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Environmental contamination of free-range hen with dioxin

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Abstract

Introduction: The transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) from a contaminated environment into the food chain is a serious consumer safety problem. As part of the Polish National Surveillance Program of PCDD/Fs and PCBs in food of animal origin, a concentration of PCDD/Fs of 4.61 ± 0.75 pg WHO-TEQ/g fat was determined in a sample of free-range eggs, which exceeded the permitted limit of 2.5 pg WHO-TEQ/g. The aim of the study was to investigate the source of the egg contamination and the risk for the eggs' consumers. **Material and methods:** Eggs, muscles, feed and soil from the place where backyard waste burning had been carried out in the past and ash from a household stove tipped onto the paddock were analysed using the isotope dilution technique with high-resolution gas chromatography coupled with high-resolution mass spectrometry. **Results:** The concentration in ash was low at 0.20 pg WHO-TEQ/g and the congener profile did not indicate the source of contamination. The dioxin content in soil from the backyard waste-burning site was 2.53 pg WHO-TEQ/g dry matter (d.m.) and the soil's profile of PCDD/F congeners matched the profile of the contaminated eggs. **Conclusion:** By reason of the congener profile similarity, the investigation concluded, that the cause of the contamination was the backyard waste-burning site soil which the animals had access to. Frequent consumption of contaminated eggs from the analysed farm could pose a health risk due to chronic exposure, especially for vulnerable consumers.

Keywords: free-range eggs, PCDD/Fs, PCBs.

Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are compounds commonly found in the environment. The sources of PCDD/Fs are industrial and combustion processes (5). Unlike dioxins, PCBs were produced at industrial scale and are found in capacitors, transformers and heating and cooling systems, from which they get into the environment as a result of leaks and improper disposal. In recent years, a decrease in dioxin and PCB emissions from industrial sources has been observed; however, uncontrolled burning processes in household furnaces and burning of organic and plastic waste in backyards contribute significantly to environmental pollution (24). Affinity for organic carbon and lipids and a relatively low volatility allow these chemicals to be retained in soils, sediments, and biota for long periods of time (29). Studies carried out for many years have shown that eggs from free-range hens which are exposed to direct contact with a contaminated environment may be a source of dioxins and PCBs in the diet of consumers (1, 3, 10, 11, 18). Poultry intake of dioxins and PCBs from various sources leads to an increase in their content in tissues and eggs (12, 14, 17, 18). Compared with hens kept on cage farms, free-range birds have greater contact with these sources due to better access to outdoor runs (15). On free-range farms, in addition to atmospheric deposition, other sources of dioxins may also include debris, shingles, building materials made from fly ash or preserved by chlorophenols, sewage sludge applied as fertilisers, and spills and erosion from nearby contaminated areas (17, 24, 29). Recognising the threat, the European Commission issued recommendations 2013/711/EU and 2014/663/EU to reduce the presence of dioxins, furans and PCBs in feed and food, which indicates the need for increased monitoring of free-range and organic eggs. The former are one of the matrices analysed in the Polish National Surveillance Program. The aim of the present study was to investigate the sources of free-range hen egg contamination on a farm and assess the risk for consumers.

Material and Methods

Sampling and sample collection. The sampling procedure was in accordance with the provisions of Regulation 2017/644/EC. Eggs, commercial feed, ash and soil samples and five hens were collected by the Veterinary Inspectorate and delivered to the National Reference Laboratory. Hens muscles were collected to assess compliance with the regulations as regards the maximum levels of the contaminants at issue. Post-mortem examinations of the hens and the removal of biological material for research were performed by a veterinarian in the laboratory. The activities were carried out as a part of an official and procedurally complete investigation aimed at the identification of food source contamination in accordance with Commission Regulation (EU) 2017/644 and Commission Recommendation 2013/711.

Sample preparation, extraction, purification and detection. To determine the content of PCDD/Fs and dioxin-like (DL-)PCBs in food, the isotope dilution technique with the use of high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS) was applied. This method allows low background levels to be monitored and congener profiles to be precisely defined in order to identify the possible source of contamination.

Eggs and muscles were freeze-dried and the feed and soil dried at 40°C for 48 h. Samples of the egg with mass of 5 ± 1 g and of the muscles with mass of 10 ± 2 g were mixed with 10–15 g of diatomaceous earth. Samples of ash with mass of 5 ± 1 g and of soil with mass of 10 ± 2 g were also prepared in the same way for extraction. Then all samples were extracted using a Dionex ASE 350 accelerated solvent extractor (Thermo Fisher Scientific, Waltham, MA, USA) at 100 bar and 120°C in three 5-min cycles. Carbon-labelled isotope $^{13}C_{12}$ PCDD/Fs and DL-PCB standards were added to the samples before extraction.

All native standards and their $^{13}C_{12}$ homologues were purchased from the Cambridge Isotope Laboratory (Andover, MA, USA) and Wellington Laboratories Inc. (Ontario, Canada). Soil extraction was performed with toluene, egg extraction with a mixture of toluene and methanol (30 : 70), muscle with dichloromethane and hexane (50 : 50) and feed toluene and acetone (70 : 30). All organic solvents were of suitable purity for residue analysis and were supplied by LGC Standards (Wesel, Germany). In order to remove interfering compounds, the samples were subjected to a multistage clean-up on chromatographic columns. In the first stage, fat was oxidised through an acidic silica gel column (Fluka, Buchs, Switzerland), and samples were eluted with n-hexane. In the next column, which contained Florisil (LGC Standards, Wesel, Germany), the PCDD/F and PCB fractions were separated. These fractions were further purified through a carbon column by eluting with toluene. PCB fractions were separated into non-ortho-PCBs (the toluene fraction) and mono-ortho-PCBs (the n-hexane fraction) through carbon/Florisil column. Before HRGC-HRMS analysis, recovery standards were added to each fraction (1,2,3,4-tetrachlorodibenzo-p-dioxin and PCB 111). Chromatographic separation and detection were performed using a Trace GC Ultra gas chromatograph (Thermo Scientific, Milan, Italy) with a DB5-MS capillary column (60 m, inner diameter 0.25 mm, film thickness 0.1 μm; Agilent J&W Scientific, Folsom, CA, USA) combined with a MAT95XP high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The mass spectrometer worked in electron ionisation mode (electron impact, EI) under conditions providing resolution exceeding 10 000. The World Health Organization Toxic Equivalents (WHO-TEQ) were calculated using the toxic equivalency factors (TEF_{2005}) (27). To calculate the concentrations of PCDD/Fs and DL-PCBs, the concept of upper bound was adopted, according to which the contribution to TEQ of each non-quantified congener is its limit of quantification.

Quality assurance and quality control. Quality assurance and quality control were achieved through analysis of blank samples, duplicates, in-house reference material (eggs) and certified reference materials (BCR-607). The relative standard deviations for all compounds were 15% for both the duplicates and the reference materials. The method performance was verified by success in the proficiency testing study organised by the European Union Reference Laboratory for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food (Freiburg, Germany).

Results

Under the Polish National Surveillance Program of PCDD/Fs and PCBs in food of animal origin, an instance of high content of PCDD/Fs in free-range eggs was found. The concentration of PCDD/Fs was 4.61 pg WHO-TEQ/g fat (in sample 205-MDZ) and exceeded the limit of 2.5 pg WHO-TEQ/g fat (Commission Regulation (EU) No 1259/2011) (Table 1). European Union legislation obligates the responsible authority, in this case the Veterinary Inspectorate, to identify the source of contamination. In view of these requirements and in cooperation with the Radiobiology Department of the National Veterinary Research Institute, the local veterinary services took appropriate action. A veterinary inspector resampled eggs (as sample number 488-D), and collected five hens, a feed sample (483-D), and paddock soil (051-BN) from the farm. The results of their analysis are shown in Table 1. The content of PCDD/Fs in the resampled eggs was over 30% higher than in the first sample (6.13 pg WHO-TEQ/g fat). In the examined muscle tissue of all five chickens, the sum of PCDD/Fs was in the range of 3.04 to 6.47 pg WHO-TEQ/g fat, which exceeds the maximum level of 1.75 pg WHO-TEQ/g fat between almost two and almost four times. The low concentrations determined in soil and feed indicated that they could not be the source of the chickens' contamination. In the feed samples, all PCDD/Fs congeners were below the limit of quantification (LOQ).

The veterinary inspectorate was asked to visit the farm and interview the farmer because the contamination source could not be identified. Potential sources were investigated (backyard burning sites, pesticide storage or home-made feed materials) that could lead to contamination of animals. In the interview, the inspector identified two potential sources. The first one was a place in the paddock where backyard waste burning had been carried out in the past. The second one was ash, which the farmer tipped onto the paddock after waste burning. Both soil (059-BN) and ash (058-BN) were taken for analysis.

Table 1. PCDD/F and DL-PCB (concentrations with uncertainty). Eggs and muscles are expressed on a fat basis, feed on 12% moisture content, soil and ash on dry matter

Sample no.	Matrix	PCDD/F	DL-PCB	∑ PCDD/F/ DL-PCB
		pg WHO-TEQ/g		
205-MDZ	eggs	4.61 ± 0.75	1.31 ± 0.25	5.92 ± 1.48
488-D	eggs	6.13 ± 1.00	1.31 ± 0.25	7.44 ± 1.86
483-D	feed	0.05 ± 0.01	0.02 ± 0.00	0.07 ± 0.02
051-BN	soil	0.43 ± 0.06	0.06 ± 0.01	0.49 ± 0.11
058-BN	ash	0.20 ± 0.03	0.03 ± 0.00	0.23 ± 0.05
059-BN	soil	2.53 ± 0.36	0.18 ± 0.03	2.71 ± 0.60
Chicken 1	muscles	6.47 ± 1.06	1.51 ± 0.28	7.98 ± 1.99
Chicken 2	muscles	4.52 ± 0.74	1.94 ± 0.37	6.46 ± 1.61
Chicken 3	muscles	3.04 ± 0.50	1.25 ± 0.24	4.30 ± 1.07
Chicken 4	muscles	4.53 ± 0.74	1.53 ± 0.29	6.06 ± 1.51
Chicken 5	muscles	4.25 ± 0.70	1.86 ± 0.35	6.11 ± 1.52

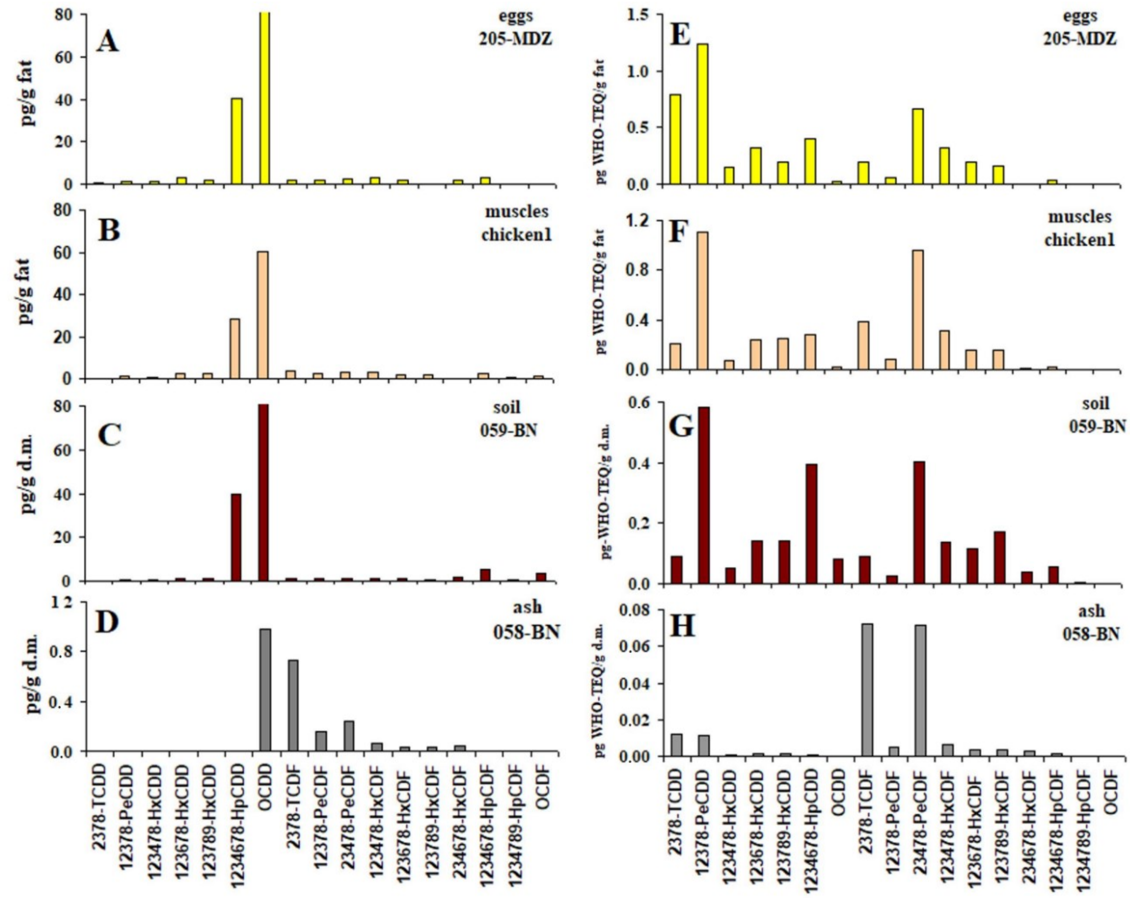

Fig 1. Congener profiles. A, B, C, D – profiles expressed as pg/g; E, F, G, H – profiles expressed as pg WHO-TEQ/g

The concentration of PCDD/Fs in ash was at a low level of 0.20 pg WHO-TEQ/g d.m. and the congener profiles did not match those of the eggs and muscles (Fig. 1 A, B, D, E, F, H). Dioxin content in soil from the waste-burning site (059-BN) was 2.53 pg WHO-TEQ/g d.m. and the profile of PCDD/F congeners was analogous to the profile of the contaminated eggs and muscles (Fig. 1 A, B, C). An analogous profile was also observed in the toxicity (Fig. 1 E, F, G). In eggs, muscles and soil the highest concentrations were reached by highly chlorinated OCDD and 1,2,3,4,6,7,8-HpCDD. As the congener with the lowest toxicity (TEF_{2005}=0.0003), OCDD contributed less than 1% to the total toxicity of the egg sample and approximately 3% to that in soil. The highest contributions to the total toxicity of eggs, muscles and soil were constituted by 1,2,3,7,8-PeCDD (TEF_{2005}=1) at over 23%, 40%, and 26%, respectively and 2,3,4,7,8-PeCDF (TEF_{2005}=0.3) at almost 14%, 18% and 16%.

Based on the congener profile similarity it could be concluded that soil from the place in the paddock where backyard waste burning had been carried out in the past was the source of dioxins for hens and eggs.

To characterise the potential health risk associated with dioxin and DL-PCB intake, we assumed consumption of eggs containing PCDD/Fs and DL-PCBs at the level of 7.44 pg WHO-TEQ/g fat. An average egg weight of 60 g was assumed. To estimate the theoretical intake amount of PCDD/Fs and DL-PCBs for adults, we used statistical data on egg consumption. According to recently published official statistical data, the average monthly consumption of eggs *per capita* in Polish households in 2018 was 11.64 (equal to around 2.7/week) (25). The calculations were performed for an adult of 70 kg and a child of 23.1 kg (6) and the potential risk associated with dioxin intake was characterised by comparing calculated intakes with the tolerable weekly intake (TWI) of 2 pg WHO-TEQ kg^{-1} body weight. These assumptions allow estimation of the exposure to dioxins for consumers of eggs from the contaminated farm. Estimated dioxin and DL-PCB intakes were 103% TWI and 313% TWI for adults and children, respectively. It should be made clear that this ingestion scenario is one where only eggs with the identified high level of dioxins are consumed; nevertheless, a situation where contaminated eggs are consumed for a period of a week or longer cannot be excluded. It is alarming that the calculated dioxin intake for children exceeds the toxicological reference values by a factor of three.

Discussion

Hens' exposure to PCDD/Fs and PCBs may come from different sources; for example, consumption of contaminated feed, the soil on which they live, or contaminated structural elements of the henhouse (9, 17, 18). Feedstuffs are the most frequently recognised source of dioxins in hen eggs (10, 13, 26). Therefore, in cases of egg contamination, the first step to find the source is to test the feed that was given to the animals. If it transpires not to be the source of contamination, soil is considered. Free-range hens may ingest dioxins when pecking in the ground for food. They ingest soil with food items such as earthworms, either intentionally or coincidentally. The amount of soil intake depends on several factors, including the number of birds per free ranging area, the time hens spend outdoors, the nature of the paddock, and the presence of soil organisms such as worms (1, 3). Uptake of the contaminants from soil and carry-over to eggs depends on their concentration and bioavailability, and the hens' foraging behaviour and time spent outdoors (3, 11). The bioavailability of dioxins from soil was estimated for laying hens at between 40 and 60% (8, 28). Hens accumulate dioxins in the liver, muscles and adipose tissue and transfer them to eggs (17, 18, 26). Many papers presented soil as a significant contributor to the dioxin contamination of eggs (3, 8, 17, 21), and the causal relationship of soil dioxin content and the significantly higher levels of PCDD/Fs and DL-PCBs generally in eggs coming from free-range and outdoor-reared hens than in those from battery-reared birds can be contended (7, 15, 17, 22). People buy organic and free-range eggs mainly because they believe that they are healthier and they expect laying hens to be kept under improved, more humane welfare conditions. Unfortunately free-range eggs could be a source of several contaminants such as dioxins and pose a risk to some groups of consumers. Surveys from other countries on the proportions of the total dietary dioxin intake for adults in different foodstuffs indicate that eggs contribute about 1% in France (23), 4–7% in the USA, Netherlands, Spain, and Germany (2, 16, 20, 22), and even 31% in China (30). For children, regardless of the country, those values are generally higher than for adults, due to the lower body mass used in the estimated dietary intake calculations. It can be said that hen eggs are not a significant dietary source of dioxins for the general population and their nutritional benefits outweigh the health risks associated with the intake of dioxins (4). However, for some vulnerable groups (children and pregnant and breast-feeding women), eggs can contribute to unnecessary increased uptake of these toxic compounds.

Taking into account that soil is a frequent source of PCDD/Fs and PCBs, it appears that allowable levels for paddock soil should be established. The evidence suggests that frequent consumption of contaminated eggs from the analysed farm could pose a health risk for consumers due to chronic exposure.

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