DIOXINS (PCDD/Fs) AND DIOXIN-LIKE PCBs (dl-PCBs) IN FREE-RANGED CHICKEN EGGS FROM TOXIC HOTSPOTS OF JAVA

Ismawati Y[1], Petrlik J[2,4], Arisandi P[3], Bell L[4,5], Beeler B[4], Behnisch PA[6], Grechko V[2] and Ozanova S[2]
[1] Nexus3 Foundation, Denpasar, Indonesia, 80223, yuyun@nexus3foundation.org; [2] Arnika – Toxics and Waste Programme, Prague, Czech Republic, CZ17000; [3] Ecoton, Gresik, Indonesia, 61177; [4] International Pollutants Elimination Network (IPEN), Gothenburg, Sweden, S-402; [5] National Toxics Network (NTN), Perth, Australia, 6054; [6] BioDetection Systems B.V., Amsterdam, The Netherlands, 1098 XH.

Introduction

Persistent Organic Pollutants (POPs) contamination in developing countries can include domestic and foreign pollution sources.

The study focused on sites potentially polluted by such sources on Java, Indonesia, in particular, areas affected by plastic and paper waste imports, secondary aluminum production, and waste incineration[1,2].

Developed countries sharply increased exports of non-recyclable plastic waste to Indonesia and other Southeast Asian countries after China closed its borders to plastic waste imports in 2018. As a result, Indonesia's plastic waste imports doubled to 320,000 tons in 2018 compared to 2017. Based on observations by Ecoton and Nexus3, between 25% and 50% of the plastic wastes imported by Indonesian plastic and paper recycling companies were mismanaged[2,3].

Aluminum is another material used widely in beverage packaging and car production. Waste from primary aluminum production and discarded aluminum scrap, often used for secondary aluminum production, is also used in smelters located in the Jombang Regency.

Many toxic additives in the plastic waste can leak into the environment when disposed of or burned, including chemicals listed in the Stockholm Convention (SC)[4] such as for example polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) or perfluorooctane sulfonate (PFOS)[4-6]. Toxic chemicals are also involved and/or generated in secondary aluminum production and contained in aluminum scrap. These include substances such as dioxins (PCDD/Fs) that the treaty requires to be continuously minimized with the objective of elimination (see Article 5 of the SC)[4]. PCDD/Fs are also by-products of burning plastic wastes in open burning of wastes and/or their use as fuel or their waste incineration. Waste incineration and metallurgy are listed as major sources of PCDD/Fs and other unintentionally produced POPs listed in Annex C to the SC[4].

This paper examined PCDD/Fs contamination of free-range chicken eggs from five sites in Indonesia: Tropodo, where plastics are burned as fuel in tofu factories; Bangun and Tangerang, where the residues of imported plastics are dumped on the ground and burned; Lakardowo, where a privately owned hazardous waste incinerator facility is located; and Kendalsari, where dozens of secondary aluminum smelters operate.

Materials and methods

Free-ranged chickens and their eggs are considered ideal "active samplers" and indicator species for POPs contamination. Eggs from contaminated areas can readily lead to exposures which exceed thresholds for the protection of human health[7-9]. Free-ranged chicken eggs from the four pooled samples (two samples from Bangun, one sample from Tropodo and one sample from Tangerang) and one pooled sample of commercial eggs (non-free-ranged) from Jakarta were analyzed for PCDD/Fs as well as for dioxin-like polychlorinated biphenyls (dl-PCBs) using the DR CALUX® method.

These samples were sent to a Dutch ISO 17025 certified laboratory BDS performing the cell-based screening analysis DR CALUX® according to the European Standard EC/644/2017. The procedure for the BDS DR CALUX® bioassay has been described in detail by Besselink, et al.[10].

Briefly, rat liver H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct were cultured in an α-MEM culture medium supplemented with 10% (v/v) FCS under standard conditions (37°C, 5% CO_2, 100% humidity). Cells were exposed in triplicate on 96-well microtiter plates containing the standard 2,3,7,8-TCDD calibration range, a reference egg sample (analyzed by GC-HRMS; for the bioassay apparent recovery), a procedure blank, a DMSO blank and the sample extracts in DMSO.

Following a 24-hour incubation period, cells were lysed. A luciferin-containing solution was added, and the luminescence was measured by using a luminometer (Mithras, Berthold Centro XS3). The DR CALUX® bioassay method has been shown to be a cost-efficient, semi-quantitative, effect-based toxicity screening analysis for all kinds of stable dioxin-like compounds (PCDD/Fs, dl-PCBs, PBDD/Fs, PBBs, and chlorinated and brominated polycyclic aromatic hydrocarbons, N-dioxins). However, for confirmation, it is recommended to go for more specific PCDD/Fs and dl-PCBs congener analyses, which also allow examination of the fingerprints of dioxins (PCDD/F congener patterns) specific to different sources of pollution.

Seven pooled egg samples from Java as well as samples of soil, ash, rice and dust were analyzed for content of individual PCDD/Fs and an extended list of PCB congeners by HR-GCMS at the accredited laboratory of the State Veterinary Institute in Prague, Czech Republic.

Samples of eggs collected in Bangun and Tropodo in May 2019 were analyzed for specific PCDD/F and dl-PCB congeners in MAS laboratory, Muenster, Germany by GC-HRMS, simultaneously for brominated dioxins. Results for PBDD/Fs are in larger report published in 2020[1].

Results and discussion

The results of the chemical analyses revealed levels of PCDD/Fs among the highest ever measured in several pooled free-ranged chicken egg samples. In the pooled samples of free-ranged chicken eggs from Tropodo, we found high levels of 200 and 140 pg WHO-TEQ g-1 fat of PCDD/Fs, respectively. The regulatory limit in Indonesia is 2.5 pg WHO-TEQ g-1 fat including both PCDD/Fs and dl-PCBs.

These are the third- and the fourth-highest levels of PCDD/Fs in eggs from Asia ever measured and the sixth- and seventh-highest levels of PCDD/Fs in eggs found globally. The chicken eggs from Kendalsari and Tangerang belong together with two pooled eggs samples from Tropodo to samples from this study, which are among 20 egg samples with the highest ever measured levels of PCDD/Fs globally.

Table 1. PCDD/Fs and dl-PCBs in chicken eggs from five toxic hotspots in Java, Indonesia.

Locality	Sample ID	Eggs in sample	Fat	PCDD/Fs	dl-PCBs	PCDD/Fs/dl-PCBs	Total PCDD/Fs/dl-PCBs - DR CALUX®
	Eggs	number	%	pg TEQ g^{-1} fat			pg BEQ g^{-1} fat
Bangun	Bangun 1	3	13	10.8	3.1	13.9	21
	BAN-E-1	3	9.5	9.5	5.1	14.6	13
Tropodo	Tropodo 1	3	15	200	32	232	560
	TROP-E-1	6	13.9	140	32	172	NA
Kendalsari	KEN 01	9	27.4	49	35	84	NA
	KEN-E-1/19	6	14.3	41	20	61	NA
Sumberwuluh	SUM-E-1, E-2	6	14.1	11.0	2.0	13.0	NA
Tangerang	SEM-E-1	3	16.2	54	18	72	88
	TAN- ESIN-01	5	13.7	20.4	7.4	28	NA
Jakarta	JAK-SUP	6	9.5	0.0012	0.0020	0.0032	<LOQ (0.6)
Indonesia limit		-	-	-	-	2.50	-
EU limits		-	-	2.50	-	5.00	1.7/3.4

Table 2. Number of eggs consumed to reach the Tolerable Daily Intake (TDI) suggested by WHO

Locality	Bangun		Tropodo		Sumber-wuluh	Kendalsari		Tangerang		Jakarta
Sample	Bangun 1	BAN-E-1	Tropodo 1	TROP-E-1	SUM-E-1 and 2	KEN 01	KEN-E-1/19	SEM-E-1	TAN-ESIN-01	JAK-SUP
PCDD/Fs + dl-PCBs (pg WHO-TEQ in one egg)	63	48	1,218	836	64	810	303	407	133	0.011
Number of eggs to reach 116 pg WHO-TEQ per day	1.83	2.41	0.10	0.14	1.81	0.14	0.38	0.28	1	10,900
Number of eggs to reach 14.5 pg WHO-TEQ per day	0.23	0.30	0.01	0.02	0.23	0.02	0.05	0.04	0	1,363

Table 2 summarized the results of calculation for TDI for PCDD/Fs/dl-PCBs as it is set by WHO globally and as it was also suggested by European Safety Authority before 2018. The calculation was made for average adult person in Asia, with a weight of 58 kg[11]. However, the TDI for PCDD/Fs/dl-PCBs was reassessed by EFSA in 2018[12], and calculation for this new TDI is included in larger report published in 2020[1].

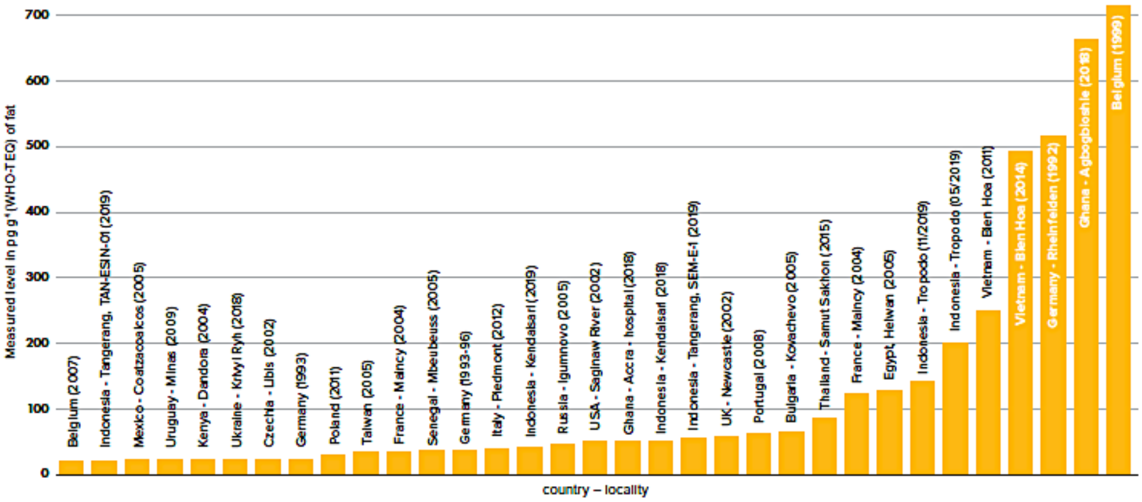

Figure 1. Maximum levels of PCDD/Fs measured in chicken eggs in different countries. Samples before 2006 are in WHO-TEQ 1998. Source: Petrlik J, et al. 2020[1].

The measured levels of POPs in chicken egg samples were compared with the legislative limits established in Indonesia and the European Union. The limits used to compare levels measured in food in many other studies, mainly in developing countries that do not have official limits for dioxins and other POPs in food. Indonesia has set a limit value for PCDD/Fs and dl-PCBs in eggs at 2.50 pg TEQ g^{-1} fat[13].
Several bio-analytical tools are accepted by international standards for measuring dioxin-like activity in environmental and food samples. Those standards are such as EC/644/2017, EPA 4435/2008, JIS 463/2009, Dutch Specie 07/2005 and the Chinese standard for Solid waste—screening of PCDD/Fs—Chemical activated luciferase expression, 2018. These methods are a more accessible and cost-efficient option for screening larger quantities of environmental, food or human samples. Many studies use them to evaluate contamination by dioxins and dioxin-like substances, e.g., food[14-16].
The four pooled egg samples in this study were analyzed using the DR CALUX® method. The highest level in BEQs was measured in the sample from Tropodo (560 pg BEQ g^{-1} fat), followed by a sample from the site affected by open burning and dumping of plastic waste in Tangerang (88 pg BEQ g^{-1} fat). Two samples from Bangun were also suspected not to meet neither the Indonesian[13] nor the EU limit[17] for PCDD/Fs/dl-PCBs, of 2.5 and 5 pg TEQ g^{-1} fat, respectively.
All sample results measured using the DR CALUX® method were also in the same order of magnitude in the chemical GC/HRMS analysis for PCDD/Fs/dl-PCBs -TEQ as follow:

- Bangun-1: DR CALUX® 21 vs chemical analysis 13.9; therefore, non-compliant according to EU guidelines;
- BAN-E-1, the second sample from Bangun: (DR CALUX® 13 vs chemical analysis 14.6; therefore, non-compliant according to Indonesian and EU guidelines;
- Tropodo-1: DR CALUX® 560 vs chemical analysis 232; therefore, non-compliant according to Indonesian and EU guidelines;
- SEM-E-1, the sample from Tangerang: DR CALUX® 88 vs chemical analysis 72; therefore, non-compliant according to Indonesian and EU guidelines;
- and, finally, the egg samples from the Jakarta supermarket, which was the only one compliant with both the Indonesian and the EU guidelines - DR CALUX® below LOQ of 0.6 vs chemical analysis 0.0032.

The differences between the results from the DR CALUX® analyses and the chemical GC/HRMS analyses could potentially be explained by more chemicals showing dioxin-like activity, which were not analyzed by any of the instrumental analyses in our study, and which were analyzed in our research but are not included in the WHO-TEQ value.
The difference can be explained by a variation in the homogeneity of the sample—however, the same homogenate is used for all analyses. A certainty of 40% was used in the chemical analyses of PCDD/Fs and dl-PCBs. Bioassay analyses of eggs and other environmental samples could be a pathway to broader monitoring of dioxin contamination in Asian countries.

Conclusion
High PCDD/Fs and dl-PCBs concentrations released by burning residues of mixed plastic wastes in open spaces or as fuel, emissions from secondary aluminum smelter, and from hazardous waste incinerators in Java poisoned the local food chain. On average, egg consumption calculated as half an egg (18 g of egg) per day for an adult person weighing 58 kg people eating free-ranged eggs would exceed the European Food Safety Authority (EFSA) tolerable daily intake (TDI) level for PCDD/Fs and dl-PCBs by 1.5- to 43-fold. Samples collected in 2019 in Tropodo, Kendalsari and Tangerang show the worst situation.

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