DOI 10.1002/mnfr.200500201 Mol. Nutr. Food Res. 2006, *50*, 908 – 914

Review

Contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls

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Dioxins and dioxin-like (DL) polychlorinated biphenyls (PCB) are persistent organic pollutants that enter the body mainly by food intake. A small margin exists between current exposure levels in the human population and the levels causing biological effects. Therefore, stringent control of concentrations of these contaminants in food and feed is needed. Eggs from free-range chicken are increasingly becoming an important part of the diet. These eggs have a higher risk of being contaminated with increased levels of dioxins and DL-PCB than barn or cage eggs. Ingestion of soil particles from environmentally contaminated areas may contribute to elevated dioxin levels in free-range chicken eggs. Available data show that current soil levels of dioxins and DL-PCB in residential and agricultural areas in Europe often appear to be too high to produce free-range eggs with dioxin levels below the current limit values in the EU. On the other hand, polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans concentrations in eggs from free-range chicken are not necessarily above the limit values. Contamination levels in soil should be kept low and should be controlled in areas with free foraging chicken although all modifying factors that influence uptake of dioxins and PCB from the environment and transfer into eggs are yet not well understood.

Keywords: Chicken eggs / Food contamination / Polychlorinated dibenzo-p-dioxins / Polychlorinated dibenzofurans / Polychlorinated biphenyls

Received: October 17, 2005; revised: January 23, 2006; accepted: January 31, 2006

1 Introduction

Chicken eggs are an important component of the human diet all over the world. Annual *per capita* egg consumption varies in Europe between 296 eggs in Czech Republic and 118 eggs in Greece. In the US, the annual egg consumption is 257, in China 232, in Brazil 94 and, in India 35 [1]. This includes shelled eggs and egg products, the latter constituting about 20% of the total egg consumption. Average EU *per capita* consumption has been rather stable since 1990, oscillating between 12.5 and 13.5 kg/year. In recent years, an increase in the *per capita* consumption has been observed. Of particular importance for the development of overall egg consumption is the growing consumption of convenience food as a result of social trends (increasing single-person households and out-of-home work of women).

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Abbreviations: **DL**, dioxin-like; **PCB**, polychlorinated biphenyls; **PCDD**, polychlorinated dibenzo-p-dioxins; **PCDF**, polychlorinated dibenzofurans; **TEF**, toxic equivalence factor; **TEQ**, toxic equivalents

A second trend in egg production is the growing awareness of animal welfare issues in Central and North-Western Europe. Demands for eggs from other than caged hens have steadily increased. This has led to changes in production methods and strictly regulated marketing standards for eggs. Alternative production methods for eggs from laying hens include "barn reared" hens that remain indoors with a stocking rate per m^2 floor space not exceeding 15 birds and 25 kg live weight. Eggs may be labeled as "free range" if the birds have had during at least half their lifetime continuous daytime access to open-air runs, comprising an area mainly covered by vegetation, of not less than 1 m^2 per chicken [2]. The evolution of alternative hen numbers in the EU increased from some 10 millions in 1991 to 39 millions in 2002, or from 3 to 14% of all hens. UK, Austria, Ireland, Denmark and The Netherlands score more than 20%. This trend is likely to rise in the future. For the UK, *e.g.* it is estimated that by 2012 the egg market will be 50% cage and 50% alternative, of which 42% free range and 8% barn [3]. The organic egg market, however, is of minor importance.

This way of production is similar to those of free-range hens, however, the guidelines for the birds and their feed are more stringent. The pullets must be raised by certified organic production methods from birth. The layers are required to have outdoor access all year round, or be fed sprouted grains for the period when indoors and all feed must be certified organic. No antibiotics or meat by-products are allowed in the feed and each bird is required to have two square feet of floor space [2].

According to a study published in 2002 production of organic eggs in 2000 reached 1.3% on EU average, Denmark being the notable exception with 15%. Except for these large production systems that are strictly regulated, there are also private owners with small numbers of free foraging chicken. These production systems are not regulated. Direct sales from farmers to consumers (organic and free-range eggs) are generally not more than 10% with the exception of Austria and Germany [3].

The different production methods have an influence on animal welfare but also on potential chemical and biological contamination of eggs that is under strict control for eggs that enter the commercial circuit.

There are a number of potential chemical hazards that may lead to egg contamination with, *e.g.* persistent halogenated compounds and pesticides like DDT, dieldrin, hexachlorobenzene, pentachlorophenol, hexachlorocycohexanes, polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). They are expected to be present to some extent in fat-rich animal feeding stuffs and via that route reach the animals [4]. In addition, other sources may contribute to feed contamination, as shown by several feeding incidents such as the Belgian dioxin crisis in 1999 when 200 L of PCB-oil, containing both dioxin-like (DL) PCB and PCDF, was used for the production of chicken and pig feed [5]. During the same year Austrian scientists discovered that some naturally occurring kaolinic clay, used for the production of feed premixes, contained high levels of dioxins and caused elevated dioxin levels in pigs (unpublished). Due to these incidents, the control of point sources has been elaborated and has been shown reasonably successful over the recent years leading to strongly reduced residue levels in commercial eggs [6]. However, some recent reports on PCB and dioxin levels in free-range chicken eggs have shown increased levels and there are strong indications that the environment rather than the feed is the most important contributor [7].

This review brings together these data. Knowledge on contamination sources and the potential implications on daily dietary intake levels of PCDD/F and DL-PCB are discussed. The focus is on PCDD/F and DL-PCB, which are subject to stringent regulations due to their well-known toxicity. These 29 different congeners have in common that they bind to the arylhydrocarbon receptor (AhR). This AhR is a ligand-dependent transcription factor that mediates toxicity and consequently these compounds share toxic effects including immuno- and reproductive toxicity, they negatively influence growth and neurobehavior, and in addition, they have carcinogenic potency [8, 9]. The effects, however, become only manifest after a certain critical body-burden level has been exceeded [7, 10]. PCDD and PCDF are by-products of industrial processes such as waste incineration, metallurgic processing, bleaching of paper pulp and the manufacturing of some herbicides and pesticides, also combustion of organic materials including municipal garbage can be local sources [11]. PCB have been manufactured since 1920 and its major uses were in electronic appliances, heat transfer systems and hydraulic systems. Based on their relative toxic potencies in animal studies and *in vitro* models, individual congeners of dioxins, furans and PCB have been assigned a toxic equivalence factor (TEF) that relates their toxicity to that of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which is the most toxic congener. Each congener concentration in a mixture is multiplied with its TEF and the resulting TCDD equivalents are added up and expressed as toxic equivalents (TEQ). Most recent TEF have been set under supervision of the WHO in 1998 and levels of DL compounds are often expressed in WHO-TEQ (TEF assignment according to WHO, 1998), whereas older data used so-called I-TEF values set by NATO and expressed the sum as I-TEQ [12]. In the following, we will explicitly mention when the I-TEF values are used, if nothing is mentioned WHO-TEF values are used. Differences between TEF may result in differences in TEQ-levels, in general the use of the most recent WHO-TEF values lead to 10–15% higher levels compared to the use of I-TEF values.

2 An overview of recent data on dioxin and DL-PCB levels in chicken eggs

A survey by the European commission of PCDD/F levels in eggs, sampled between 1987 and 1999, reported by eight countries showed levels ranging from 0.5–7.3 pg I-TEQ/g fat. Data on DL-PCB were scarce. The few data submitted to the EU by the participating countries revealed a ratio of PCDD/F and DL-PCB of roughly 2:1 to 1:1.5 [13]. More recent data provided by the member states on chlorinated dioxins, furans and DL-PCB in food [14] showed an average of 0.63 and 0.56 pg TEQ/g fat for PCDD/F and DL-PCB (68 samples) with maximum levels up to 7.7 and 3.93 pg TEQ/g fat, respectively. More detailed analysis of these data indicated increased levels of PCDD/F in eggs from free-range chicken compared to levels in conventionally produced cage eggs (Table 1). Cage eggs are usually far below the current limit value for dioxins of 3 pg TEQ/g fat (EU council regulation No 2375/2001), but almost 10%

Table 1. EU reported levels in eggs from caged and free-range chicken [14]

	PCDD/F pg TEQ/g fat		DL-PCB pg TEQ/g fat	
	Median	95th percentile	Median	95th percentile
Cage	0.38	0.83	0.29	1.74
Free range	0.85	3.36	0.34	3.97

of the eggs produced in alternative systems exceed this limit value. In addition, the levels of DL-PCB are on average higher in free-range and organic-farmed chicken eggs than in cage eggs. In general, data on free-range chicken in these databases are too scarce to give proper insight to this issue.

Individual more targeted studies have given more details on increased levels of dioxins/furans and DL-PCB in eggs from free-range chicken (Table 2). These data clearly show that average levels are increased and that a fraction of the eggs (10–15%) does not comply with the current limit of 3 pg TEQ/g fat for dioxins, and future limit of 6 pg TEQ/g fat for total TEQ (dioxins and DL-PCB). The major question is what are the sources of the contamination and whether these can be removed.

Potential pathways for accumulation of dioxins and PCB in poultry include uptake from commercial feed, drinking water, bedding, soil, vegetation and annelids like earthworms. Concentrations of dioxins in feed and particularly feed ingredients have to comply with the strict EU regulation and therefore are checked regularly. As a result, levels in feed will be several factors below the feed limit for dioxins of 0.75 ng TEQ/kg (EU regulation 466/2001). Drinking water contains virtually no PCB and dioxins because these compounds are lipophilic with an octanol water partition coefficient above 4.

As shown below, several studies have shown that soil is a potential source of contamination with dioxins and PCB. Many variables influence uptake of the contaminants from soil and transfer to eggs. These are, *e.g.* the concentration of the chemicals in soils, the congener pattern, the bioavailability, which is a function of the soil composition, soil coverage with vegetation or other materials, the presence of organisms in soil, the time the chickens are kept outdoor, the availability of feed, and the foraging behavior of the hens.

3 Uptake from feed

Feed may be an important source of contamination. During the Belgian dioxin crisis, *e.g.* feed contaminated with dioxins and PCB resulted in dioxin levels of eggs up to 713 pg I-TEQ/g fat, as well as even higher levels of DL-PCB. The indicator PCB pattern reflected the Aroclor1260 congener pattern [5, 15]. If feed from the Belgian dioxin crisis was experimentally administered to laying hens, levels of dioxins and PCB in eggs started to increase rapidly during the 7-day period in which these hens received the contaminated feed. When administration of this diet was terminated, the dioxin levels decreased to about 40% after 21 days on clean feed [16]. The use of contaminated kaolinic clay, as an anti-caking agent in feeding stuffs, resulted in an elevated contamination of 13 pg I-TEQ/g fat in chicken eggs [17]. Due to incidents like these, PCDD/F levels in commercial feed are now strictly regulated. Data on contamination levels indicate that feed with PCDD/F concentrations below the EU feed limit of 0.75 ng PCDD/F TEQ/kg in general does not yield concentrations in eggs above the EU limit of 3 pg TEQ/g fat [18]. However, experimental studies with laying hens showed that levels in eggs eventually exceeded the limit if the hens were fed feed contaminated with levels as low as 0.4 ng TEQ/kg. Consequently, lower limits are

Table 2. Overview of concentrations of PCDD/F and DL-PCB in egg samples from free-range chicken

	Number of egg samples	pg TEQ/g fatPCDD/F		pg TEQ/g fatDL-PCBs		Country	Ref.
		Mean	Min-max	Mean	Min-max		
Free range	8		2.3–19			Switzerland	[24]
Free range			0.4–22.8			Germany	[36]
Free range	53	4.4	<DL-22.8			Germany	[37]
Organic	6		0.7–7.7		0.7–5.8	Netherlands	[25]
Free range	19	12.9	2.1–26I-TEQ			United Kingdom	[21] After fly ash removal
Free range	11		6.3–121.6		0.35–46.4	France	[22] MSWI surroundings
Free range	15	9.9				Belgium	Private gardens [24]
Free range	35		0.4–18		0.4–270	Belgium	Private farms
Free range	16	0.5	<DL-0.8	0.3	0.4	Ireland	[27]
Organic	4	1.3	2.7	1.4	3.9	Ireland	[27]

required to guarantee that egg levels remain below the limit (Hoogenboom, L. A. P., Kan, C. A., Zeilmaker, M. J., van Eijkeren, J. C. H., *et al. Food additives and Contaminants*; submitted). This experiment showed no equilibrium of the levels in eggs during the exposure period of 56 days. Between 5 and 30% of any congener ingested during that period was excreted in the eggs. Bioconcentration and carry-over factors were higher for the lower chlorinated congeners than for the higher ones, which may be of relevance when dealing with, *e.g.* the higher chlorinated congeners in pentachlorophenol related incidents. After termination of administration of the diet, both dioxins and PCB showed a biphasic elimination rate with half-lives of 2–3 days for the first phase and 30–60 days for the second phase (Eijkeren van, J. C. H., Zeilmaker, M. J., Kan, C. A., Traag, W. A., Hoogenboom, L. A. P., *Food Additives and Contaminants*; submitted).

This study highlights the necessity for strict control of feed and in particular feed ingredient levels, thus insuring that feed levels will in general be well below the limits.

4 Uptake from soil

Bioavailability of dioxins from soil was demonstrated in experimental studies in which chicken received PCDD/F-contaminated soil (pentachlorophenol source) mixed in their diet for 178 days [19]. Uptake and transfer of PCDD/F from soil to the eggs was observed. In this case, the levels in eggs reached equilibrium after 30–60 days.

The influence of environmental soil contamination on levels of PCDD/F in free-range chicken eggs was demonstrated in several studies. Elevated levels in eggs have been recorded in areas with increased soil contamination. In a UK survey, concentrations of PCB and PCDD/PCDF in duck and bantam eggs from smallholding farms close to a chemical waste incinerator, were considerably higher than those found elsewhere [20]. Chicken kept in pens in which incinerator ash has been used showed elevated levels of PCDD/PCDF in eggs [21]. The congener pattern in 17 of the 19 egg samples was similar to that in ash, indicating a link with the use of the incinerator ash. Twenty months after removal of the contaminated fly ash, average levels in eggs were reduced from 16.4 pg/g I-TEQ to 9.4 pg/g I-TEQ, which is still much higher than levels found in barn eggs. In a study in the Northern France, eggs collected from chicken kept for foraging in private gardens at ten sites between 1000 and 1500 m away from a municipal waste incinerator under the prevailing wind stream showed all egg samples with total TEQ values above 3 pg TEQ/g fat while soil levels were between 4 and 61 pg total TEQ/g dry matter [22]. On a contaminated area close to a pentachlorophenol wood treatment facility in Oroville (USA), soil concentrations of around 6 ng I-TEQ/kg (range 1.5–46) resulted in egg levels of 20–50 pg I-TEQ/g fat (range 0.8–140). The fraction of eggs exceeding 10 pg I-TEQ/g was around 70–90% [23].

However, apart from these rather local and restricted contamination sites, also at rural sites with low soil contamination elevated concentrations in eggs were sometimes observed. In a Swiss study, in which soil levels were between 1.3 and 11 pg PCDD/F TEQ/g dry matter, only one of the eight egg samples tested was below the current limit of 3 pg PCDD/F TEQ/g fat [24]. Similar to previous observations (Hoogenboom, L. A. P., Kan, C. A., Zeilmaker, M. J., van Eijkeren, J. C. H., *et al. Food additives and Contaminants*; submitted), congeners with a lower chlorine content were transferred easier into eggs than congeners with more chlorine atoms. Transfer rates decreased by more than one order of magnitude from the PCDD/F to OCDD/F. This can be explained by the decreasing bioavailability of congeners with higher chlorine content.

A study in The Netherlands showed elevated PCDD/F concentrations in egg samples from three out of seven organic farms, soil and straw samples from one of the affected farms showed total TEQ levels of, respectively, 1.3 and 3 ng TEQ/kg, feed and drinking water samples did not contain significant levels [25]. In the same study, eggs from six free-range farms, where chicken were allowed but not forced to go outside, did not show elevated levels. A more elaborate study by the Dutch Food Inspection Service confirmed that the problem was more widespread and affected about 9% of the farms [26,27]. In particular, the smaller farms produced eggs that exceeded the limit [24,27]. Thirteen percent of the eggs coming from 24% of the farms were affected with levels occasionally up to 10–15 pg TEQ/g fat [27]. This indicates that not only chicken from smaller farms but also in particular chicken from private owners may contain the highest levels. Feed levels were low, pointing to soil as the major source of contamination of the eggs. However, there was no clear relation between levels in soil or levels in worms and levels in eggs, indicating that other factors influencing the actual soil uptake are more important. Soil levels were in general very low in the Dutch study and exclude that the contamination is restricted to contaminated areas only.

Eggs laid by free-range hens kept in private gardens in the Northern part of Belgium showed an average level of 9.9 pg TEQ/g fat. In the same study, samples from eggs produced in organic farms were below the EU action limit of 2 pg TEQ/g fat. Soil samples from the same district showed levels up to 6 ng/kg dry matter. The soil profile was dominated by the OCDD congener, which was also the dominant congener in the eggs [28]. Also, egg samples taken from free-range chicken, in seven "green areas" in the Northern part of Belgium, which had low dioxin deposition levels (below ≤ 6 pg WHO-TEQ/m^2/day) showed levels up to 18 pg PCDD/F TEQ/g fat, one sample showed extremely high levels of non-ortho DL-PCB TEQ (270 pg TEQ/g fat) (Schoeters, 2005 unpublished results).

In farms from South Germany [29] egg levels around 8 pg PCDD/F TEQ/g fat and chicken body fat levels up to 50 pg PCDD/F TEQ/g were measured. A very peculiar contamination case was described, showing high levels of higher chlorinated dioxins in eggs of up to 88 pg TEQ/g fat due to the use of wood shavings that were contaminated with pentachlorophenol [30]. These data show that the problem is not restricted to certain countries and that increased and targeted monitoring of eggs produced by chicken that really forage outside is likely to show the more general nature of this phenomenon.

Ingestion of soil may be partly responsible for the observed elevation in dioxin and DL-PCB levels. Assuming a soil contamination of 10 ng TEQ/kg dry matter, which is an upper level found in rural areas in Western Europe, and an average daily intake of soil of 10 g/day by free foraging chicken (average daily feed intake 100 g [19]), a complete absorption of the PCDD/F present in the soil sample and 30% transfer into the egg (Hoogenboom, L. A. P., Kan, C. A., Zeilmaker, M. J., van Eijkeren, J. C. H., *et al. Food additives and Contaminants*; submitted) would result in a contamination level as high as 5 pg TEQ/g fat (6 g of fat/egg), being well above the current EU limit for PCDD/F concentrations in eggs. The assumptions may be conservative, but we cannot exclude that at many locations current PCDD/F levels in soil are not fully compatible with having laying hens foraging outdoor. Other parameters such as the number of animals per square meter may also have some influence. Chicken eggs showed higher levels of contamination if the chicken had more space available [24]. Except for soil particles that are a supply for minerals, chicken like to eat also grains, grass, insects and annelids, which provide them with calcium and proteins. Their consequence for egg contamination with dioxins and PCB is unknown. In addition, other variables such as foraging behavior, age of the chicken, laying frequency, type of domestic heating, frequency of backyard household waste incineration may contribute as well. The time spent outdoors, which is at least 8 h/day for organic chicken, may influence the PCDD/F concentration in eggs as well. The study of Pirard [22] examined some of these parameters but could not explain the contamination levels in the eggs; the study design may not have been sufficient to investigate the role of all these parameters.

Free-range chicken eggs have not *a priori* higher levels of PCDD/F and DL-PCB than eggs from caged chicken. A recent Irish survey [31] showed no significant differences in levels of PCDD/PCDF and dioxin like PCB between eggs from free-range chicken and from caged chicken. Sixteen samples were analyzed each time. In the same study, eggs from organic farming showed elevated levels (5.2 pg total TEQ/g fat) although only four samples were analyzed. There was a close correlation between the age of the laying hens and the levels of dioxins and DL-PCB found in the eggs. Seven organic egg samples from free-range chicken analyzed in a study in Belgium did not show elevated levels of dioxins [32].

5 Congener pattern

In general, the congener profile of dioxins/furans is comparable for free-range eggs and cage eggs, indicating the influence of a general background contamination. OCDD is the dominant congener in egg samples but also in most of the soil samples tested. In cases of accidental contamination, the congener profile in eggs reflect the source of contamination. In the Belgian dioxin crisis, furans were, dominant [5].

The few data submitted to the EU by the participating countries and reported in the EU SCOOP report revealed a ratio of PCDD/F and DL-PCB of roughly 2 : 1 to 1 : 1.5 [13]. Data collected in the EU between 1997 and 2004 [14] showed that DL-PCB contributed on average 48% to total TEQ values in eggs. The Irish study [31] showed that non-ortho and mono-ortho DL-PCB contributed between 40 and 47% to the total TEQ measured in the case of battery, free-range and barn eggs and slightly above 50% in the case of the organic-egg samples. In this study, PCB 77 was the most abundant non-ortho DL-PCB detected, at levels of 5–20 times that of PCB126, depending on egg type, while PCB118 was the most abundant of the DL mono-ortho PCB [31].

6 Contribution to human body burden

Dietary fat intake is considered the major source of dioxins and DL-PCB in humans. Different surveys in Europe on food consumption patterns reveal that eggs contribute about 2–4% to daily fat intake [33, 34]. Based on mean concentrations of dioxins and DL-PCB in eggs between 0.6 and 2.4 pg TEQ/g fat it was shown that eggs in general contribute about 4% to the daily intake of PCDD/F and DL-PCBs [33, 34]. Average daily dietary intake levels were 95.4 pg TEQ PCDD/F in Catalonia [33] and 84 pg TEQ for the sum of PCDD/PCDF and DL-PCB in The Netherlands [34]. This is in the same range as reported for other countries.

It may well be anticipated that home produced eggs are eaten regularly by the same local consumers. Daily consumption of one egg containing 10 pg TEQ/g fat means an intake of 60 pg TEQ. For an adult person of 70-kg body weight this represents already 42% of the proposed tolerable weekly intake (PTWI) of 14 pg/kg body weight for dioxins as proposed by the European Scientific committee for food in 2001 [7]. In addition, children may consume these eggs regularly. Due to the low body weight, their risk for exceeding the PTWI of 14 pg/kg body weight is high.

Impact of increased concentrations of dioxins in eggs on body burden has been observed in the USA, where elevated serum levels of tetra, penta and hexa dioxin levels were found in residents consuming home-produced eggs in an area, which was contaminated due to accidental burning of pentachlorophenol. An apparent dose-response relationship existed with diet and/or length of exposure. Levels in eggs were between 3.5 and 14 pg EPA TEQ/g fat [35].

7 Conclusion

Different studies show that eggs from free-range chicken, and in particular organically and home-raised ones, have a higher risk of being contaminated with dioxins and DL-PCB. These contaminants are transferred from feed, soil or organisms living in the soil into fat from chicken eggs. Dioxin levels in feed and feed ingredients are under strict control and feed, which complies with the stringent regulatory limits, is not assumed to be the source of elevated levels in chicken eggs except for accidental contaminations of feed such as occurred in the Belgian dioxin crisis. Dioxins present in soil may be the major source of the elevated dioxin levels in eggs from chicken that forage outside. Current soil levels of dioxins and DL-PCB in residential and agricultural areas in Europe often appear to be too high to produce free-range eggs with dioxin levels below the current limit values in the EU.

On the other hand, PCDD/F concentrations in eggs from free-range chicken are not necessarily above the limit values, indicating that sources or other relevant factors may be adequately controlled if they are elucidated. No systematic analysis has been reported yet identifying the factors from the external environment that influence and modify the dioxin and PCB levels in eggs from free-range chicken. If this information becomes available, appropriate measures can be taken to protect consumers of free-range and in particular organic or home-produced eggs similar to consumers of barn or cage eggs. In particular, consumers that eat their own eggs or buy from local small farms may be at risk of having an elevated dioxin intake.

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