PCDD/Fs and PCBs in eggs – data from China, Kazakhstan and Thailand

Petrlik J[1,2], Teebthaisong A[3], Bell L[2,4], Behnisch PA[5], Da M[6], Saetang P[3], Ritthichat A[3], Kalmykov D[7]

[1] Arnika, Delnicka 13, Prague, Czech Republic, CZ17000; [2] IPEN, Göteborg, Sweden, SE-4035; [3] Ecological Alert and Recovery – Thailand (EARTH), Nonthaburi, Thailand, TH-11000; [4]National Toxics Network, Perth, Australia; [5]BioDetection Systems BV (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands; [6]Nature University, Beijing, China; [7] Karaganda Regional Ecological Museum, Karaganda, Kazakhstan, 100000

Introduction

There are a range of studies on PCDD/Fs and PCBs in eggs[1-7]. Eggs have been found to be sensitive indicators of PCDD/F and PCB 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[1-3]. 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 China, Kazakhstan and in Thailand. It is based on larger reports released in 2016/2017, where more information about the sites can be found[7-9].

Materials and methods

At the first stage, 21 pooled egg samples were analyzed by bioassay analysis to receive information about PCDD/Fs and dl-PCBs levels. They were collected from 11 localities in China (5) and Kazakhstan (6). In addition, one sample was bought from a supermarket in Beijing. At the second stage, 29 pooled egg samples were analysed for PCDD/Fs, dl-PCBs and 6 indicator PCB congeners (i-PCBs) by instrumental analysis, and an additional 3 pooled samples for i-PCBs only. The eggs were collected at the same localities where possible plus additional samples in Thailand. We were not able to repeat sampling at certain localities in China for instrumental analyses. Free range chicken eggs were collected from 19 localities in China (4), Kazakhstan (8) and Thailand (7). In addition, one pooled sample was bought from supermarkets in each of three countries. Eggs from supermarkets in Beijing (3 eggs), Karaganda (6 eggs) and Bangkok (6 eggs) were analysed to help establish background levels. There were following ranges of eggs in pooled samples from different countries: China between 2 – 8 eggs/sample[7], Kazakhstan - between 2 – 10 eggs/sample[8], and Thailand 3 – 5 eggs/sample[9]. 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 mainly from Beihai, China. Samples were collected within the three year period 2013 – 2016[7-9]. The choice of sampled sites were prioritised according to industrial or other human activity criteria. The sites with potential releases of POPs had priority, especially sites with industries listed as major sources of PCDD/Fs under Annex C to Stockholm Convention, however some sites chosen as potentially 'clean' were sampled as well. Contaminated sites and their neighbouring localities were also included into our sampling scheme. We followed a similar model of selection of sites as in IPEN's previous free range eggs study[5].

Bioassay. 22 samples were analyzed 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[11]. [10].The procedure for the BDS DR CALUX® bioassay has previously been described in detail[11] but, briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in α-MEM culture medium supplemented with 10% (v/v) FCS under standard conditions (37ºC, 5% CO_2, 100% humidity). Cells were exposed in triplicate on 96-well microtiterplates containing the standard 2,3,7,8-TCDD calibration range, a DMSO blank Following a 24 hour incubation period, cells were lysed. A luciferine solution was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

Instrumental analysis. 31 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[11].

Results and discussion

Bioassay analyses of dioxin toxicity. The BEQ levels in chicken eggs from 21 sites near industrial facilities or contaminated sites in China and Kazakhstan were found to be between 3.8 pg BEQ/g fat and 101 pg BEQ/g fat in egg samples from Ekibastuz – Soyuz (near PCBs contaminated site) and Balkhash – north (close to non-ferrous metals production facility) respectively. All samples exceeded by 3 – 84 times the reference level of 1.2 g BEQ/g fat found in eggs from a supermarket in Beijing, and 19 of 21 samples were at levels suspected to be above EU standard for eggs. Results for all samples are summarized in graph at Figure 1.

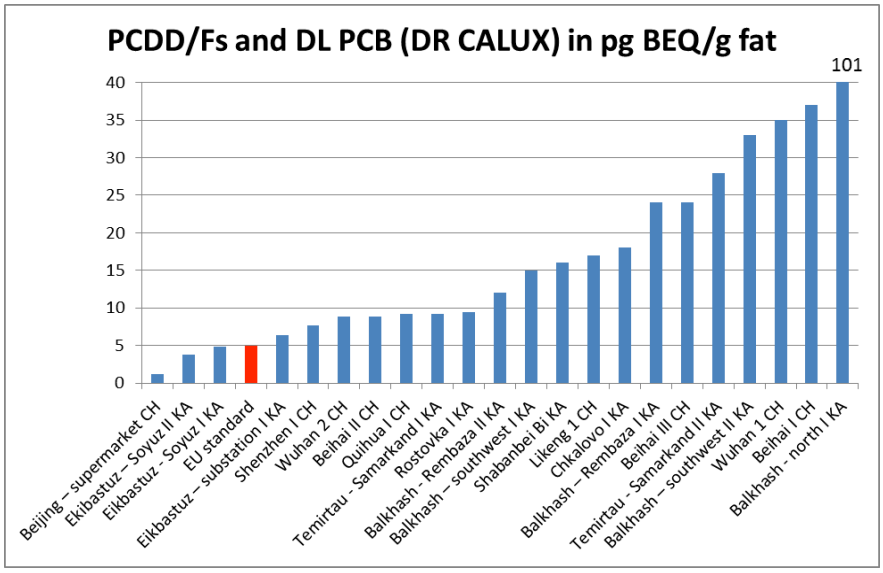

Figure 1: Graph summarizing results of DR CALUX® analyses of free range chicken eggs from 21 sites, 11 localities in China and Kazakhstan.

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 are at the beginning of Table 1. Lowest reference levels for PCDD/Fs, dl-PCBs and i-PCBs were measured in a pooled egg sample from a supermarket in Bangkok. The highest level and third highest levels of PCDD/Fs + dl-PCBs were found in two pooled egg samples from Shabanbei Bi, Kazakhstan (both are from the same farm but in different years), a site on the edge of natural preserved area in Kazakhstan where such a high level was not expected as no obvious source of contamination was present. We assume that the contamination source is probably some abandoned old equipment containing PCBs to which chickens had access based on result for i-PCBs in the same sample. We didn't locate that specific source. The second highest level was observed in a sample from Samut Sakhon, Thailand, a site with a small artisanal 'recycling' workshop and open burning of e-waste[9]. At this site 19 out of the total 29 pooled egg samples exceeded the EU standard set for PCDD/Fs + dl-PCBs in eggs at a level of 5 pg TEQ/g fat[12]. The lowest level of PCDD/Fs+dl-PCBs was found in egg samples from Koh Samui, Thailand collected downstream from the waste landfill in the middle of island.

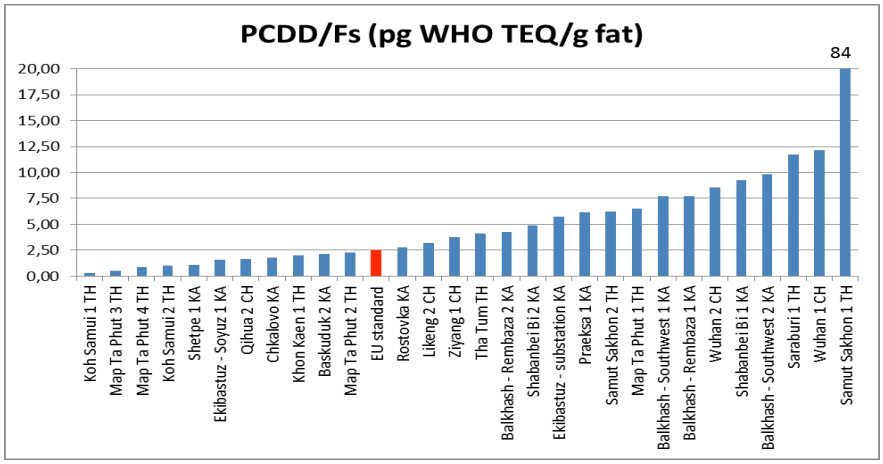

Figure 2: PCDD/Fs levels in 29 pooled egg samples from China, Kazakhstan and Thailand.

Levels of PCDD/Fs measured in 29 egg samples are summarized in graph at Figure 2. In total 18 egg pooled samples exceeded the EU standard set for PCDD/Fs at a level of 2.5 pg TEQ/g fat[12]. The highest level was measured in a sample from Samut Sakhon described above. All free range egg samples were above (reference) levels in eggs from supermarkets. The lowest level was in samples from Koh Samui exceeding reference level only 3.6 times.

i-PCBs: 6 out of 31 samples exceeded the EU standard for i-PCBs in eggs set at a level of 40 ng/g fat[12]. The extremely high level of 1,976 ng/g fat was measured in eggs from Shabanbei Bi, Kazakhstan. High contamination of selected localities in Kazakhstan by PCBs in general is demonstrated also by the prevalence of dl-PCBs in total WHO-TEQ levels (see Figure 3.), while in samples from China and Thailand PCDD/Fs prevailed.

Dietary exposure from consuming free range eggs contaminated by PCDD/Fs and PCBs was evaluated for each hotspot and summarized in published reports for Kazakhstan and Thailand [7-9]. Calculations for PCDD/Fs + dlPCBs were compared with tolerable weekly intake (TWI) suggested by Scientific Committee on Food (SCF) at a level of 14 pg WHO-TEQ/kg of body weight per week = 2 pg WHO-TEQ/kg of b. w. per day[13-14]. In cases of the most polluted samples, eating of half an egg can reach and/or exceed several times the level of 140 pg TEQ which is the daily portion of TWI per adult person. The most critical situation is in Samut Sakhon (Thailand), Wuhan, Beihai (both China), Shabanbei Bi, Chkalovo, Rostovka, Balkhash and Temirtau (all Kazakhstan).

Conclusions

Results of conducted analyses for PCDD/Fs and PCBs in free range chicken eggs from selected hotspots in 3 Asian countries have shown that some of them are seriously contaminated. In Kazakhstan, high levels of PCBs were found at certain places most likely influenced by PCB oils used in obsolete equipment. This finding is in agreement with similar studies focused on camel milk contamination in this country[14,15]. PCDD/Fs prevailed in egg samples from Thai and Chinese hotspots, as well as at a site with influence of a metallurgical plant in Kazakhstan. Potential sources at Thai and Chinese hotspots were waste incineration, metallurgy and open burning of wastes. Relatively high levels of PBDD/Fs were found in addition to PCDD/Fs + dlPCBs in eggs from Wuhan, China and Samut Sakhon, Thailand[16,17]. These findings 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] and to set stricter rules to control POPs releases in wastes[16,19].

Acknowledgements: The study was financially supported by the Government of Sweden through IPEN, Global Greengrants Fund and Thai Health Foundation. European Commision's grants via EU Aid support for actions in China, Kazakhstan and Thailand made possible chemical analyses of egg samples.

References

1.Hoogenboom LA, Kan CA, Zeilmaker MJ, et al. (2006): *Food Addit Contam.* 23, 518-527.
2. Kijlstra A, Traag WA, Hoogenboom LA. (2007): *Poult Sci.* 86, 2042-2048.
3. Hoogenboom R, ten Dam G, Immerzeel J, Traag W (2014): *Organohalogen Compounds* 76, 1707-1710.
4. Arkenbout A (2014): *Organohalogen Compounds* 76, 1700-1703.
5. DiGangi J, Petrlík J (2005): The egg report – Keep the promise eliminate POPs Report. IPEN.
6. Malisch et al (2006): CVUA Freiburg Statusbericht zu Dioxinen in Eiern. 05. April 2006.
7. Petrlik J (2015): *POPs in Chicken Eggs from Hot Spots in China*. Beijing-Gothenburg-Prague.
8. Petrlik J, Kalmykov D, Behnisch PA, Vachunova Z (2017): *Chicken eggs as the indicator of the pollution of environment in Kazakhstan.* Prague - Karaganda - Aktau, Arnika/EcoMuseum/Eco Mangystau: 46.
9. Petrlik J, Teebthaisong A, Ritthichat A (2017): *Chicken Eggs as an Indicator of POPs in Thailand.* Arnika/EARTH
10. Besselink H, Pijnappels M, Swinkels A, Brouwer B, (2004) Organohalogen Compounds 66, 677-681
11. European Comm. Regulation (EU) No 252/2012 of 21 March 2012 (OJ L 84, 23.3.2012, p. 1–22.
12. European Comm. Regulation (EU) No 1881/2006 of 19/12/2006. OJL 2006R1881-EN-01.04.2016-020.001:1-40.
13. European Commission (2001). Community strategy for dioxins, furans and polychlorinated biphenyls. (COM(2001) 593 final). OJL/C 322: 2-18.
14. Gies A, Neumeier G, Rappolder M, Konietzka R. (2007): *Chemosphere* 67(9):344-349.
15. Petrlik J, Kalmykov D, Zatloukalova K. (2016): *Toxic pollutants in camel milk from the Mangystau Region of Kazakhstan.* Arnika, EcoMuseum; Karaganda: Prague - Aktau. p. 50
16. Weber R et al. (2015): *Organohalogen Compounds* **77:**615-618.
17. Weber R et al. (2015): *13th International HCH & Pesticides Forum, 3-6 November 2015*; 204-208.
18. Stockholm Convention. (2008): Guidelines on Best Available Techniques and Guidance on Best Environmental Practices Relevant to Article 5 and Annex C of the Stockholm Convention.
19. Bell L et al. (2016): *Assessment of POPs contaminated sites and the need for stringent soil standards for food and feed safety*. EG of the Stockholm Convention on BAT/BEP Guidelines and Toolkit, Bratislava.

Table 1: Summary of results of instrumental analyses for PCDD/Fs and PCBs per localities in each of three countries plus supermarkets serving to establish reference background levels of POPs contaminants.

Country (other specification)	Supermarkets			China			
Locality	Bejing	Bangkok	Karaganda	Likeng	Qihua	Wuhan	Ziyang
Industrial activity/contaminated site	-	-	-	Municipal WI	PVC plant	Municipa WI	PCB stockpile
Number of pooled samples	1	1	1	1	1	2	1
PCDD/Fs (pg WHO TEQ/g fat)	0.2	0.08	0.9	3.22	1.63	12.17 / 8.59	3.77
dl-PCBs (pg WHO TEQ/g fat)	0.28	0.0009	0.0003	0.95	0.99	3.79 / 4.70	1.4
PCDD/F + dl-PCBs (pg WHO TEQ/g fat)	0.48	0.08	0.9	4.17	2.62	15.96 / 13.29	4.82
6 i-PCB (ng/g fat)	2.1	0.22	0.99	0.79	0.60	1.03 / 5.29	1.8

Country	Thailand						
Locality	Khon Kaen	Koh Samui	Map Ta Phut	Praeksa	Samut Sakhon	Saraburi	Tha Tum
Industrial activity/contaminated site	Mixed industry	Waste landfill	Chemical industry	Waste landfill	Metallurgy & waste recycling	Cement kilns	Mixed industry
Number of pooled samples	1	2	4	1	2	1	1
PCDD/Fs (pg WHO TEQ/g fat)	1.99	0.29 / 0.99	0.51 - 6.53	6.17	6.23 / 84.04	11.73	4.14
dl-PCBs (pg WHO TEQ/g fat)	0.83	0.007 / 0.001	0.73 - 1.92	3.41	6.00 / 11.67	6.71	3.94
PCDD/F + dl-PCBs (pg WHO TEQ/g fat)	2.82	0.30 / 0.99	1.57 - 8.45	9.58	12.23 / 95.71	18.44	8.09
6 i-PCB (ng/g fat)	1.00	0.72 / 1.07	0.50 - 3.54	2.56	7.08 / 11.40	2.23	0.39

Country	Kazakhstan							
Locality	Balkhash	Baskuduk	Ekibastuz	Chkalovo	Rostovka	Shabanbei Bi	Shetpe	Tauchik
Industrial activity/contaminated site	Metallurgy	Waste landfill	PCB cont.	Former chemical plant		PCB contamin.	Cement kiln	Mining
Number of pooled samples	4	1	2 (3)	1	1	2	1 (2)	(1)
PCDD/Fs (pg WHO TEQ/g fat)	4.25 - 9.81	2.16	1.57 / 5.73	1.82	2.79	9.26 / 4.90	1.7	NA
dl-PCBs (pg WHO TEQ/g fat)	2.88 - 22.33	11.48	2.89 / 6.45	25.94	26.51	28.62 / 150.37	5.34	NA
PCDD/F + dl-PCBs (pg WHO TEQ/g fat)	12.70 - 30.06	13.64	4.46 / 12.18	27.76	29.30	37.88 / 155.27	6.41	NA
6 i-PCB (ng/g fat)	10.12 - 58.84	26.85	10.45 - 22.98	360.44	275.47	1,975.97 / 204.00	11.35 / 9.73	8.67

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