# PCDD/F, PBDD/F AND PCB CONTAMINATION IN EGGS AS SENSITIVE INDICATOR FOR SOIL CONTAMINATION AROUND POLLUTION SOURCES

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## Abstract

Contamination of eggs with polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F), polybrominated PBDD/F or polychlorinated biphenyls (PCBs) have been found around larger emission sources in different countries. The DRCALUX have been used as screening tool for monitoring dioxin-like toxicity in eggs. Contamination have been found around (former) PCB-using industries, metal industries, incinerators and e-waste treatment sites. A preliminary screening of eggs around the production site and landfill site at the Sabiñanigo HCH production indicates that no larger PCDD/F contamination is present around these sites. The low PCDD/F levels in soil at which eggs from free range chicken can be contaminated above regulatory limits demonstrate that regulatory soil limit values need to be re-assessed.

**Key words:** Dioxin; PCDD; PCDF; PBDD; PBDF; contaminated sites; monitoring; egg; DRCALUX; bioassay

## Introduction

There have been an increasing number of reports on contamination of eggs with PCDD/Fs and particularly dl-PCBs in last decade[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 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, eggs were sampled at sites suspected of being impacted by PCDD/Fs or PCBs in Germany, Spain and Thailand and the relationship between the sources and contamination levels has been examined.

## Materials and methods

Eggs were sampled at potential hot spots in Germany, Spain and Thailand. The first site in Germany was close to a condenser factory and the second site close to a hazardous landfill site. For both sites two individual chicken flocks were sampled. The eggs in Spain were sampled in the vicinity of INQUINOSA lindane factory and Sardas and Bailin HCH landfills. 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 Thailand and Spain 2 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[9]. The procedure for the BDS DR CALUX bioassay has previously been described in detail[8] 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 for the control of levels of PCDD/Fs and dl-PCBs for levels in certain foodstuffs (Commission Regulation (EC) No 252/2012)[9]. The sample from Thailand was 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[10].
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[11] 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 dioxin toxicity around hot spots of a former HCH-production site and related landfills (Spain)***

Chicken eggs were sampled from six flocks in the vicinity of the former HCH production site in Sabiñanigo and related landfills. The screening with DRCALUX assay showed that in none of the analysed flocks high PCDD/F or dl-PCB levels were present. Only in the egg sample around the Sardas landfill (1 Km from the landfill, 1.4 Km from the INQUINOSA factory) the Bio-TEQ levels were at the regulatory limit for PCDD/F (2.5 pg BEQ/g fat) with low levels of dl-PCB (0.6 pg TEQ/g fat). Overall the preliminary study indicates that there seems no major PCDD/F or dl-PCB contamination around the sites but that further assessment is needed around the Sardas landfill. The results are in agreement with a preliminary study of PCDD/F in wastes and soils were PCDD/F levels of 20 ng TEQ/kg were detected in soils and 500 ng TEQ/l were detected in leachates near the landfill, which would be problematic for accumulation of PCDD/F in chicken eggs (see below).

HCH determination by GC-MS made on some of these samples in the Bailin landfill laboratory yield values below the detection limits (<0.1 µg/g for each isomer of HCH).

***Levels of dl-PCBs and chlorinated and brominated dioxins/furans in eggs at a metal recycling site of informal sector (Thailand)***

Egg samples in Thailand were taken at a site where metals containing waste including e-waste were recovered by simple approaches including open burning. Such recycling frequently take place in developing countries and in countries of economies in transition.

The bio-TEQ in eggs from the site showed extreme high levels of 100 pg BEQ/g fat with 83 pg TEQ/g in the dioxin-fraction and 17 pg TEQ/g in the PCB-fraction. The instrumental analysis for PCDD/F showed 55.6 pg TEQ/g fat and for PBDD/F 22.9 pg TEQ/g fat. This demonstrate that also PBDD/F can be transferred into eggs and accumulate.

PBDD/F are formed from the brominated flame retardants present in e-waste plastic[13]. Such pollution can be expected at sites with e-waste plastic or cable burning and likely result in contamination of free range animals at these sites. It has recently been established in a UK food survey that PBDD/F can also contribute significantly to total dioxin exposure for the UK population[14]. 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.

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

The chicken flocks investigated need further assessment. E.g. the PCDD/F, PCB and PBDD/F levels in soils at the site in Thailand have not yet been measured as well as other sites of the study of IPEN.

While the high levels of PCDD/F and PBDD/F in eggs in Thailand indicates high contamination levels at the site, the carry-over of PBDD/F compared to PCDD/F or PCBs has not yet been assessed and need detailed carry-over studies. Since a large number of e-waste recycling sites exists with increasing recycling volumes and contamination, a detailed assessment of these sites and exposure of the people living at and around these sites are needed including chickens and cattle raised at and around these sites.

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 might exceed 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 such hazardous 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)[15] in an eel from a creek receiving drainage water from the former German capacitor 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 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 became clear that low levels of PCDD/F and dl-PCBs contamination in soils can result in chicken eggs being contaminated above EU 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 seems affected at distances of up to approx. 50 km from a PCB production facility[16]. 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 IPEN global egg studies on PCDD/F levels from developing countries sampled eggs around industrial emission sources including e.g. non-BAT incinerators and metal industries 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 generating larger datasets of egg levels and related soil contamination are recommended for statistically determining problematic soil 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/F 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[17] 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 Thailand show that brominated PBDD/F 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 in the material and waste flow.

***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 re-evaluation of soil limit values and compensation of farmers and private owners***

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.

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