PCDD/Fs, PCBs AND HCB IN EGGS – DATA FROM ARMENIA, BELARUS, CZECH REPUBLIC AND UKRAINE

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Introduction

There is a range of studies on PCDD/Fs and PCBs in eggs[1-4]. Eggs have been found to be sensitive indicators of POPs 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 [2, 5, 6]. Chickens and eggs might therefore be considered ideal "active samplers" and indicator species for POPs contaminated sites but there are, as yet, few systematic studies linking pollution sources, related exposures and concentrations of contaminants in eggs.

In this study, we sought to broaden the available data and eggs were sampled at sites suspected of being impacted by POPs in countries of Central and East European Region: Armenia, Belarus, Bosnia and Herzegovina, Czech Republic, Montenegro, Serbia and Ukraine. It is based on larger reports released in 2015 - 2018, where more information about the sites can be found[7-11].

Materials and methods

Free range chicken eggs were collected from 11 localities in Armenia (1), Belarus (1), Bosnia and Herzegovina (2), Czech Republic (2), Montenegro (1), Serbia (1) and Ukraine (3). Additional pooled samples were taken in order to obtain information about reference levels of POPs in chicken eggs: In three countries one pooled sample was bought from supermarkets (Armenia, Czech Republic, and Ukraine). Two pooled samples were taken at remote locality of Plužine in Montenegro as background locality for all three Balkan states. Sample from supermarket in Ukraine can serve as reference level also for Belarus. 22 pooled free range chicken egg samples were analysed for PCDD/Fs, dl-PCBs, 6 indicator PCB congeners (i-PCBs) and hexachlorobenzene (HCB) by instrumental analysis, and an additional 2 pooled samples for i-PCBs and HCB only.

There was following number of individual eggs in pooled samples from different countries: Armenia between 3 – 4 eggs/sample[9], Belarus – 3 eggs/sample, Bosnia and Herzegovina 5 – 11 eggs/sample[7], Czech Republic 3 – 5 eggs/sample[11], Montenegro 2 - 3 eggs/sample[7], Serbia 2 – 6 eggs/sample[7] and Ukraine 5 – 16 eggs/sample[8]. These ranges were dependent on eggs available at the sampled sites at the time of sampling. Pooled samples with only 2 eggs were the exception and this was only from Pljevlja, Montenegro, and one site near Obrenovac, Serbia. Samples were collected within the five years period 2014 – 2018[7-11]. The choice of sampled sites were prioritised according to industrial or other human activity criteria. The sampled sites were chosen according industrial or other human activity criteria. The sites with potential releases of POPs had priority, in particular such with industries listed as major sources of PCDD/Fs under Annex C to Stockholm Convention. Also contaminated sites and their neighbouring localities were included into our sampling scheme. We followed similar model of selection of sites as in previous IPEN's free range eggs study[1].

Bioassay. 3 samples were analysed at Bio Detection System for dioxin-like activity according to the standard procedures of the DR CALUX® following the European Union's methods of analysis for the control of levels of PCDD/Fs and dl-PCBs for levels in certain foodstuffs in Commission Regulation (EC) No 252/2012[12].The procedure for the BDS DR CALUX® bioassay has previously been described in detail[13], and has been used also in our previous studies[14].

Instrumental analysis. 24 samples were analysed by gas chromatography high resolution mass spectrometry (GC/HRMS) in ISO 17025 accredited laboratories with a resolution >10,000 using ^{13}C isotope labelled standards for PCDD/F and dl-PCB analysis based also on EC/252/2012[12]. The samples were also analysed for their content of indicator congeners of PCBs (iPCBs), and HCB in a certified Czech laboratory (University of Chemistry and Technology Prague, Department of Food Chemistry and Analysis). The analytes were extracted by a mixture of hexane: dichloromethane (1:1). The extracts were cleaned by means of gel permeation chromatography (GPC). The identification and quantification of the analyte was conducted by gas chromatography coupled with tandem mass spectrometry detection in electron ionization mode.

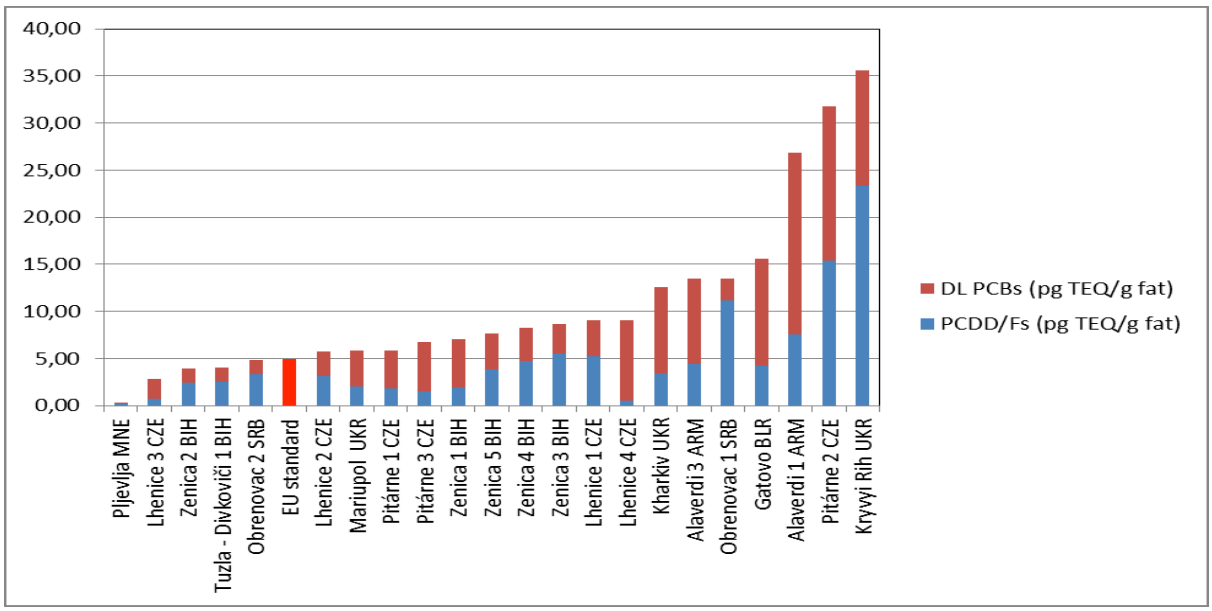

Figure 1: Graph summarizing results of analyses of free range chicken eggs from 22 sites, 11 localities in Armenia (ARM), Belarus (BLR), Bosnia and Herzegovina (BIH), Czech Republic (CZE), Montenegro (MNE), Serbia (SRB) and Ukraine (UKR) for PCDD/Fs and dl-PCBs.

Results and discussion

Levels of PCDD/Fs and dl-PCBs in eggs. Results of instrumental analyses for PCDD/Fs, dl-PCBs and i-PCBs are in Table 1 summarized for each locality. Results for supermarkets and reference site for three western Balkan states are at the beginning of Table 1. Lowest reference level for PCDD/Fs was measured in a pooled egg sample from a supermarket in Prague, while for dl-PCBs, i-PCBs and HCB were lowest levels measured in samples from supermarkets in Yerevan and Kiyv (see Table 1). The highest level and third highest levels of PCDD/Fs + dl-PCBs were found in two pooled egg samples from the vicinity of metallurgical plants in Kryvyi Rih, Ukraine and Alaverdi, Armenia respectively. Second highest level was measured in pooled egg sample from Pitárne, Czech Republic. Balance between PCDD/Fs and dl-PCBs in total TEQ is different in these three samples (see graph at Figure 1). Only 5 out of 22 samples were below EU standard of 5 pg WHO-TEQ/g fat for PCDD/Fs + dl-PCBs in eggs[15]. The lowest level of PCDD/Fs+dl-PCBs was found in pooled egg sample from Pljevlja, Montenegro where the coal burning power plant is located. Levels of all analysed POPs in this study were in sample from Pljevlja comparable to levels found in reference egg samples from this region. In several cases mainly dl-PCBs were major contributor to total TEQ, in the vicinity of metallurgical plants, car shredder and by obsolete PCBs contaminated site in particular.

Levels of PCDD/Fs measured in 22 egg samples are summarized in graph at Figure 2. In total 14 egg pooled samples exceeded the EU standard set for PCDD/Fs at a level of 2.5 pg TEQ/g fat[15]. The highest level was measured in a sample from Kryvyi Rih described above. All free range egg samples were above (reference) levels in eggs from supermarkets. Sample from Pljevlja contained PCDD/Fs at level of reference sample from Plužine, both from Montenegro, and it was also the lowest level of PCDD/Fs in free range chicken eggs in this study.

i-PCBs: 3 out of 23 samples exceeded the EU standard for i-PCBs in eggs set at a level of 40 ng/g fat[15]. The highest level of 113 ng/g fat was measured in eggs from Pitárne, Czech Republic. Second a third highest levels of i-PCBs were in eggs from Gatovo (BLR), a site near car shredder, and from Lhenice 4 (CZE), a site with soil contaminated by PCBs respectively. Last two samples exhibited also high levels of dl-PCBs contributing to total TEQ (see graph at Figure 1).

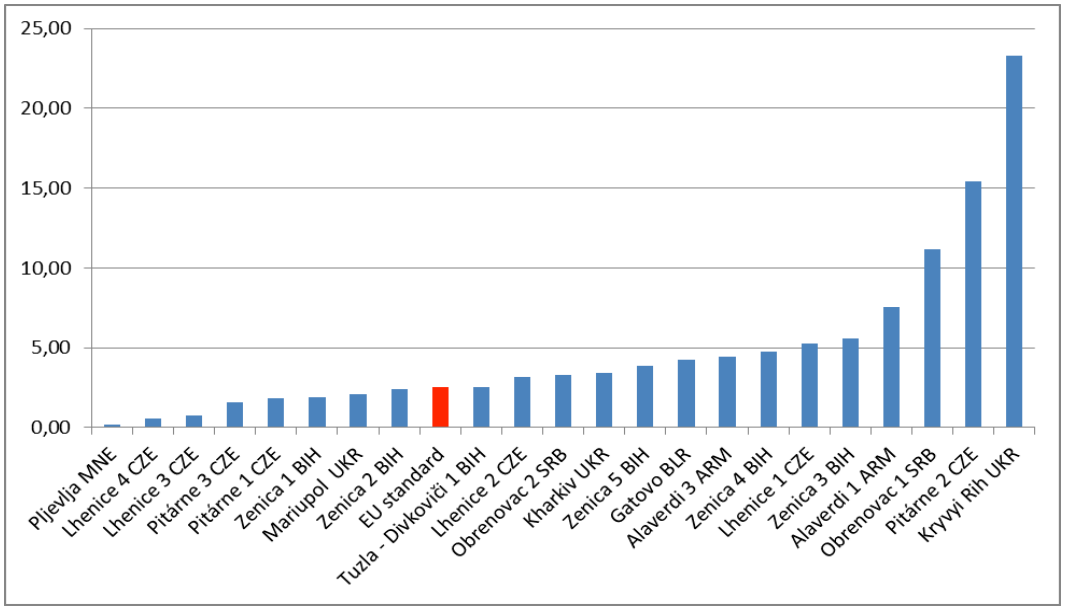

Figure 2: PCDD/Fs levels in 22 pooled egg samples from CEE countries.

Table 1: Summary of results of instrumental analyses for PCDD/Fs, PCBs and HCB per localities in each of seven countries plus supermarkets serving to establish reference background levels of POPs contaminants. Note: Plužine is remote locality in Montenegro, where free range chicken eggs were sampled and analysed by DR CALUX® for dioxin-like activity only, so the levels in table are in BEQs for this locality as no instrumental analysis for PCDD/Fs was used for samples from this locality.

Country (other specification)	Supermarkets/background				Armenia	Ukraine		
Locality	Yerevan	Prague	Plužine	Kiyv	Alaverdi	Kharkiv	Mariupol	Kryvyi Rih
Industrial activity/contaminated site	-	-	-	-	Metallurgy	Metallurgy	Metallurgy	Metallurgy
Number of pooled samples	1	1	2	1	2	1	1	1
PCDD/Fs (pg WHO TEQ/g fat)	0.20	0.03	0.34*	0.25	4.5 / 7.5	3.4	2.1	23,3
DL PCBs (pg WHO TEQ/g fat)	0.14	0.20	NA	0.03	9.1 / 19.3	9.2	3.7	12,3
PCDD/F + DL PCBs (pg WHO TEQ/g fat)	0.34	0.23	0.98*	0.28	13.5 / 26.9	12.6	5.8	35,6
6 PCB (ng/g fat)	0.50	13.0	0.59 / 3.0	0.69	8.3 / 16.2	30.7	9.0	27,3
HCB	< 0.1	0.81	1.4 / 2.3	0.95	1.7 / 0.9	3.8	1.7	4,5
Country	Belarus	Bosnia and Herzegovina		Montenegro	Serbia	Czech Republic		
Locality	Gatovo	Zenica	Tuzla	Pljevlja	Obrenovac	Lhenice	Pitárne	
Industrial activity/contaminated site	Car shredder	Metallurgy	Coal power plant / chlorine industry	Coal power plant	Coal power plant	Contaminated site (PCBs and OCPs)	PVC recycling	
Number of pooled samples	1	4 (5)	1 (2)	1	2 (3)	4	3	
PCDD/Fs (pg WHO TEQ/g fat)	4.25	1.9 - 5.6	2.51	0.20	3.3 / 11.1	0.55 - 5.3	1.6 - 15.4	
DL PCBs (pg WHO TEQ/g fat)	11.33	1.6 - 5.2	1.56	0.06	1.6 / 2.4	2.2 - 8.5	4.0 - 16.4	
PCDD/F + DL PCBs (pg WHO TEQ/g fat)	15.58	4.0 - 8.7	4.07	0.26	4.9 / 13.5	2.9 - 9.1	5.8 - 31.8	
6 PCB (ng/g fat)	52.92	2.3 - 13.0	15.1 / 19.4	< 0.6	1.1 - 2.9	9.2 - 41.6	13.1 - 113	
HCB	8.1	1.1 - 2.7	1.5 / 1.6	0.43	1.7 - 5.4	4.0 - 11.3	5.9 - 8.0	

Numbers of pooled samples in brackets stand for pooled samples analyzed for i-PCBs and HCB, un-bracketed figures are pooled samples analyzed for both PCDD/Fs + dl-PCBs, i-PCBs and HCB. * Result of bioassay analysis.

HCB: Levels of HCB in 23 samples of pooled free range eggs were within the range of 0.4 – 11.3 ng/g fat. Highest level was measured in eggs from Lhenice (CZE) and can be considered as potential result of contamination of the site due to longterm storage of obsolete pesticides. Reference levels in supermarket eggs were within the range from < 0,1 (LOQ) to 0.95 ng/g fat. Levels from reference site in Montenegro were between 1.4 – 2.3 ng/g fat which can be due to influence of household heating at the site. In general levels observed in this group of samples did not include very high levels of HCB, in comparison with highest level of 250 ng/g fat measured in free range eggs in Liberec (CZE) in one of previous studies by IPEN[1] or recently measured level of 481 ng/g fat in eggs from Wuhan, China in the vicinity of waste incinerator[16].

Dietary exposure from consuming free range eggs contaminated by PCDD/Fs and PCBs was evaluated for each hotspot and summarized in published reports for Armenia, Western Balkan countries and Ukraine, however it was not done for samples from Belarus and Czech Republic yet. For more information we refer to published reports [7-9]. Very critical situation is in Kryvyi Rih (Ukraine), Alaverdi (Armenia), and Zenica (Bosnia and Herzegovina), all three sites are influenced by metallurgical industry in particular. Evaluation for all sites needs to be done in the light of reassessment of tolerable intake for PCDD/Fs and dl-PCBs by EFSA in 2018[17].

Conclusions

Results of conducted analyses for PCDD/Fs and PCBs in free range chicken eggs from selected hotspots in Central and East European countries have shown that some of them are seriously contaminated.

High levels of PCDD/Fs and dl-PCBs in free range chicken eggs from neighbor environment of metallurgical plants underline the need for enforcement of implementation of existing tools to control dioxin releases as set in Article 5 of the Stockholm Convention such as e.g. BAT/BEP Guidelines[18]. Significant levels of dioxins and dioxin-like substances were found also close to car shredder, contaminated site and PVC recycling plant which highlights the need to set stricter rules to control POPs transfers in wastes in agreement with some previous studies[16,19].

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