

# Persistent Organic Pollutants in Free-Range Chicken Eggs from Moldova

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## 1 Introduction

This study is focused on the evaluation of the levels of persistent organic pollutants (POPs) measured in three pooled egg samples of free-range chicken eggs from three Moldovan villages, Ciobanovca, Balti, and Dumbrava that are potentially affected by near potential POPs sources such as landfills, chemical industry using combustion technology and many obsolete pesticide warehouses. Our investigation is focused on the assessment of contamination by chemicals which were either listed in the Annexes to the Stockholm Convention on POPs or fall into the larger group of chemicals with POP characteristics. The chemicals that were investigated fall into three groups:

1. additives to plastics, textile consumer products and paper food packaging such as brominated flame retardants (BFRs), per- and polyfluoroalkyl substances (PFAS), short-chain chlorinated paraffins (SCCPs)
2. unintentionally produced POPs (UPOPs) as polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (PCBs), pentachlorobenzene (PeCB), and hexachlorobenzene (HCB)
3. intentionally used POPs such as organochlorine pesticides (OCPs) including DDT, hexachlorocyclohexane (HCH) etc. and/or polychlorinated biphenyls (PCBs).

## 2 Materials and Methods

Pooled samples of six individual egg samples were collected at each of the selected sampling sites in the villages of Ciobanovca (near the Tintareni landfill), Balti (near the Balti landfill), and Dumbrava (near Vatra industrial area with tire pyrolysis technology and asphalt production) in order to obtain more representative samples. We also used a sample of pooled eggs sample from Kyiv – a supermarket in Kyiv as a reference sample to exhibit background levels of POPs, following precedents from other studies <sup>8,9</sup> [8, 9].

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 <sup>13</sup>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 to the EU regulation<sup>10</sup> [10]. The results are presented in pg WHO TEQ/g of fat. TEFs defined in 2005<sup>11</sup> [11] were used to evaluate dioxin toxicity in samples. Analyses of PBDEs, HBCD, 17 PFAS, including PFOA, PFOS and PFHxS, organochlorine pesticides (DDT and its metabolites, three HCH isomers), PeCB, HCB, hexachlorobutadiene (HCBd), and seven indicator PCB congeners were conducted in a Czech certified laboratory at the University of Chemistry and Technology in Prague. The identification and quantification of the analyte was conducted by GC-MS/MS detection in electron ionization mode for OCPs, HCB, PeCB, HCBd, and indicator PCBs. The identification and quantification of SCCPs were performed via GC/TOF-HRMS in the mode of negative chemical ionisation (NCI). The analysis of PBDEs were conducted by GC-MS-NICI. The identification and quantification of HBCD isomers and selected PFAS were performed by UHPLC-MS/MS-ESI.

## 3 Results

Levels of the individual and grouped POPs measured in three free-range chicken eggs from three studied locations Ciobanovca, Balti, and Dumbrava are summarized in the Table 1. It also shows the results of measurements for reference sample from the supermarket in Kyiv. PCDD/Fs were measured together with twelve dl PCBs and expressed in toxic equivalence levels (TEQ) in all the egg samples and compared with the EU standards for food. The analytical results show low levels or levels below LOQ for PBDEs, SCCPs, and HCBd, HBCD, 6 novel BFRs and PFAS.

Table 1: Summary of the results for the individual and grouped POPs measured in three pooled egg samples from Moldova, and the reference egg sample from the supermarket in Kyiv with the EU maximum residue levels (EU MRL) for the selected OCPs values

Locality		Ciobanovca	Balti	Dumbrava	Reference
Sample ID	Units	TIN-EGG-1	BAL-EGG-1	VAT-EGG-1	Kyiv – sup.
Fat content	%	11.5%	11.6%	11.7%	10.2%
PeCB	ng/g fat	16.57	0.60	0.42	<0.10
HCB	ng/g fat	2.31	1.73	1.59	0.95
HCBD	ng/g fat	<0.10	<0.10	<0.10	<0.10
Sum HCH	ng/g fat	3,005	3.84	5.56	1.70
Sum DDT	ng/g fat	554	47	50	0.25
6 iPCBs*	ng/g fat	144	2.2	209	0.69
7 iPCBs	ng/g fat	222	3.08	229	0.69
SCCP C10-C13	ng/g fat	<50.0	<50.0	<50.0	NA
Sum PBDEs	ng/g fat	<LOQ	<LOQ	<LOQ	<LOQ
Sum HBCD	ng/g fat	4.77	<LOQ	14.75	NA
6 nBFRs	ng/g fat	<LOQ	0.341	<LOQ	<LOQ
Sum PFASs	ng/g	0.76	0.26	0.38	NA
PCDD/Fs	pg TEQ/g fat	5.09	1.29	4.81	0.25
dl PCBs	pg TEQ/g fat	51.95	3.13	12.7	0.03
Total PCDD/F + dl PCBs	pg TEQ/g fat	57.04	4.42	17.51	0.28
					EU MRL
HCB	ng/g	0.27	0.20	0.19	20
α-HCH	ng/g	156	0.12	0.16	20
β-HCH	ng/g	177	0.28	0.41	10
γ-HCH	ng/g	13.03	0.04	0.08	10
sum 4 DDT	ng/g	63.62	5.47	5.80	50

NA = not analysed; <LOQ = below level of quantification; for PCDD/F and dl PCB congeners half of LOQ was counted in total levels in the case of congeners below LOQs; \* sum of PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180; PCB 118 is in 7 iPCBs congeners in addition.

The Figure 1 shows the percentage contribution of PCDD/Fs congeners in the 3 samples of free-chicken eggs. The dioxin congener profiles for the eggs from Ciobanovca, Balti, and Dumbrava vary greatly between the samples, showing that the potential sources of pollution with these chemicals are most probably also different at all three sites.

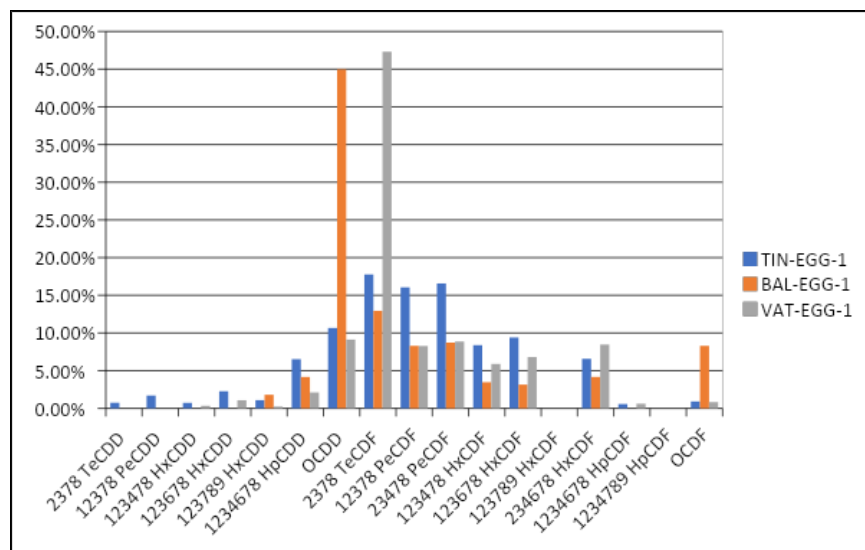


Figure 1: Graph showing the percentage contribution of PCDD/F congeners to their total level, expressed in pg/g fat.

#### 4 Discussion

**PCBs:** The level of six indicator PCB congeners (iPCBs) in the free-range chicken eggs from Ciobanovca and Dumbrava exceeded the EU maximum limit of 40 ng/g fat<sup>19</sup> [19] by almost four- and more than fivefold, respectively (see Table 1). In the eggs from Dumbrava it is well below that limit and it is slightly above the level in the reference egg sample from Kyiv; however, it is lower in comparison to the level of 13 ng/g fat in eggs from a large farm obtained in a supermarket in Prague<sup>20</sup> [20]. The levels of iPCBs in the eggs from Ciobanovca and Dumbrava are comparable to those observed in eggs from some locations in central Kazakhstan, e.g. Rostovka or Shabanbai Bi<sup>21,22</sup> [21, 22], but are much higher than the levels found in eggs from certain localities in Ukraine, Armenia, and Balkan countries<sup>23</sup>[23] or south-western Kazakhstan<sup>24</sup>[24]. PCBs were not found in the transformers in Moldova that were checked; however, they can rather be found in old capacitors, according to the Moldovan NIP from 2004<sup>4</sup> [4]. The levels of PCBs in the eggs from Ciobanovca and Dumbrava indicate the influence of some potential PCB sources near the sampling sites.

**Dioxins (PCDD/Fs) and UPOPs:** Dioxins exceeded the EU standard set at the level of 2.5 pg TEQ/g fat<sup>19</sup> [19] twofold in the samples from Ciobanovca and Dumbrava, while in the sample from Balti they reached half of it. There were very high levels of dl PCBs, with almost 52 pg TEQ/g fat measured in the sample from Ciobanovca, and the total TEQ level exceeded the EU standard of 5 pg TEQ/g fat<sup>19</sup>[19] by more than ten times in this sample. The sample from Dumbrava also had a relatively high content of dl PCBs and contributed significantly to a 3.5-fold exceeding of the EU standard. The sample from Balti did not exceed the EU standard for PCDD/Fs + dl PCBs. All three free-range chicken eggs samples had PCDD/Fs and dl PCBs levels that were many times higher in comparison with the reference samples from supermarkets in Kyiv and Prague<sup>20</sup> [20]. The indicator and dioxin-like PCB levels in Ciobanovca seems to be somewhat similar to the Shabanbai Bi location in Kazakhstan, where we also found high levels of both indicator and dl PCB congeners in free-range chicken eggs.

The sample of eggs from Balti, with a dominant OCDD congener, is very similar to the profile observed in eggs from Bangun, Indonesia<sup>26</sup>[26], where plastic waste is often burned. The profile of PCDD/F congeners in the eggs from Dumbrava is closer to the waste incineration one demonstrated in a study from China<sup>27</sup>[27]. We consider tire pyrolysis to be a potential source of the contamination of the eggs from Dumbrava.

An increased level of PeCB (16.57 ng/g fat) was measured in the eggs from Ciobanovca, much higher in comparison with the eggs from Balti and Dumbrava (see Table 1). This level is close to the 22 ng/g fat of PeCB measured in eggs from the Agbogloshie scrapyards in Ghana in 2018<sup>28</sup> [28]. This relatively high level can also be the result of a potential hidden hotspot of obsolete pesticides, as PeCB was used for pesticide production in the past as well. PeCB is also present at low levels as an impurity in several herbicides, pesticides, and fungicides<sup>29</sup>[29]. The PeCB levels in the eggs from Balti were lower than the level of PeCB measured in eggs from a supermarket in Karaganda<sup>22</sup>[22].

**OCPs:** The high levels of some OCPs measured in the egg sample from Ciobanovca, where the highest level was observed for the sum of three HCH isomers in that sample, among which the  $\alpha$ - and  $\beta$ - isomers had much higher levels than the  $\gamma$ - isomer (lindane). The  $\beta$ -HCH level of 1,536 ng/g fat in free-range chicken egg from Ciobanovca is comparable to the level of 1,800 ng/g fat measured in free-range chicken eggs from the vicinity of the obsolete plant for the production of lindane in Porto Romano, Albania<sup>12</sup> [12].

The sum of DDT and its metabolites (554 ng/g fat) was also very high in the egg sample from Ciobanovca, ten times higher than in the eggs from Balti and Dumbrava and was comparable to the levels of 547 and 491 ng/g fat measured in samples of eggs from Kovachevo, Bulgaria in 2005<sup>13</sup> [13] and Kryvyi Rih, Ukraine in 2018<sup>14</sup> [14]. The level of the sum of DDT in the eggs from Ciobanovca is lower in comparison to those from some other locations, such as e.g. Peshawar in Pakistan, Helwan in Egypt, or Lysa nad Labem in the Czech Republic, where the levels of the sum of DDT were above 1,500 ng/g fat<sup>15</sup>[15]. The levels of the sum of DDT in the eggs from Balti and Dumbrava are up to 200 times above the reference sample from the supermarket in Kyiv, indicating that continuing contamination by DDT has remained present in the Moldovan environment since the time when it was applied in the last century in large volumes<sup>4</sup> [4]. However comparable levels of 26 and 30 ng/g fat to those in the eggs from Balti and Dumbrava were measured in eggs from large farms obtained in Prague<sup>16</sup>[16] and Beijing<sup>17</sup> [17].

We compared the measured levels of OCPs per gram of fresh eggs with the maximum residue levels (MRL) set by the EU (see Table 1). The level of DDT metabolites in the eggs from Ciobanovca (63.6 ng/g fresh weight) exceeded the EU maximum residue level set for eggs, 50 ng/g fresh weight. It also exceeded the EU MRL for lindane and, by several times, the levels for  $\alpha$ - and  $\beta$ - HCH isomers.

It is obvious from the analytical results that the levels of OCPs in the eggs from Ciobanovca were caused by some local pollution hotspot, while the levels of OCPs in the other two samples from Moldova may result from the remaining overall contamination of the Moldovan environment. Over 1000 warehouses for pesticides had been built in Moldova by the early 1990s, according to the National Implementation Plan for the Stockholm Convention<sup>4</sup> [4], so any agricultural structure and/or building can be suspected of being a former warehouse for the POP pesticides that were widely used in Moldova. Another source of contamination might be that the Tintareni Landfill became the destination for obsolete POP pesticides. According to the project focused on sustainable management of POP stockpiles funded by the Global Environment Facility (GEF), more than two tonnes of unidentified POP pesticides were transferred from Tintareni to central storage before the year 2005<sup>5</sup> [5].

**Other measured POPs:** The levels of HBCD, six novel BFRs, or PFAS were below LOQ or low in comparison with studies from other countries<sup>30</sup> [30]. The level of the sum of HBCD isomers of 14.75 ng/g fat in the egg sample from Dumbrava is higher in comparison to the levels in the samples from Ciobanovca or Balti; however, it is still a very low level when compared to samples from Kazakhstan or Thailand<sup>30</sup> [30]. The sum of HBCD isomers was also below LOQ in the reference sample from Prague<sup>16</sup> [16]. The levels of PFAS in all three pooled egg samples were lower than, for example, in eggs from the vicinity of plastic waste yards in Java, Indonesia<sup>26</sup> [26] and comparable to the levels observed at some other locations in Java<sup>26</sup> [26] or to the levels observed in reference egg samples from other countries<sup>31</sup> [31].

## 5 Conclusions

Under this study the POPs presence was analysed in samples of free-range chicken eggs, collected in Moldova at three locations potentially affected by different POPs sources. The results of the findings can be summarised as follows:

- **Ciobanovca/Tintareni Landfill:** The contamination with HCH (3,005 ng/g fat) and PeCB (16.57 ng/g fat) in the eggs from Ciobanovca is most probably related to a hidden stockpile of obsolete OCPs or a building remaining contaminated after it was used as a warehouse for OCPs in the past. The level of DDT metabolites and HCH isomers in the eggs from this location exceeded the EU MRL set for eggs. High levels of dl PCBs, with almost 52 pg TEQ/g fat and the total TEQ level exceeded the EU standard of 5 pg TEQ/g fat by more than ten times in this sample and also twofold exceeded the level set for dioxins. The very high levels of 6 measured PCBs (144 ng/g fat) in the sample from this village also show a potential source of contamination with technical PCBs.
- **Dumbrava/Vatra industrial zone:** The levels of UPOPs in the eggs from Dumbrava also show that tire pyrolysis or another industrial source in Vatra can be a significant source of the release of dioxins and dl PCBs. The egg sample collected in this location exceeded twofold the EU standard for dioxins and 3.5-fold the dl PCBs standard.
- **Balti/Balti Landfill:** The POP levels in the eggs from Balti collected in the very near vicinity of the Balti Landfill were not so high in comparison with those in the eggs from Ciobanovca or Dumbrava and the dioxin profile shows the open burning of mainly plastic waste at the landfill as a potential source of contamination, although the level of PCDD/Fs in the eggs was below the EU standard for eggs as food.

There is a lack of data about POPs in the Moldovan environment. Our study shows the importance of filling this gap and the need also to better evaluate new potential sources of UPOPs, including combustion processes. Tire pyrolysis in Vatra belongs to this group of sources. Tire pyrolysis is a known priority source of UPOPs identified in Annex C to the Stockholm Convention. The low levels of other POPs such as PBDEs or PFAS in the eggs show that waste containing these POPs has probably not reached the landfills in Moldova yet, and/or that free-range chicken eggs did not become the final destination of these POPs.

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