HIGH LEVELS OF PCDD/F, PBDD/F AND PCB IN EGGS AROUND POLLUTION SOURCES DEMONSTRATES THE NEED TO REVIEW SOIL STANDARDS

Weber R[1]*, Watson A[2,3], Petrlik J[2,4], Winski A[5], Schwedler O[6], Baitinger C[6], Behnisch P[7]

[1]POPs Environmental Consulting, D-73527 Schwäbisch Gmünd, Germany; [2]International POPs Elimination Network (IPEN), Göteborg, Sweden: [3]Public Interest Consultants, Uplands Court, Eaton Crescent, Uplands, Swansea, Wales, UK SA1 4QR; [4]Arnika, Chlumova 17, Prague, Czech Republic; [5]BI Sauberes Grundwasser, Siedlung 7, D-79331 Teningen, Germany, [6]Bund für Umwelt und Naturschutz (BUND) Nordrhein-Westfalen, Germany; [7]BioDetection Systems BV (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands.

Introduction

There have been an increasing number of reports on contamination of eggs with PCDD/Fs and particularly dl-PCBs in recent years[3-7]. 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 and eggs from contaminated areas can readily lead to exposures which exceed thresholds for the protection of human health [1-4]. Chickens and eggs might therefore be ideal "active samplers" and indicator species for contaminated soils but there are, as yet, few systematic studies linking pollution sources, related soil exposures and concentrations of contaminants in eggs.

In this study, therefore, eggs were sampled at sites suspected of being impacted by PCDD/Fs or PCBs in Germany and in China and the relationship between the sources and contamination levels has been examined. Furthermore it has recently been established in a UK food survey that polybrominated dibenzo-p-dioxin and dibenzofurans can also contribute significantly to total dioxin exposure for the UK population[8]. This is possibly linked to the UK having set exacting flammability standards for furniture and thus having been a major user of brominated flame retardants including PBDEs. China is currently the largest producer of brominated flame retardants and is a major importer of BFR treated plastic for recycling. Therefore PBDD/Fs were measured in the eggs from China in addition to PCDD/Fs and dl-PCBs.

Materials and methods

Eggs were sampled at two potential hot spots in Germany. The first site was close to a condenser factory and the second close to a hazardous landfill site. For both sites two individual chicken flocks were sampled.

The two sites from which eggs were sampled in China were both from flocks from close to one municipal waste incinerator. Reference samples of eggs were also analysed after being purchased from a Chinese supermarket.

The egg samples were pooled in each case. For each of the German sites 10 to 20 eggs were pooled and for each flock in China 3 or 4 eggs were pooled for analysis.

Bioassay. All samples were analyzed at Bio Detection System for dioxin-like activity according to the standard procedures of the DR CALUX® method from BDS[10]. The procedure for the BDS DR CALUX bioassay has previously been described in detail[9] 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 containing solution (Glow Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

Instrumental analysis. All samples were analysed by gas chromatography high resolution mass spectrometry (HRGC/HRMS) in ISO 17025 accredited laboratories with a resolution >10,000 using ^{13}C isotope labelled standards. PCDD/F and dl-PCB analysis followed 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[9]. Selected samples from China were also analysed for PBDD/F using ^{13}C isotope labelled standards.

Results and discussion

Levels of PCB in chicken eggs around a former capacitor factory (Germany)

Hens eggs were sampled from two private chicken farmers (A and B) close to a factory in Teningen, a small town in South-West Germany which was used for the production of capacitors from 1932 on including PCB containing capacitors for some time. The groundwater below the former production site and the associated dumping area is contaminated with PCBs and a PCB plume contaminates the ground water of the nearby town[11].

Bioassay screening for dioxin-like toxicity was conducted for eggs from the two flocks/sites with DR CALUX. The BEQs from the bio-assays were 18 pg BEQ/g fat at both sites. PCBs were the main contributor to the BEQ for both samples and the levels considerably higher than the regulatory limit of 5 pg TEQ/kg fat for the sum of PCDD/F and dl-PCB. For confirmation the eggs were also analysed by instrumental analysis (HRGC/HRMS). The same egg samples from chicken holder A were highly contaminated (36.4 pg TEQ/kg fat) mainly from dl-PCB (25 pg TEQ/g fat). The levels in eggs from chicken farmer B were nearly as highly contaminated with 31.9 pg TEQ/kg fat. Again this was mainly due to dl-PCB (25.5 pg TEQ/g fat).
The competent authority tested the soils in the area for PCDD/F and PCB and found 2.3 ng PCB-TEQ (0-5 cm) and 3.3 ng/kg (5-10 cm) at farm A and 4.4 ng/kg PCB-TEQ (0-5 cm) and 3.8 ng/kg (5-10 cm) at farm B - significantly above German background levels of approx. 0.5 ng PCB-TEQ/kg. The upper levels of the soil contamination would be sufficient to explain the PCB-contamination in the chicken eggs via exposure from soil ingestion at high soil intakes.

Levels of PCB in soil and chicken eggs around a hazardous landfill (Germany)

The BEQ levels in chicken eggs from two farms (C and D) close to the Eyller Berg hazardous waste landfill close to the city of Kamp-Lintfort in Germany were found to be 7.1 pg BEQ/g and 6.4 pg BEQ/g fat in a screening with DR CALUX. Both samples exceeded the EU limit for egg consumption of 5 pg TEQ/g fat. The instrumental analysis (HRGC/HRMS) in these cases samples confirmed the contamination with levels of 10.4 and 8.7 pg TEQ/g fat (sum of PCDD/F and dl-PCB) in the two pooled egg samples respectively.
The competent authority of the federal state had already conducted a soil screening for PCDD/F around the hazardous landfill in 2012 and found dl-PCBs between 3.1 und 6.6 ng WHO-PCB-TEQ/kg dm[12] which was therefore about 6 to 10 times above background soil levels in German pasture land. These PCDD/F and PCB levels in the soils were sufficiently elevated to explain the contamination levels in the eggs (see below).

Levels of dl-PCBs and chlorinated and brominated dioxins/furans in eggs around an incinerator (China)

The egg sample from a Chinese supermarket showed low levels both in the DR CALUX bioassay (1.2 pg BEQ/g fat) and from instrumental analysis (total TEQ of PCDD/F and dl-PCB of 0.66 pg TEQ/g fat) and were comparable to, for example, the background levels in Europe.
Eggs in China were sampled from two private chicken farms in the vicinity of an incinerator Guoding Shan in Wuhan. The closer location (site E) was <0.3 km away from the incinerator and the second (site F) was approx. 1 km Northwest of the incinerator. The bio-TEQ in eggs from site F was 8.8 pg BEQ/g fat and the samples were therefore also analysed by instrumental analysis. The instrumental analysis showed levels of 13.3 pg TEQ/g fat for the sum of PCDD/F and dl-PCB with major contribution coming from PCDD/F with 8.6 pg TEQ/g fat.
The bio-TEQ in eggs from site E was 35 pg BEQ/g fat and instrumental analysis showed levels of 16 pg TEQ/g fat for the sum of PCDD/F and dl-PCB with major contribution coming from PCDD/F with 12.2 pg TEQ/g fat.
Due to the discrepancy between the BEQ and the TEQ the 17 brominated 2,3,7,8-PBDD/F congeners were also screened in these eggs and high levels of PBDD/F contamination were found (29 pg TEQ/g fat). This additional PBDD/F contamination is likely to be responsible for part of BEQ in the free-range eggs from site E and explains the large gap between BEQ and instrumental PCDD/F and dl-PCB TEQ in this case.
This example demonstrates also that also PBDD/F are bio-accumulating in eggs.

Need for further assessment and management - currently assessed sites and potential contamination around emission sources

The surroundings of the four sites investigated need further assessment. The PCDD/F, PCB and PBDD/F levels in soils around the Chinese sites have not yet been measured. As the sites were both close to incinerators and no other obvious potential pollution sources it is likely that the PCDD/F contamination detected in the eggs arose from the incinerator. However the PBDD/F seems only present at the flock of site F and is unlikely to stem from the incinerator. Therefore further studies are needed to clarify the contamination levels in the respective soils before any definitive conclusion can be reached about whether the incinerator was the source. It remains also possible that the contamination of the chickens and their eggs could have originated from their feed or bedding.
The case study around the German factory demonstrate that soils around PCB using industries are likely to be polluted with PCBs at levels where eggs contamination exceeds regulatory limits and is thus of concern for human health impacts. The egg contamination around the hazardous waste landfill site indicates that PCB and PCDD/F levels in soil around these landfills might be impacted by these contaminants at levels of concern for human exposure via chicken/egg pathways.
For both sites in Germany, however, further assessment of the scale of the pollution is needed. For the former production site a key question is the extent of the pollution of the soils and the distance over which soils have

been impacted by either atmospheric PCB deposition over the decades of production and/or also by migration of PCBs in the ground water. It therefore needs to be established at what distance from the site the soil is polluted to the extent that chickens (and other livestock) can not be safely kept - or can only be kept with particular management measures including, for example, special feeding regimes, restrictions on movement or substitution of the upper soil layer. Another study showed high level of PCBs (259 pg PCB-TEQ/g)[13] in an eel from a creek receiving drainage water from the former German factory. Fish and eels from the creek are consumed by members of a local fishing club which is worrying when it is considered that a single (200 g) portion of eel would exceed the Tolerable Daily Intake (TDI) for a whole year in a 70 kg adult. This case also demonstrates that in spite of contamination of the site being well known for 35 years - and whilst Germany has had adequate PCDD/F and PCB monitoring capacity for more than 30 years - there has still been no assessment of potential human exposure through the multiple pathways from this high risk PCB site.

It is clear that low levels of PCDD/F and dl-PCBs contamination in soils can result in chicken eggs being contaminated above regulatory limits and above levels relevant to TDI and health. This means that chickens around present and former PCDD/F and PCB emission sources are likely to be the most sensitive exposure pathways for contamination of humans and exposure assessments are urgently needed for many of these sites. A recent assessment of a former factory in Slovakia has shown that humans were affected at distances of up to approx. 50 km from a PCB production facility[15]. Therefore the distances of concern could be very large depending upon the source strength and the local dispersion. A recent German study showed that more than 50% of smaller chicken flocks raised in an industrialised areas in South Germany had PCDD/F and PCB levels above EU limits while most of the flocks from rural areas were significantly below regulatory limits[2] with only two exemptions both of which were likely a result of high PCB levels from point sources at the farms[2]. Another study in the Netherlands similarly warns that PCB contamination from historic PCB use in open applications such as paints and sealants can be responsible for exceedance of regulatory limits in eggs and potential on farm contamination sources should be carefully assessed[6]. Therefore when assessing contamination sources for a flock, potential local sources on the farm should be considered together with larger emission sources in the vicinity. It is therefore useful to assess at least two independent flocks around pollution sources together with detailed soil investigations including assessments of fingerprints of sources and soils before any firm conclusions are reached.

Indication of critical soil levels from other studies and consequences for soil limit values

The initial IPEN global egg study on PCDD/F levels from developing countries sampled eggs around industrial emission sources including incinerators and revealed that in many areas soils are already polluted with PCDD/F levels at which eggs can be highly contaminated. Other studies on chicken eggs such as those in the Netherlands have indicated that eggs from free-range chicken on soils with levels of 2 to 4 ng PCDD/F-TEQ/kg dm frequently exceed EU limits[4]. Calculations taking into account the soil intake of chicken (up to 36 g/day) and the regulatory levels of the eggs indicate that soil levels around and even below 2 ng TEQ/kg for PCDD/F or dl-PCB can be sufficiently high to reach the EU standards of 2.5 pg TEQ/g fat for PCDD/F or 5 pg TEQ/g fat for the sum of PCDD/F and dl-PCB. This is particularly relevant for flocks of chickens spending a lot of time outside with associated higher soil exposures/intake.

The soil-chicken-egg exposure pathway is therefore probably the most sensitive exposure path for PCBs and PCDD/Fs from soil to humans. This pathway is relevant in many contaminated sites in both developing and industrial countries and it needs to be carefully considered in the development of regulatory soil limits for PCDD/Fs and PCBs. People – and especially young children - consuming contaminated eggs can easily exceed health based standards and may be subject to very high exposure levels. In conclusion the contamination levels in soil used for the production of free-range eggs should ideally be less than 2 ng TEQ/kg dm for the sum of PCDD/F and dl-PCBs (and certainly less than 5 ng TEQ/kg dm). Further studies for generating larger datasets are recommended for determining statistically validated limits. There are different bio-accumulation factors for dl-PCBs and PCDD/F in eggs and the current EU legislation for eggs has an individual limit for PCDD/F but a combined limit for PCDD/DF and dl-PCB. Therefore soil limits for PCDD/F and dl-PCB might have to be determined individually for PCDD/F and for dl-PCB. Furthermore the particular sensitivity of dl-PCB accumulation in beef[14] reinforces the importance of defining dl-PCBs limits in soils independently of PCDD/F limits and not just as the sum of both. To our knowledge there is not yet any soil standard for dl-PCBs.

Monitoring approach using bioassay

This study demonstrates the utility of using bioassay for monitoring of chicken eggs. Specifically bioassays have the dual benefit of being both a cheap and useful tool to measure PCDD/F and PCB in eggs and are also a

sensitive tool to measure pollution in soils via the egg levels. Furthermore the bioassay approach can also detect PBDD/F and mixed-halogenated PXDD/F in eggs (and associated soils). Due to the complexity of instrumental analysis of the mixed halogenated PXDD/F currently only total dioxin-toxicity measured by appropriate bioassays can adequately address this challenge. Therefore the bioassay approach is the only method yet available to assess overall environmental and food contamination with dioxin and dioxin-like contamination in a comprehensive way at reasonable costs.
The chicken eggs from China show that brominated dioxins, in particular, can be a main contributor to total Dioxin-toxicity. PBDD/F and PXDD/F are not yet regulated in foodstuffs or soils and this is a major and serious regulatory omission which needs to be addressed especially considering the increase in PBDD/F precursors.

Consequences for industrial emissions and for controlling ashes from thermal processes

The low PCDD/F and PCB levels in soil at which chicken/eggs can become contaminated above regulatory limits and health based limits highlights the need to strictly control industrial and other emissions. It is also particularly important to ensure the safe treatment and disposal of residues from waste incinerators and even ashes from residential sources where waste plastics/PVC or contaminated wood are co-incinerated. Residual ashes with contamination levels as low as 50 ng TEQ/kg can be a risk sources. Even if such ash is "diluted" on soils the PCDD/F can re-accumulate over time with repeated applications. In this respect it needs to be highlighted that the current provisional low POPs limit established by the Basel Convention for dioxin contaminated waste of 15,000 ng TEQ/kg is much too high and needs urgently to be re-evaluated and reduced. A single kilogram of ash meeting the Basel "low POPs" level could contaminate 7 tonnes of soil to a level where eggs would not meet EU regulatory limits if laying chickens were kept on it.

Need for compensation of farmers and private owners considering polluter pays principle

A major challenge is that the levels of contamination in the soil which result in excessive levels of contamination of chicken/egg (and other livestock) are below the current regulatory soil limits. In Germany, for example, the regulatory limit for soil for residential areas/private estate is 1,000 ng PCDD/F-TEQ/kg dm. If chickens were kept on land with these levels this could result in eggs with approx. 800 pg TEQ/g fat! For a 16 kg child a single egg (10 g fat) would exceed the TDI by 250 times. Farmer and private owners have legitimate grounds to expect the original polluters to compensate them for loss of the use of land and in some cases for historic (and current) exposure. The regulatory framework therefore needs to be updated by the establishment of much lower thresholds for soil contamination reflecting the levels at which land uses need to be restricted if excessive exposure via soil-chicken-egg pathways are to be reduced. More stringent emission standards and residue treatment can reduce long-term costs associated with the loss of productive land close to emission sources.

Acknowledgements: The analyses of chicken eggs from China were undertaken within a project financed by EU Aid Non-state Actors Programme in China, and by Global Greengrants Fund.

References

1. Hoogenboom LA, Kan CA, Zeilmaker MJ, Van Eijkeren J, Traag WA. (2006) *Food Addit Contam.* 23, 518-27.
2. Kijlstra A, Traag WA, Hoogenboom LA. (2007) *Poult Sci.* 86, 2042-2048.
3. Hoogenboom R, ten Dam G, van Bruggen M, Zeeilmaker M, Jeurissen S, Traag W, van Leeuwen S (2014) Dioxines en PCB's in eieren van particuliere kippenhouders. RIKILT-rapport 2014.012.
4. Hoogenboom R, ten Dam G, Immerzeel J. Traag W (2014). *Organohalogen Compounds* 76, 1707-1710.
5. Arkenbout A (2014) Organohalogen Compounds 76, 1700-1703.
6. DiGangi J, Petrlík J (2005) The egg report – Keep the promise eliminate POPs Report. IPEN.
7. Malisch et al CVUA Freiburg (2006) Statusbericht zu Dioxinen in Eiern. 05. April 2006.
8. Mortimer D, Acheampong R, Fernandes A, Rose M (2013) *Organohalogen Compounds* 75, 1138-1141.
9. Commission Regulation (EU) No 252/2012 of 21 March 2012 (OJ L 84, 23.3.2012, p. 1–22.
10. Besselink H, Jonas A, Pijnappels M, Swinkels A, Brouwer B. (2004) Organohalogen Compds. 66:677-681.
11. Bürgerinitiative Sauberes Grundwasser http://www.sauberes-grundwasser.de/
12. LANUV Nordrhein-Westfalen (2012a) Abschlussbericht zur weiterführenden Probenahme und Untersuchung von Böden in der Umgebung der Deponie Eyller Berg im Januar 2012.
13. Badische Zeitung (2013) Vom Verzehr wird abgeraten. Untersuchungen von drei Fischen aus dem Köndringer Dammgraben weist hohe PCB Werte auf. 17.09.2013.
14. Weber R, Albrecht M, Ballschmiter K, Berger J, Bruns-Weller E, Kamphues J, Körner W, Malisch R, Nöltner T, Schenkel H, Severin K, Vossler C, Wahl K (2014) Organohalogen Compounds 76, 815-818..
15. Wimmerová S, Watson A, Drobná B, Šovčíková E, Weber R, Lancz K, Patayová H, Jurečková D, Jusko TA, Murínová L, Hertz-Picciotto I, Trnovec T (2015). Environ Sci Pollut Res Int. (accepted).