled samples of free-range hens' eggs from Alaverdi, Sanahin and Mets Ayrum communities and compared with the eggs from the Yerevan supermarket, considered as the background level in Armenia.

The calculation of daily intake levels was performed by using the following formula:

$$DI_{adult} = \frac{\left(\left(\frac{c \cdot F}{100}\right) \cdot ADC\right)}{70}$$

$$DI_{child} = \frac{\left(\left(\frac{c \cdot F}{100}\right) \cdot 18\right)}{35}$$

where DI = daily intake (pg kg^{-1} bw); C = POPs concentration (pg WHO TEQ g^{-1} fat), and F = fat content in the sample (%); ADC = average daily consumption of the food type.

The results were compared with the tolerable daily intake (TDI) newly established by the European Food Safety Authority (EFSA) at a level of 0.25 pg WHO-TEQ kg^{-1} bw day^{-1} [17].

Average consumption of eggs (1/3 of an egg per day) would lead to the exceedance of TDI for PCDD/Fs/dl PCBs by 260% and 520% for a 70 kg adult man and a 35 kg 10 year old child respectively. This calculation was made from levels in all 5 pooled egg samples from Alaverdi. For the eggs sample with the highest level, the exceedance would be much higher at levels of 457% and 915% respectively. Eggs from Mets Ayrum and supermarket in Yerevan reached 23% and 3% of TDI for an adult. The percentage is doubled for a 10 year old child. The result is alarming if we consider that PCDD/Fs and dl PCBs are certainly also present in other food consumed by the inhabitants of Alaverdi. Contamination with PCDD/Fs and dl PCBs should be addressed in this town and its neighborhoods.

Conclusions

Levels of PCDD/Fs and dl PCBs measured in free range chicken eggs from Alaverdi and its vicinity exceeded safe levels. An adult man and 10 year old child in Alaverdi consuming an average portion of free range eggs usual for Armenians would exceed TDI for PCDD/Fs and dl PCBs set by EFSA by 260% and 520%. The copper smelter seems to be an obvious source of contamination with PCDD/Fs and dl PCBs as their levels are decreasing in the eggs with distance from the smelter (see map at Figure 1). Their level at a 14 km distant location is several times lower than levels found in eggs from Alaverdi. The levels of contamination of free range eggs in Alaverdi with PCDD/Fs/dl PCBs is comparable to other locations in the vicinity of metallurgical plants, e.g. in Beihai, China or Balkhash, Kazakhstan. It shows that parties to the SC should rapidly implement best available techniques (BAT) and best environmental practices (BEP) for these technologies. Introduction of limits for air releases from metallurgical plants is desired. Metallurgical plants have proven to be significant source of PCDD/Fs/dl PCBs.

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