KYIV–PRAGUE 2018

USE OF FREE-RANGE POULTRY EGGS AS AN INDICATOR OF THE POLLUTION IN EASTERN UKRAINE

Results of sampling conducted in 2018

ARNIKA

UCT PRAGUE

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TRANSITION

This report is based on the results of environmental sampling conducted in Ukraine in May 2018 as a part of the project named Stop poisoning Ukraine: Coalition for Clean Air, financed by the Transition Promotion Programme of the Ministry of Foreign Affairs of the Czech Republic. The views expressed herein do not necessarily reflect the official opinion of the donor.

Use of free-range poultry eggs as an indicator of the pollution in Eastern Ukraine
Results of sampling conducted in 2018
This report is published in English and Ukrainian.

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Contents

1. Introduction

In this study, we present the results of the monitoring of free-range poultry eggs from Kharkiv, Mariupol, and Krivyi Rih, cities in the eastern part of Ukraine, which were considered to be potentially contaminated by persistent organic pollutants (POPs). Free-range poultry eggs were used for monitoring levels of contamination by POPs in various locations in many previous studies [1-6]. Eggs have been found to be sensitive indicators of POP contamination in soils or dust and are a significant 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 [7-9]. Poultry and their eggs might therefore be ideal "active samplers": an indicator species for the evaluation of levels of contamination in sampled areas by POPs, particularly by dioxins (PCDD/Fs) and PCBs. On the basis of this assumption, we have chosen a sampling of free-range poultry eggs and their analyses for selected POPs as one of the monitoring tools within the project "Stop poisoning Ukraine: Coalition for Clean Air" (further information about the project can be found at https://english.arnika.org/ukraine)

The data and analyses of free-range poultry eggs discussed in this report were obtained during field visits in May 2018 as a result of the above-mentioned joint project of Ukrainian and Czech NGOs. A general description of the samples and of the localities from which the samples were collected can be found in Chapter 2.

1.1 Acknowledgements

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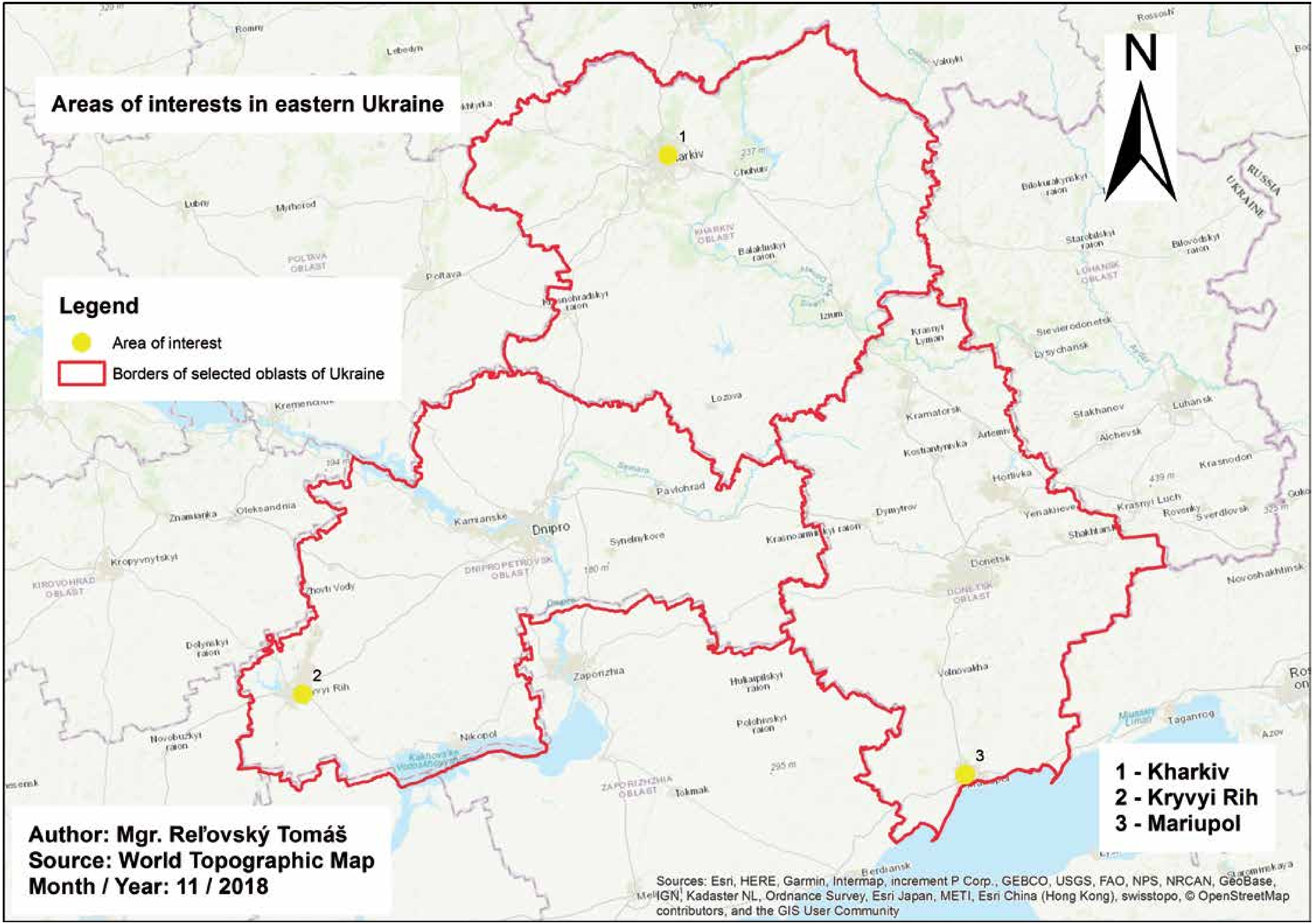
Abbreviations

BDS – BioDetection Systems (laboratory in the Netherlands)
BEQ – bioanalytical toxic equivalent
CALUX – chemically activated luciferase gene expression
CAS – chemical abstracts service registry number (a unique numerical identifier assigned to every chemical substance described in the open scientific literature)
DDD – dichlorodiphenyldichloroethane (a metabolite of DDT)
DDE – dichlorodiphenyldichloroethylene (a chemical compound formed by the loss of hydrogen chloride from DDT)
DDT – dichlorodiphenyltricholoroethane (pesticide)
DI – dietary intake
DL PCBs – dioxin-like PCBs
d.w. – dry weight
EFSA – European Food Safety Agency

EU – European Union
f.w. – fresh weight
GC – gas chromatography
GEF – Global Environment Facility
GPC – gel permeation chromatography
GPS – global positioning system
HCB – hexachlorobenzene
HCBD – hexachlorobutadiene
HCHs – hexachlorocyclohexanes (pesticides and their metabolites)
HpCDD – heptachlorodibenzo-p-dioxin
HpCDF – heptachlorodibenzo-p-furan
HRGC-HRMS – high-resolution gas chromatography – high-resolution mass spectroscopy
HxCDD – hexachlorodibenzo-p-dioxin
HxCDF – hexachlorodibenzo-p-furan
IPEN – International POPs Elimination Network
IARC – International Agency for Research on Cancer
INC – Intergovernmental Negotiating Committee (normally set up for negotiations of new international convention)
iPCBs – indicator PCBs (this mostly covers six PCB congeners (PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180)
LOD – limit of detection
LOQ – limit of quantification
MAC – maximum acceptable (allowable) concentration
ML – maximum level
MRL – maximum residue level
NA – not analysed
NGO – non-governmental organization (civil society organization)
NIP – National Implementation Plan of the Stockholm Convention
OCPs – organochlorinated pesticides
OCDD – octachlorodibenzo-p-dioxin
OCDF – octachlorodibenzo-p-furan
PBDD/Fs – polyfrominated dibenzo-p-dioxins and furans
PCBs – polychlorinated biphenyls
PCDD/Fs – polychlorinated dibenzo-p-dioxins and furans
PCDDs – polychlorinated dibenzo-p-dioxins
PCDFs – polychlorinated furans
PeCB – pentachlorobenzene
PeCDD – pentachlorodibenzo-p-dioxin
PeCDF – pentachlorodibenzo-p-furan
POPs – persistent organic pollutants
SC – Stockholm Convention on Persistent Organic Pollutants
TCDD – tetrachlorodibenzo-p-dioxin
TCDF – tetrachlorodibenzo-p-furan
TDI – tolerable daily intake
TEF – toxic equivalency factor
TEQ – toxic equivalent
TWI – tolerable weekly intake
UNDP – United Nations Development Programme
UNECE – United Nations Economic Commission for Europe
U-POPs – unintentionally produced POPs (by-products of different processes including incineration and/or burning of halogenated materials)
US EPA – United States Environmental Protection Agency
WHO – World Health Organization
WHO-TEQ – toxic equivalent defined by a WHO expert panel in 2005
w.w. – wet weight


Areas of interests in Ukraine
N
Legend
State border
Area of interest
1
2
3
1 - Kharkiv
2 - Kryvyi Rih
3 - Mariupol
UKRAINE
MOLDOVA
Kiev
Kharkiv
Kryvyi Rih
Mariupol
Dnipro
Odesa
Kherson
Mykolaiv
Zaporizhzhia
Poltava
Kremenchuk
Cherkasy
Chernihiv
Sumy
Zhytomyr
Rivne
Lutsk
Lviv
Ternopil
Khmelnytskyi
Vinnytsia
Chernivtsi
Uzhhorod
Kropyvnytskyi
Nikopol
Melitopol
Berdiansk
Simferopol
Sevastopol
Donetsk
Luhansk
Kramatorsk
Horlivka
Taganrog
Kishinev
Tiraspol
Azov Sea
Author: Mgr. Reľovský Tomáš
Source: World Topographic Map
Month / Year: 11 / 2018
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community


Areas of interests in eastern Ukraine
Legend
Area of interest
Borders of selected oblasts of Ukraine
1
2
3
1 - Kharkiv
2 - Kryvyi Rih
3 - Mariupol
N
Author: Mgr. Reľovský Tomáš
Source: World Topographic Map
Month / Year: 11 / 2018
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community
Kharkiv
Chuhuiv
KHARKIV OBLAST
Balakliiskyi raion
Izium
Lozova
Poltava
POLTAVA OBLAST
Lubny
Myrhorod
Romny
Lebedyn
Valuyki
Bilokurakynskyi raion
Starobilskyi raion
Bilovodskyi raion
LUHANSK OBLAST
Sievierodonetsk
Lysychansk
Krasnyi Lyman
Kramatorsk
Kostiantynivka
Artemivsk
Horlivka
Stakhanov
Luhansk
Alchevsk
Krasnodon
Krasnyi Luch
Rovenky
Sverdlovsk
Yenakiieve
Shakhtarsk
Donetsk
DONETSK OBLAST
Dymytrov
Krasnoarmiiskyi raion
Pavlohrad
Dnipro
Kamianske
Synelnykove
DNIPROPETROVSK OBLAST
Kremenchuk
Znamianka
Oleksandriia
Kropyvnytskyi
KIROVOHRAD OBLAST
Zhovti Vody
Dolynskyi raion
Novobuzkyi raion
Nikopol
Zaporizhzhia
Hulaipilskyi raion
Polohivskyi raion
ZAPORIZHZHIA OBLAST
Tokmak
Volnovakha
Taganrog
Azov
Novoshakhtinsk
Berdiansk
RUSSIA
UKRAINE

2. Sampling

Samples of free-range poultry eggs were collected in three East Ukrainian industrial cities: in Kharkiv, Krivyi Rih, and Mariupol. One sample was taken from a supermarket in the city of Kyiv, considered as a background sample for Ukraine, as suggested e.g. by Dvorská [10]. The localities that were chosen were expected to be influenced by pollution from the metallurgical industry, coke production, and some other industrial activities, as well as potentially by inappropriate handling of waste. Several types of metallurgical industry facilities are listed as potential sources of dioxins (PCDD/Fs) and dioxin-like PCBs [11-16], and coke plants are included as well [14].

2.1 Description of the sites

2.1.1 Kharkiv

Kharkiv is the second largest city in Ukraine. Kharkiv is the administrative centre of the Kharkiv Region. The population of the city is about 1,440,000 inhabitants. The residents of the Kharkiv Region have suffered from air pollution for many years. About 5.3 thousand inhabitants of this region die of cancer each year, most often from lung cancer.

There are many industrial plants in the city. Among the main polluters of the city are Thermal Power Station-3, Thermal Power Station-5, the Kharkiv Tractor Plant, and the State Enterprise "Malyshev plant". The area most affected by pollution is the Novobavarskyi district in the western part of the city. There are two big industrial concerns located in this district, in the valley at the confluence of the Udy and Lopan Rivers. The first of them, Termolife Private JSC, a mineral wool plant, was put into operation in 2006. The second one, the PJSC Kharkiv Coke Plant, was built in 1932 as the state-owned Diprocoks coke research centre. The experimental laboratory ceased operation in 1952, but new owners began coke production with obsolete technology under the current name Kharkiv Coke Plant in 2003.

2.1.2 Mariupol

Mariupol is a city of regional significance in south-eastern Ukraine, situated on the north coast of the Sea of Azov at the mouth of the Kalmius River, in the Pryazovia Region. It is the tenth largest city in Ukraine, with a population of 449,498 inhabitants. A full 10% of all Ukrainian industrial production comes from Mariupol. During the 20th century, the iron and steel industries predominated in the city.

There are more than 50 large enterprises in the city. The two large metallurgical plants based in Mariupol are the Azovstal Iron and Steel Works and the Ilyich Iron and Steel Works. Established during the Soviet era, in the early 1930s, both plants are technologically obsolete, with outdated equipment lacking environmental safety controls. The Azovstal Iron and Steel Works is located right on the shore of the Sea of Azov, in the centre of Mariupol.

2.1.3 Kryvyi Rih

Kryvyi Rih is a city in the Dnipropetrovsk Region and the eighth most populous city in the country. It is a large industrial city and the development centre of the Kryvyi Rih iron ore basin. Historically, the city has been connected with metallurgy and iron mining; it is often called the metallurgical heart of the country. It produces up to 80% of Ukraine's iron ore and smelts a significant part of the iron and steel from the total amount produced in Ukraine. The total area of the city of Kryvyi Rih is 410 square kilometres, and the industrial areas occupy more than a quarter of the city.

Kriviyi Rih

Kriviyi Rih

Kharkiv

Mariupol

Kriviyi Rih

Kharkiv

Mariupol

Mariupol

Kharkiv

Kriviyi Rih

Nowadays there are five mining and processing plants, a number of quarries and mines, the largest metallurgical plant in Ukraine – PJSC ArcelorMittal Kryvyi Rih, machine-building factories, and chemical and food industries in the city. The PJSC ArcelorMittal Kryvyi Rih metallurgical plant is the source of 80% of the atmospheric emissions in the city and 40% of those in the whole Dnipropetrovsk Region. The plant was built in 1934 as the Kryvorizhstal complex (Kryvyi Rih Metallurgical Works), privatised in June 2004, and then sold in June 2005 to the global company Mittal Steel. In 2007, the plant was renamed PJSC ArcelorMittal Kryvyi Rih. Exporting to more than 160 countries, Ukraine was the world's sixth largest steel exporter in 2016.

2.2 Sampling and Analytical Methods

To obtain representative samples, pooled egg samples were collected at each of the selected sampling sites. All the sampled eggs originate from free-range poultry, with the exception of the eggs bought in the supermarket. Table 1 summarizes the basic data about the sample size and measured levels of fat content in each of the pool samples.

Three pool samples of eggs were taken and analysed in total, plus the one sample taken in Kyiv, where we bought hens' eggs in a supermarket. The last one of the above-mentioned samples is used to exhibit background levels of POPs, as suggested by Dvorská [10]. All the samples were taken in May 2018. Two samples from Kharkiv and Kryvyi Rih were from free-range hens, and one sample from Mariupol was of turkey eggs. This difference could have influenced the final results as different species might have different uptakes of certain contaminants, which is an aspect that has been studied more in relation to the milk from ruminants [17-19] than in poultry eggs or meat [20].

Table 1: Overview of poultry egg samples from selected sites in Ukraine.

No	Sample(s)	Locality	Month/Year of sampling	Eggs in pooled samples	Fat content
1	KH-E-01, KH-E-02	Kharkiv	05/2018	16	13.0
2	MA-E-01	Mariupol	05/2018	10	12.4
3	KR-E-01	Kryvyi Rih	05/2018	5	9.9
4	Kyiv – supermarket	Kyiv – supermarket	05/2018	9	10.2

We sampled eggs from two free-range hen anciers in Kharkiv, eight eggs from each. Since both were very close to each other (approximately 200 metres), we decided to perform an analysis of all the eggs as one pooled sample together. They were taken approximately at a distance of 1-1.5 km from the Kharkiv coke plant, in a south-westerly direction from the plant.

Ten turkey eggs from one poultry fancier in Mariupol were sampled. It location was approximately seven to eight hundred metres north of the Azovstal iron and steel works, just across the valley of the Kalmius River.

Five free-range hens' eggs were sampled in Kryvyi Rih from a family raising hens in their garden. The location of the sampling site was 0.8-1 km to the west or slightly south-west of the ArcelorMittal steelworks.

The eggs that were sampled were collected into typical plastic egg packaging. They were boiled for approximately seven minutes right after the sampling. The cooled eggs were then stored in a refrigerator and also kept in cold conditions during their journey to the laboratory. In the first laboratory the edible parts of the eggs were homogenized and the same pool sample of homogenized eggs was used for analyses in both laboratories.

All the samples were analysed for their content of individual PCDD/Fs and dioxin-like PCBs (DL PCBs) by GC/HRMS in an ISO 17025 accredited laboratory at the State Veterinary Institute, an accredited laboratory in Prague, Czech Republic, with a resolution >10,000 using ^{13}C isotope labelled standards. The PCDD/F and dl-PCB analysis followed the European Union's methods of analysis for checks on levels of PCDD/Fs and dl-PCBs for levels in certain foodstuffs in Commission Regulation (EC) No 252/2012 [21].

The samples were also analysed for their content of indicator congeners of PCBs (iPCBs), OCPs, PeCB, and HCBD in a certified Czech laboratory (Institute of Chemical Technology, Department of Food Chemistry and Analysis). The analytes were extracted by a mixture of organic solvents, 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.

3. The Ukrainian, Russian, and EU Limits for POPs in Eggs

Poultry eggs are a common component of the diet in almost every country of the world. Their proportion within the dietary basket differs from country to country. Ukraine belongs among those countries with a higher level of egg consumption (1.7%) in comparison with other countries, e.g. Armenia or Kazakhstan, where the proportion is below 1% of the total dietary basket. Their share of the diet increased between 1997 and 2007 [22]. It is necessary to notice that we do not have more recent data, so we use the figure for 2007, which is 39 g of egg per person per day. It is also common practice for Ukrainian people to raise their own poultry.

There are set limit values for certain POPs in poultry eggs in Ukraine [23, 24], more specifically for PCDD/Fs, PCBs, DDT, and lindane. The limits for PCDD/Fs and PCBs are the same as those set in the EU [25]. For comparison with some other countries from the former Soviet Union, we also used the limits set in Armenia. The limit values we used for free-range poultry eggs are summarized in Table 2.

Table 2: Limit concentration values for OCPs, PCBs, and PCDD/Fs TEQs in poultry eggs.

	Hens' eggs			
	Ukrainian[1,2]	**Armenian[3]**	**EU ML[4]/MRL[5]**	
Unit	pg g^{-1} fat	pg g^{-1} fat	pg g^{-1} fat	ng g^{-1} fat
WHO-PCDD/Fs TEQ	2.5[1]	3.0	2.5	–
WHO-PCDD/Fs-dl-PCB TEQ	5.0[1]	None	5.0	–
PCBs[6]	40 (ng g^{-1})[1]	None	–	40
	ng g^{-1} fresh weight			
DDT[7]	100[2]	100	–	50 (fresh) [9]
γ-HCH (lindane)	100[2]	None	–	10 fresh
α-, β-HCH[**]	None	100	–	20, 10
HCH[10]	None	None	–	None
HCB	None	None	–	20 (fresh)

1 Державні гігієнічні правила і норми «Регламент максимальних рівнів окремих забруднюючих речовин у харчових продуктах», наказ Міністерства охорони здоров'я України 13.05.2013 № 368. [24]

2 «Медико-биологические требования и санитарные нормы качества продовольственного сырья и пищевых продуктов», утверждено заместителем Министра здравоохранения СССР Кондрусев А. И. от 01.08.1989 г. № 5061-89. [23]

3 Hygienic Requirements for Food Raw Material and Food Value: Hygienic Guidelines N 2-III-4.9-01-2010 [26].

4 EU Regulation (EC) N°1259/2011 [25] sets maximum levels for dioxins, dioxin-like PCBs, and non-dioxin-like PCBs in foodstuffs.

5 Regulation (EC) N°149/2008 [27]. The maximum residue level (MRL) means the upper legal level of a concentration for a pesticide residue in

or on food or feed set in accordance with the Regulation, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers.

[6] sum of PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180.

[7] sum of p,p´-DDT, o,p´-DDT, p,p´-DDE, o,p´-DDE, p,p´-DDD, and o,p´-DDD.

[8] p,p´-DDT.

[9] sum of p,p´-DDT, o,p´-DDT, p,p´-DDE, and p,p´-DDD.

[10] sum of HCH-alpha, HCH-beta, HCH-gamma, and HCH-delta.

** for each isomer is MRL set separately.

4. Results

The pooled samples of eggs were analysed for OCPs and U-POPs. GCMS-HRMS analyses were chosen for the confirmation of contamination by dioxins and dioxin-like PCBs of the sampled poultry eggs. The same samples were also analysed for other POPs (including indicator PCBs) and OCPs: hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), and DDT and its metabolites. HCB is also considered to be unintentionally produced POP (U-POP) via the same processes as dioxins and DL PCBs [13], although it is commonly measured together with other OCPs. Also, two other U-POPs, pentachlorobenzene (PeCB) and hexachlorobutadiene (HCBD), were analysed in all the samples. The results for U-POPs and OCPs are summarised in Table 3.

4.1 Unintentionally produced POPs

4.1.1 Dioxins (PCDD/Fs) and PCBs

Dioxins belong to 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). The other PCBs do not exhibit dioxin-like toxicity but have a different toxicological profile and are referred to as 'non-dioxin-like PCB' (NDL PCBs) [25]. Levels of PCDD/Fs and DL PCBs are expressed in total WHO-TEQ calculated according to their toxic equivalency factors (TEFs), set by a WHO expert panel in 2005 [28]. These new TEFs were used to evaluate dioxin-like toxicity in samples of poultry eggs from Ukraine in this study.

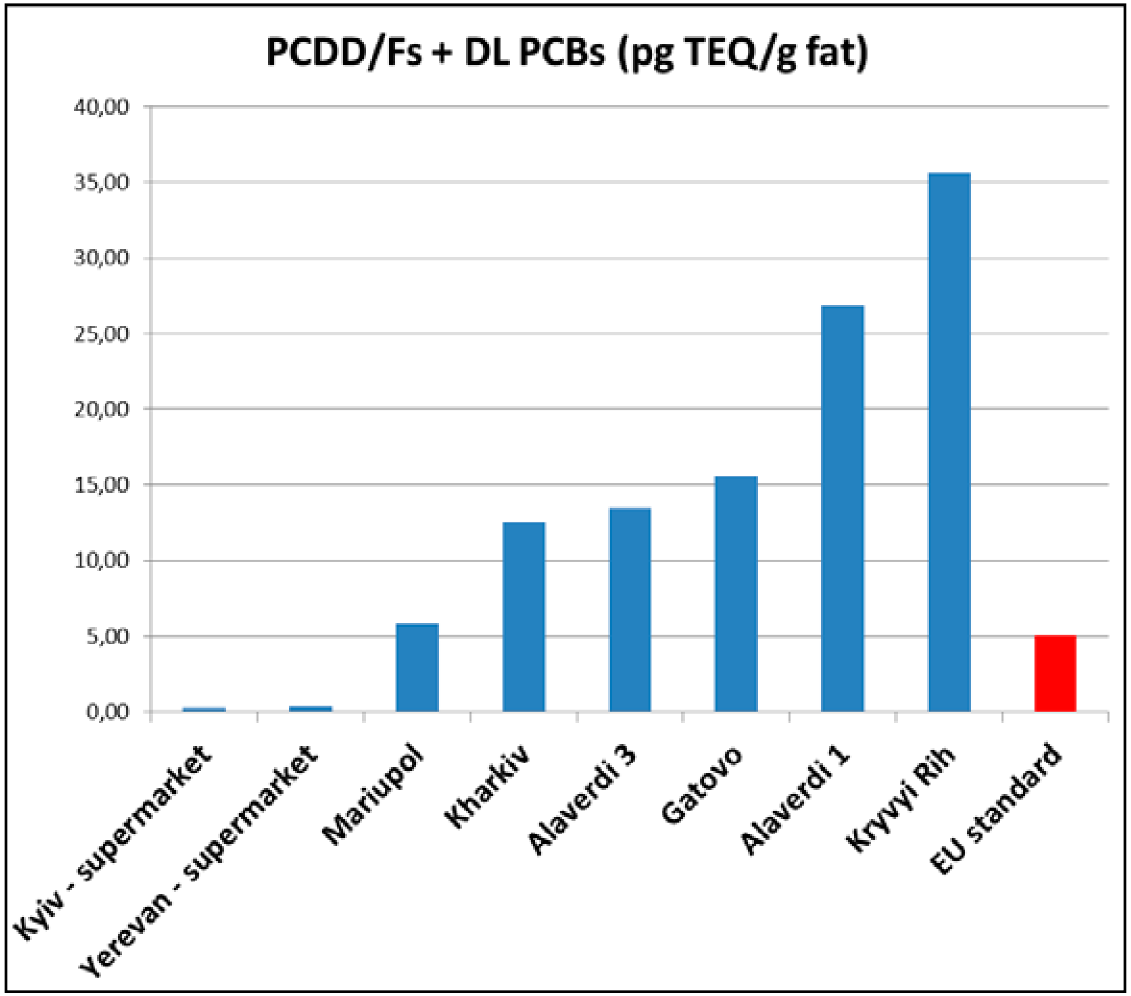
Table 3: Summarised results of analyses for POPs for four pooled poultry eggs samples from Ukraine, sampled in May 2018.

Locality	Kharkiv	Mariupol	Kryvyi Rih	Kyiv	Ukraine/EU standards/ limits
Sample	KH-E-01/KH-E-02	MA-E-01	KR-E-01	Kyjev – supermarket	
Fat content (%)	13.0	12.4	9.9	10.2	-
PCDD/Fs (pg WHO TEQ g^{-1} fat)	3.39	2.07	23.30	0.25	2.50
DL PCBs (pg WHO TEQ g^{-1} fat)	9.16	3.75	12.32	0.03	-
Total PCDD/F + DL PCBs (pg WHO TEQ g^{-1} fat)	12.56	5.82	35.62	0.28	5.00
HCB (ng g^{-1} fat)	3.76	1.68	4.52	0.95	-
PeCB (ng g^{-1} fat)	0.65	< 0.1	0.729	< 0.1	-
HCBD (ng g^{-1} fat)	< 0.1	< 0.1	< 0.1	< 0.1	-
7 PCB (ng g^{-1} fat)	46.37	12.07	39.69	0.69	-
6 PCB (ng g^{-1} fat)	30.67	9.05	27.29	0.69	40.00
Sum of HCH (ng g^{-1} fat)	22.78	9.90	90.58	1.70	-
Sum of DDT (ng g^{-1} fat)	244.23	116.22	491.84	0.25	-

ND – not defined

All three free-range poultry egg samples from Ukraine exceeded the ML of PCDD/Fs and DL PCBs, expressed as WHO TEQ in poultry eggs (see Table 3 and the graph in Figure 1); [24, 25]. The samples of hens' eggs from Kharkiv and Kryvyi Rih also exceeded ML of PCDD/Fs, which is set at 2.5 pg WHO-TEQ g^{-1} fat. The background levels for PCDD/Fs and DL PCBs measured in poultry eggs from a supermarket in Kyiv were 0.25 and 0.03 pg WHO-TEQ g^{-1} fat, respectively. The highest levels of dioxins (23.30 pg WHO-TEQ g^{-1} fat) and DL PCBs (12.32 pg WHO TEQ g-1 fat), respectively, were measured in eggs from Kryvyi Rih, sampled near the ArcelorMittal metallurgical plant.

Figure 1: The graph compares levels of PCDD/Fs and DL PCBs in pooled egg samples from three different localities on which this study focused and also in eggs from Alaverdi, Armenia [29] and Gatovo, Belarus [30], which were analysed recently. The PCDD/Fs + DL PCBs content in the eggs is expressed in pg WHO-TEQ/g fat. In Alaverdi there is a metallurgical plant, while in Gatovo there is a car shredder.



The total WHO-TEQ level of PCDD/Fs and DL PCBs in the sample from Kharkiv is comparable with the levels in the samples from the Alaverdi 3 or Gatovo sites. It is also at a comparable level to that observed in eggs from the vicinity of a waste incinerator in Wuhan, China [31]. The level in Kryvyi Rih is the highest among the chosen group[1] of samples from the former Soviet Union countries in the graph in Figure 1. It also belongs among the significantly high levels in free-range hens' eggs generally, and shows comparable levels of PCDD/Fs + DL PCBs to eggs from areas similarly polluted by the metallurgical industry in Beihai, China (12-37 pg BEQ/g)[2] [32]or in Balkhash, Kazakhstan (13-30 pg WHO-TEQ/g fat) [31]. The level in the eggs from Kryvyi Rih of almost 36 pg WHO-TEQ/g fat represents a rather higher level in comparison with the results presented in IPEN's global Egg Report [3], and can be compared with levels observed in such hot spots as very wild landfills in African countries at Mbeubeuss, Senegal [33] or Dandora in Nairobi, Kenya [34], with levels of 39 and 31 pg WHO-TEQ/g fat respectively. It is also close to the level observed recently in free-range poultry eggs at a site seriously contaminated by wood treated with pentachlorophenol in Poland [35, 36]. PCDD/Fs occurred as by-products in pentachlorophenol production and then also contaminated treated wood [35, 37].

The level of PCDD/Fs and DL PCBs in the eggs from Mariupol is somewhat lower in comparison with the other samples, although it was sampled close to metallurgical plants, as were the other samples. We have to bear in mind that these are not hens' but turkey eggs, and turkeys might show different uptake of POP contaminants and dioxins and dioxin-like PCBs in particular. Peterson et al. [38] found, for example, a difference between the toxicity of PCBs for chicken and turkey embryos: "*Tetrachlorobiphenyl (TCB) was 20-100 times more toxic in chicken embryos than in turkey embryos when injected into eggs.*" This difference shows that there might be more differences regarding the availability of POPs and their impact on different poultry species. The bioavailability is also influenced by different foraging habits or the ability of the birds to excrete and/or bioaccumulate POPs [39, 40].

All the free-range poultry egg samples from East Ukrainian industrial cities had levels of PCDD/Fs and DL PCBs higher than those observed in the pool sample of eggs bought in a Kyiv supermarket (see also the graph in Figure 1 for comparison), which is used as a control sample showing background levels in poultry eggs from Ukraine for this study. On this topic, see also the discussion about background levels in other studies focused on POPs in free-range poultry eggs [3, 30].

4.1.2 Hexachlorobenzene, pentachlorobenzene, and hexachlorobutadiene

Hexachlorobenzene (HCB), pentachlorobenzene (PeCB), and hexachlorobutadiene (HCBD) are three further chemicals listed as unintentionally produced POPs under Annex C to the Stockholm

[1] We have chosen results for samples taken by different organisations participating in IPEN and published in previous studies within the last three years.

[2] Result of analyses by bioanalytical methods. ''Bioanalytical methods'' means methods based on the use of biological principles such as cell-based assays, receptor assays, or immunoassays. They do not give results at the congener level but merely an indication of the TEQ level, expressed in Bioanalytical Equivalents (BEQ) to acknowledge the fact that not all the compounds present in a sample extract that produces a response in the test may obey all requirements of the TEQ-principle. 21. European Commission, *Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods for the sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs, and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006 Text with EEA relevance* European Commission, Editor. 2012: Official Journal of the European Communities. p. L 84, 23.3.2012, pp. 1–22.

Convention. They are also listed under Annex A to the Convention as they were also produced intentionally and mostly used as pesticides in the past [12]. Only HCB and PeCB were measured at levels above LOQ in the pooled egg samples from Ukraine in this study, while HCBD was below LOQ in all samples (see Table 3). The levels of pentachlorobenzene do not seem to be significant, although there is not much data available about PeCB in poultry eggs. Observed levels of HCB are discussed in Chapter 4.3, Organochlorinated pesticides.

4.2 Polychlorinated biphenyls (PCBs)

A basic description of PCBs is given in Chapter 4.1.1. They are listed under the Stockholm Convention in two different annexes because this group includes both unintentionally produced chemicals (12 PCB congeners) and those produced and used intentionally in a variety of applications, among which those best known are PCBs containing oils used in transformers and capacitors. Unintentionally produced PCBs are listed under Annex C to the Stockholm Convention, and intentionally produced PCBs under Annex A, and they are forbidden to be newly produced and used in new applications and appliances [12]. Contamination by intentionally produced and used PCBs is represented by the level of what are termed indicator congeners of PCBs or NDL PCBs (see Chapter 4.1.1 for an explanation).

Indicator PCB congeners are present at levels higher than observed at industrial sites in, for example, Thailand [31]. They are comparable with the levels at some sites in Kazakhstan, in the Mangystau region in particular [30]. None of the egg samples from Eastern Ukraine exceeded the EU limit set for six indicator PCBs in eggs. However, the samples from Kharkiv and Kryvyi Rih reached ¾ of ML, which is 40 ng g-1 fat [24, 25]; see Table 3. They are also higher than the level observed recently in a sample from the Alaverdi 1 site in Armenia [29] but much lower than the samples from the most contaminated sites in Central Kazakhstan [31]. Analysis found the highest level of indicator PCBs in the eggs from Kharkiv, almost 31 ng/g fat.

4.3 Organochlorinated pesticides

Dichlorodiphenyltrichloroethane (DDT) and its metabolites, HCB and the hexachlorocyklohexane (HCH) isomers α-HCH, β-HCH, and γ-HCH were chemicals from the group of organochlorinated pesticides that were analysed in pooled egg samples in this study. The results are summarised in Tables 3 (expressed per gram of fat) and 4 (expressed per gram of fresh weight of eggs). The pooled egg sample from Kryvyi Rih nearly exceeded the EU limit for DDT and its metabolites (see Table 4). The level of β-HCH almost reached the EU limit value in the same egg sample but it is well below the Armenian limit for the sum of HCH, as well as below the Ukrainian or EU standards for lindane (γ-HCH).

Table 4: Summarised results of analyses for OCPs for four pooled egg samples from Ukraine. Comparison with EU [27], Ukrainian [23], and Armenian [26] limit values. These results are expressed in ng g^{-1} fresh weight because all the legislative limits are set for fresh weight for OCPs.

Locality	Kharkiv	Mariupol	Kryvyi Rih	Kyiv	EU*/Ukrainian/ Armenian** standard
Sample	KH-E-01/KH-E-02	MA-E-01	KR-E-01	Kyiv – supermarket	
HCB	0.49	0.21	0.45	0.10	20.0*
α-HCH	0.24	0.19	0.19	0.11	20.0*
γ-HCH	0.06	0.03	0.15	0.00	10.0*(100)
β-HCH	2.65	1.01	8.63	0.07	10.0*
sum-4DDT (EU)	31.63	14.46	48.64	0.03	50.0*
sum HCH	2.95	1.23	8.97	0.17	100.0**
sum DDT	31.63	14.46	48.69	0.03	100.0

DDT had a long history in former Soviet Union countries, and in Ukraine as well. It was also produced in Ukraine: "*DDT was among the pesticides most widely used in agriculture and medicine in all the oblasts of Ukraine from the late fifties to 1990. DDT was manufactured at the RADICAL plant in Kiev in 1954-1975. Its DDT (active ingredient) manufacturing capacity was:*

1,000 tons per year in 1954-1960
4,000 tons per year in 1960-1970
7,500 tons per year in 1970-1975.

DDT-containing substances manufactured at the RADICAL plant were supplied to the Ukrainian agricultural sector, as well as to the former Soviet republics in Central Asia and abroad. The use of DDT in medicine was prohibited from 1989 by an Order of the Ministry of Health of the USSR. According to 2006 inventory data, a total of 1,744.2 tons of DDT are stored in various oblasts of Ukraine. The largest amount of DDT (800 tons) is stored in the Odessa oblast." [16].

HCB is another OCP followed in eggs from Ukraine in this study. Its levels in eggs were well below the EU MRL. The levels in hens' eggs from

Kryvyi Rih and Kharkiv were somewhat higher than in turkey eggs from Mariupol. The levels in eggs from Kryvyi Rih and Kharkiv were also comparable to those observed in free-range hens' egg samples from some Kazakhstani hot spots analysed in Arnika's study in 2017 [30].

5. Discussion about potential exposure to dioxins and dioxin-like PCBs from poultry eggs

The share of eggs in total food consumption in Ukraine in 2007 was close to 1.7% of the total food basket per day, according to the World Atlas – Food Security data[3] [22]. Its share changed rapidly between 1997 and 2007; it increased. Since we do not know about further developments, we used the data valid for 2007, which was 36 g of eggs per person per day. We have to take it as one of the limitations of this study. If we count 50 g per poultry egg as average weight, it would mean the consumption of 2/3 of an egg per person per day as a general consumption pattern for the current Ukrainian population.

We tried to calculate the dietary intake for the group of PCDD/Fs plus DL PCBs contaminants per day for the three pooled free-range poultry egg samples from industrialised areas in Eastern Ukraine and compare it with eggs from the Kyiv supermarket, considered as the background country level in Ukraine. The calculation of daily intake levels was performed by using the following formula:

$$DI_{adult} = (((C \, . \, F\%)/100) \, . \, 36)/70;$$
$$DI_{child} = (((C \, . \, F\%)/100) \, . \, 36)/35,$$

where DI = daily intake; C = concentration of certain groups of chemicals (PCDD/Fs, DL PCBs etc.), and F% = fat content in sample. Explanation of the figures used in the calculation of the formula: 36 stands for 36 g of eggs consumed per day in Ukraine, 70 and 35 are the body weights for an adult person and child respectively. The results are summarised in Table 7.

[3] The food consumption refers to the amount of food available for human consumption as estimated by the FAO Food Balance Sheets. However the actual food consumption may be lower than the quantity shown as food availability, depending on the magnitude of wastage and losses of food in the household. Food consumption per person is the amount of food, in terms of quantity, for each individual in the total population. Food from eggs also includes the quantity of eggs used for the preparation of food such as bakery products.

Table 7: Summarised results of the calculation of dietary intake of selected POPs by eating a daily portion of eggs (36 g) from poultry raised in Kharkiv, Mariupol, and Kryvyi Rih and eggs bought in a supermarket in Kyiv from poultry raised on a commercial farm.

Locality	Kharkiv	Mariupol	Kryvyi Rih	Kyiv – supermarket	Suggested levels
Fat content	13.0	12.4	9.9	10.2	–
PCDD/Fs + DL PCBs (pg WHO-TEQ g^{-1})	12.56	5.82	35.62	0.28	5*
PCDD/Fs + DL PCBs (pg kg^{-1} bw) – adult	0.84	0.37	1.81	0.01	0.29**
PCDD/Fs + DL PCBs (pg kg^{-1} bw) – child	1.67	0.74	3.63	0.03	0.29**
Percentage of derived TDI – adult	292.67%	130.21%	634.71%	5.14%	–
Percentage of derived TDI – child	585.34%	260.42%	1269.42%	10.28%	–

* EU Regulation (EC) NºNo 1881/2006 as amended by later regulations [41] sets maximum levels for dioxins, dioxin-like PCBs, and non dioxin-like PCBs in foodstuffs.

** TDI derived from TWI suggested by EFSA [42] – calculated as one seventh of the 2 pg WHO-TEQ/kg body weight/week, although in the text of the EFSA opinion 0.25 pg WHO-TEQ pg/kg bw/day is suggested.

The results were then compared with the tolerable daily intake (TDI) derived from the tolerable weekly intake newly established in the EU by EFSA CONTAM at a level of 2 pg WHO-TEQ/kg bw/week [42]. The consumption of dioxins and dioxin-like PCBs in all three egg samples leads to the exceeding of the suggested TWI by their consumers. This is a very alarming result if we consider that PCDD/Fs and DL PCBs are surely also present in other food consumed by the inhabitants of these industrialised cities. The sources of PCDD/Fs and DL PCBs should be addressed in these regions.

The level by which the derived TDI level is exceeded in the sample from Kryvyi Rih is outrageous, although we know that this pooled sample does not represent all the poultry raised in the city area. However, it requires further research, at least including measurement or expert estimation of dioxin releases from local industries.

There is a mixture of potential sources of PCDD/Fs and DL PCBs in each of the cities that were studied, so it can be hard to find the most significant ones, but the metallurgical industry, and iron sintering plants and smelters are listed among the major potential sources of dioxins and other unintentionally produced POPs in Annex C to the Stockholm Convention [11-16]. Coke plants can contribute to dioxin contamination as well [14]. The open burning of waste or using waste in stoves for heating in the area should also be researched. However, we do not consider it a significant factor in the contamination of the sampled eggs as we managed to avoid sampling in households where such practices occur. There is also a large cement kiln in Kryvyi Rih, so checks should be performed on whether co-incineration of waste is a potentially valid source of dioxins there. Cement kilns co-incinerating hazardous wastes are also listed among the major sources of unintentionally produced POPs according to Annex C of the Stockholm Convention [12].

"Total annual amounts of PCDDs/PCDFs released in Ukraine are estimated as 2,516.5 g of TEQ in 1990 and 1,451.4 g of TEQ in 2002, of which ferrous and non-ferrous metallurgy, electricity, and heat power production make up 95%." [16]

6. Conclusions and recommendations

This study has discovered serious contamination of free-range poultry egg samples by dioxins and dioxin-like PCBs in the industrial cities of Kharkiv, Mariupol, and Kryvyi Rih. The most serious situation seems to be that in Kryvyi RIh; however, our sampling was like screening rather than real monitoring because of the financial limitations of the project. To get a more complex picture requires broader sampling in the area.

We also found increased levels of some OCPs in free-range hens' eggs, and DDT and its metabolites in particular. This is another issue which should be addressed. Potential sources have to be found and better control of animal feed and food sources for OCPs is needed.

It can be expected that contamination with POPs may also occur in other home-grown food sources in Eastern Ukraine and it can pose a serious risk to the health of the population in industrial cities in particular. Of course, this assumption has to be confirmed by much broader monitoring of PCDD/Fs and DL PCBs in local food.

Sources of pollution by dioxins and dioxin-like PCBs should follow some basic suggestions for improvement of the technology at least. The best available techniques and best environmental practices in the metallurgy sector, as well as other sectors, are described in the BAT/BEP Guidelines for Annex C major dioxin sources [13]. Reducing dust emissions is one of the first steps.

Other POPs such as PeCB, HCB, HCBD, or HCH analysed in poultry eggs from the cities that were studied were not found in levels raising serious health concerns for the local population.

We used poultry eggs as they are a proven indicator of potential contamination within the food chain. We did not sample meat but the results of some other studies demonstrated the simultaneous contamination of poultry eggs and meat from contaminated sites [43, 44].

7. Limitations of the study

The major limitations of the study were the limited financial, temporal, and personnel resources. Therefore, only a limited number of poultry egg samples could be taken, and only a limited scale of analyses could be conducted. We could not include more localities in this study, for example, or conduct broader sampling in the cities that were studied. We preferred to focus on one locality and to take pooled egg samples, which gives a better picture than analysing single eggs when financial resources for analyses are scarce.

The comparison of pollutant concentration levels found in the samples with legal standards also has its limitations. Each of the legal standards is defined in a different way, and for a different purpose. In addition, there are no existing legal standards for some of the pollutants and some legal limits.

The TDI level for PCDD/Fs and DL PCBs has changed recently in the EU; however, it is not established in Ukraine or its neighbouring countries. The standards set for certain types of food do not necessarily follow this new concept set by EFSA CONTAM during the time when we worked on this study, and published in November 2018 [42].

The estimation of the potential risk to humans and the environment cannot be conducted only by consulting legal standards; an extensive risk analysis based on a sufficient number of samples and a detailed description of the state of the area and the potential risk receivers is crucial. We tried to draw up a basic evaluation of the health risk expressed as the daily intake of PCDD/Fs and DL PCBs through the consumption of free-range poultry eggs from three sites in Eastern Ukraine, in order to give at least a basic idea about the level of human exposure to these pollutants.

We believe that it is of the utmost importance to begin to address the overall pollution by such contaminants as PCDD/Fs or PCBs in Ukraine.

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