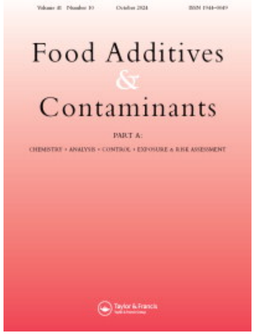

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Polychlorinated dibenzo-*p*-dioxin and dibenzofuran contamination of free-range eggs: estimation of the laying hen's soil ingestion based on a toxicokinetic model, and human consumption recommendations

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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are ubiquitous in the environment. The main route of human exposure is through food consumption. Soil contamination can be problematic for sanitary safety depending on the usage of the soil, such as farming. In case of environmental soil contamination with PCDD/Fs, hen's eggs may be contaminated due to soil ingestion by hens. For this reason, it is important to understand the parameters that influence eggs' contamination when hens are raised in contaminated areas. After the discovery of a contaminated area in Lausanne (Switzerland), we collected hens' eggs from ten domestic-produced eggs and one farm. Based on PCDD/F measurements of eggs and soil, and a toxicokinetic model, we estimated individual hen's soil intake levels and highlighted appropriate parameters to predict the dose ingested. Recommended weekly consumption for home-produced eggs was calculated based on the tolerable weekly intake proposed by EFSA in 2018. The most important parameter to assess the soil ingestion does not seem to be the soil coverage by vegetation but rather the hen's pecking behaviour, the latter being difficult to estimate objectively. For this reason, we recommend using a realistic soil ingestion interval to assess the distribution of egg PCDD/F concentration from free-range hens reared on contaminated soil. The addition of soil contamination in the toxicokinetic model can then be used to recommend to the general population weekly consumption of eggs. The consumption by adults of free-range eggs produced on land with soil containing >90 ng toxic-equivalent (TEQ)/kg dry soil should be avoided. Even with a low level of soil contamination (1–5 ng TEQ/kg dry soil), we would recommend consuming not more than 5 eggs per week for adults and no more than 2 eggs for children below 4 years old.

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Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) are ubiquitous environmental contaminants encompassing 210 aromatic organochlorine congeners. These chemicals are produced during combustion and thermal processes, unintentionally released, and carried in the environment by combustion fumes mostly from anthropogenic emissions like municipal solid waste incinerators, paper-pulp chlorine bleaching processes, and pesticide production (Weber et al. 2018; Petrlik et al. 2022). Once deposited after their atmospheric transport, PCDD/Fs tends to accumulate in topsoil due to their low mobility and high persistence with a half-life estimated at 25 to 100 years, depending on the soil's properties and the PCDD/F congeners (Bunge and Lechner 2009; Nhung et al. 2022).

Acute manifestations of exposure to PCDD/Fs in human include liver damage and chloracne. On a chronic level, PCDD/Fs exposure has been associated to immune system suppression, reproductive toxicity, and cancer (Watanabe et al. 1999; Popp et al. 2006; Mocarelli et al. 2008; Simon et al. 2009). Most of the adverse effects may be enhanced by aryl hydrocarbon receptor (AhR)

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binding of the PCDD/Fs (Safe et al. 2018). The most sensitive effect was observed on the reproductive system, specifically sperm quality. A recent study in young adults showed an association between high PCDD/F serum concentration and low sperm quality (Mocarelli et al. 2008; Mínguez-Alarcón et al. 2017). The no observed adverse effect level (NOAEL) found in the Minguez-Alacron study was used by the European Food Safety Authority (EFSA) to propose a tolerable weekly intake (TWI) of 2 pg toxic-equivalent (TEQ)/kg body weight (bw)/week (Mínguez-Alarcón et al. 2017; Knutsen et al. 2018). This level should prevent adverse effect on sperm production.

Since PCDD/Fs are usually present as a mixture of the different congeners with different toxicity, a cumulative sum should be used as a reference. The principle of the TEQ is based on the mode of action (mediated though the AhR activation) of persistent organic pollutants (POPs). It compares the relative effective potencies of individual compounds (PCDD/F congeners or dioxin-like compounds) to activate the AhR relative to the referent compound: 2,3,7,8-TCDD. The PCDD/F total TEQ is the sum of the PCDD/F congener concentrations, each multiplied by their respective toxic equivalent factor (TEF) (Van den Berg et al. 2006; IARC 2018). The TEF values established for each compound have been harmonized by WHO since the early 1990's and have been updated three times (TEQ-1993, -1997, -2005, and recently -2022) (DeVito et al. 2024).

The primary pathway for environmental contaminants entry into the terrestrial food chain is by atmospheric deposition, pesticide use or sewage sludge dispersion on soil then ingested by farm animals (Van Overmeire et al. 2009; Domingo 2014; Shen et al. 2017; Weber et al. 2018; Lambiase et al. 2022; Petrlik et al. 2022). Having entered the animal, PCDD/Fs are distributed mainly into fat-rich tissues (adipose tissues) and excreta (egg yolk and milk) due to their high lipophilicity. Therefore, it is important to improve our knowledge on the pathway between environmental contamination and the food products of animal origin. Free-range eggs represent a potential risk of human health due to their higher sensitivity to contamination by environmental pollutants. Hens' intake of contaminants happens mainly due to their pecking behaviour. The soil ingestion is supposedly accidental although some studies consider that it could act as a medication (De Vries et al. 2006; Lambiase et al. 2022). In addition to soil ingestion, an additional source of contamination is due to pedofauna (worms and insects) ingestion (Schuler et al. 1997; Kijlstra 2004). The digestive absorption of PCDD/Fs is an important aspect driven by bioaccessibility and bioavailability factors, which can be specific to individual congeners (Notenboom et al. 2023). Once absorbed, the PCDD/Fs are distributed to fat-rich tissues (i.e. adipose tissues) that account for most of a hen's body burden, which may decrease by metabolism (especially in the liver) or elimination *via* fat-rich excreta, mainly the eggs' yolks in laying hens (Amutova et al. 2021). A two-compartments dynamic model was proposed to describe the toxicokinetics of PCDD/Fs in hens and their transfer to eggs for the sum TEQ (Van Eijkeren et al. 2006) or at the individual congener level (Notenboom et al. 2023). The results can vary notably when considering only the total sum-TEQ compared to the specific congeners (Lorber 2002). The current model was optimized based on a laying hen toxicokinetic study dealing with artificial PCDD/F exposure *via* a contaminated or spiked feed (Notenboom et al. 2023). It is important to implement the soil ingestion component to the current model. Although the free-range eggs production method is beneficial in many aspects (chicken wellbeing, antibiotic use), these eggs showed higher PCDD/Fs contamination since the chickens have access to soil potentially contaminated with PCDD/Fs even at low levels compared to eggs produced in battery-rearing (Efsa 2012; Weber et al. 2018).

The objectives of this study were (1) to implement a factor in the existing toxicokinetic model when hens are reared on contaminated soil linked to the soil ingestion, based on joint measurements of PCDD/F concentrations in eggs and soil, and (2) on this basis, to assess the maximum frequency of egg consumption by adults and children to avoid exceeding the TWI proposed by EFSA (EFSA, 2018). To address those objectives, egg's PCDD/Fs contamination coming from a hen's exposure from soil ingestion in a home-produced henhouse in Lausanne (Switzerland) was investigated in this study. The Lausanne area is affected by a high PCDD/Fs contamination due to an old incinerator emitting probably until the late 1990s. The contamination was only discovered recently in December 2020 after a routine sampling campaign to analyse common pollutants. Unexpected high concentrations were measured, resulting in pollution mapping of the urban and peri-urban area, and the human PCDD/F exposure assess for targeted risk management (Vernez et al. 2023).

Materials and methods

On-field study design

After the discovery of the soil contamination, the initial risk assessment highlighted the ingestion of contaminated eggs as one of the main PCDD/F exposure routes for the inhabitants of the Lausanne area (Vernez et al. 2023). Following the soil contamination mapping, ten domestic hen owners and one farm were located in the contaminated area with soil contamination between 9 to 92 ng TEQ/kg dry soil (detailed information is provided in Supplementary Table S1). The flock size varied between 2 and 250 chickens. A total of 27 egg yolk samples were analysed for PCDD/Fs, the samples being the results of pooled egg yolks (several eggs from different hens but the same henhouse pooled and analysed together). At four different locations (within the 11 henhouses investigated), we sampled eggs at different time points. We also sampled several individual eggs at an individual location on a single day and analysed them separately. The soil PCDD/F concentration was measured on sample either taken directly in the outdoor run paddock ($n = 8$) or in a plot nearby not accessible to the hens ($n = 3$).

Soil and egg samples collection

The methodology for soil sampling consisted of stratified random sampling in accordance with the Soil Sampling Manual (FOEN 2003). Areas of $10\,m \times 10$ m square were marked out, then divided into 16 sub-squares of equal size ($2.5\,m \times 2.5$ m), and a sub-sample taken randomly from each. If the configuration of the sampling area did not allow a $10\,m \times 10$ m square to be set up (due to obstacles, land shape or access) an area of approximately 100 m^2 was defined and divided into 16 sub-sectors to sample in a similar way. Soil sub-samples were taken from the 0–20 cm top-layer using a hand auger. The 16 individual soil sub-samples collected were then pooled and homogenised by hand in a plastic bucket to form a composite sample for each area. The organic debris present in the collected samples was preserved. Once the composite soil sample was sufficiently homogeneous, an aliquot was removed and placed in a polyethylene container to be sent to the analysis laboratory. The soil samples were dried in an oven at 40 °C until their weight stabilized. Once dried, the samples were sieved to 2 mm.

The eggs were collected and analysed between September 2021 and January 2023. Egg sampling was performed differently across the sites and time. Individual egg yolks from a same henhouse and day were pooled (from 2 to 12 eggs) for the PCDD/F analysis in most of the case. At the professional farm, 10 eggs laid on the same day were analysed separately to assess inter-individual variability between hens. The analysis was conducted by two laboratories, one in Switzerland and one in Germany (22 and 5 samples, respectively).

PCDD/F analysis

Soil sample extraction was performed using two different methods. Over the 11 sites, nine soil samples were extracted using the Soxhlet method and two using the Accelerated Solvent Extraction (ASE) system (Dionex ASE). The samples were quantified using high resolution gas chromatography (HR GC-MS). In total, 17 PCDD/Fs were quantitated using the validated method (norm DIN EN 16190: 2019-10). The effectiveness of the two soil extraction methods was compared in a previous independent study (unpublished). In the specific context of contamination in the city of Lausanne, an average increase of 42.5% was observed between samples extracted using the ASE method and those extracted using the Soxhlet method. This factor was used to adjust the TEQ concentration (Mayer and Hamm 2021) and can only be used with the TEQ concentration. We used the TEF from WHO-2005 and only included the 17 PCDD/Fs congeners. We did not correct the concentration for the PCDD/Fs congeners between the two analytical methods for the soil sample.

The analytes were cleaned up by a liquid-liquid extraction for the egg yolks (Schmid et al. 2002). The PCDD/Fs were measured by high resolution gas chromatography combined with triple quadrupole mass spectrometry in the daughter ion scan mode (MS/MS; Xevo-TQ-XS APGC from Waters) (Driesen et al. 2022). The concentrations in the eggs were reported normalized on the basis of yolk lipids. For the calculation of TEQ concentrations, the Toxic Equivalent Factor (TEF) from WHO-2005 were used (van den Berg et al. 2017).

Assessment of the exposure to PCDD/Fs through soil ingestion

Toxicokinetic model of PCDD/F fate in laying hen

Simulation of PCDD/F transfer from the soil to the hen's body and egg was based on the two-compartments toxicokinetic model initially described by Van Eijkeren et al. (2006) (Figure 1). The contaminant enters the hen's body through the ingestion of contaminated soil. Once absorbed, the contaminant is distributed between the central (A_c) and the body fat (A_f) compartments (Figure 1). The contaminant can further be metabolized from the central compartment through hepatic clearance (A_{el}) or be excreted through the egg yolk lipids (A_{yel}).

The set of Equations (1–5) define the dynamic mass balances, whereas the physiological constants and other variables are reported in Table 1. The model was mathematically formulated as two mass balances changing over time in the two compartments. The model was run for the sum TEQ concentration (Van Eijkeren et al. 2006) and then for the congener-specific analysis (Notenboom et al. 2023). The kinetic constants (the mass transfer rates from the central to the fat compartment (q_c) or from the fat to the central compartment (q_f), the hepatic clearance rate (k), and the excretion rate to the egg yolk fat (ε_y)) were taken from Notenboom et al. (2023) and Van Eijkeren et al. (2006). These parameters (q_c, q_f, ε_y, and k), were previously optimized for each congener for the congener-specific modelling approach (Notenboom et al. 2023). Briefly, the parameters linked to the distribution between the fat and the central compartment can be uniquely estimated. The other parameters can be estimated as an optimal solution based on the lower and upper boundaries (i.e. k_{min} and k_{max}). Parameters of egg weight, fraction of yolk weight to egg weight and proportion of lipid into the

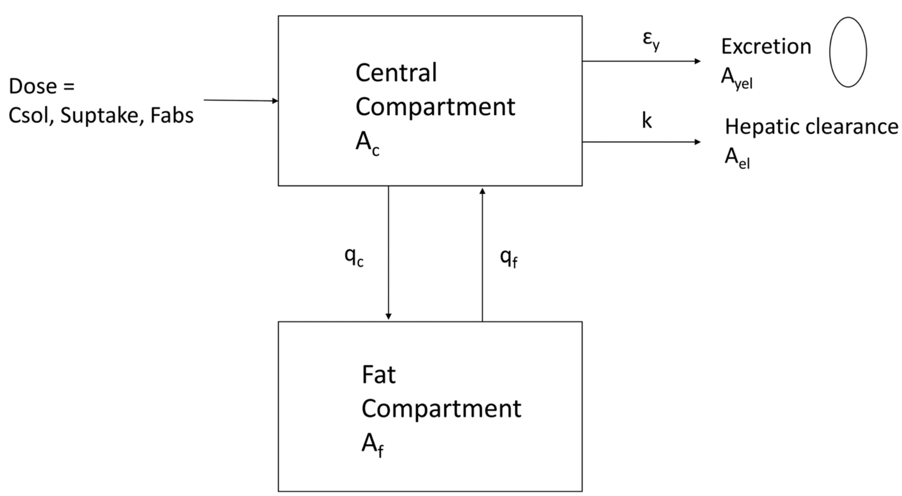

Figure 1. Two compartments and kinetic variables considered in the TK model for the distribution of PCDD/Fs in laying hen. A defines the amount of contaminants in the compartments (A_f and A_c, respectively fat and central compartment). The transfer rate from the central to the fat is define as q_c, and vice versa for the fat to the central (q_f). The dose absorbed is depends on the soil concentration (Csol), the soil ingestion (Suptake) and the fraction of contaminant absorbed (Fabs). The elimination is either done through the hepatic clearance (k) or the excretion with egg yolk lipids (ε_y).

Table 1. Physiological constants and other variables used in the toxicokinetic model.

Acronym	Description	Units	Value
Physiological constants – flux			
q_c	PCDD/Fs transfer from central compartment (Ac) to the fat compartment (Af)	day^{-1}	0.168 or congener-specific
q_f	PCDD/Fs transfer from Af to Ac	day^{-1}	0.0776 or congener-specific
k	Clearance elimination rate	day^{-1}	0.0056
E_y	Elimination through the egg's yolk	day^{-1}	0.0489 or congener-specific
Parameters eggs			
W_{egg}	weight of the egg	g	60
F_{yolk}	fraction of yolk in the egg	–	0.32
F_{Yfat}	fraction of fat in the yolk	–	0.3
ε	Laying efficiency	–	0.9
Exposure			
C_{soil}	Soil PCDD/Fs concentration	ng/kg dry matter	Variable
S_{uptake}	Ingested soil mass per day	g dry soil / day	5-30
$B_{availability}$	Bioavailability constant	–	0.5 or congener-specific
$B_{accessibility}$	Bioaccessibility constant	–	congener-specific
$T_{exposure}$	Exposure duration	day	Variable

yolk were used to calculate the PCDD/Fs egg yolk concentration and taken from the publish literature (Gilbert 1971; Van Eijkeren et al. 2006). Since we assumed that there was no feed contamination, the absorbed dose was calculated as a function of the soil concentration (C_{soil}), the amount of soil ingested by the hen (S_{uptake}), and the fraction of PCDD/Fs absorbed when carried from soil (F_{abs}).

The model is described with the following set of equations:

$$Dose = C_{sol} * S_{uptake} * F_{abs}, \text{ where } F_{abs} = B_{accessibility\_soil} * F_{abs\_free} \quad (1)$$

$$(dA_c)/dt = Dose \ (q_c + k + \varepsilon_y) * A_c + q_f * A_f \quad (2)$$

$$(dA_f)/dt = q_c * A_c - q_f * A_f \quad (3)$$

$$(dA_{yel})/dt = \varepsilon_y * A_c \quad (4)$$

$$(dA_{el})/dt = k * A_c \quad (5)$$

Parameters including age of the hens, and amount of time (in days) spent on the contaminated soil, were collected during the study by a questionnaire to the hens' owner and reported in Table S2. The laying rate was estimated to be constant over the different sampling sites and was set at 90 or 100% (i.e. nine or ten eggs produced per hen over ten days). The weight of the egg (W_{egg}), the fraction of yolk in the egg (F_{yolk}) and the fraction of lipids in the yolk (F_{yfat}) was also assumed constant. The weight of yolk lipids (W_{yf}) was calculated with Equation (6) below:

$$W_{yf} = F_{yfat} * F_{yolk} * W_{egg} \quad (6)$$

The fraction of absorbed PCDD/Fs is congener specific and is dependent on two factors. The soil bioaccessibility ($B_{accessibility\_soil}$) is the fraction available from the soil in the digestive tract for the absorption process, and the absorption fraction (F_{abs_free}) includes the transfer rate though the digestive tract to the organs (Wittsiepe et al. 2007; Roberts et al. 2019). In the initial model described by Van Eijkeren et al. (2006), the fraction absorbed from a free matrix (spiked feed that is assumed to be 100% bioaccessible) was set at 0.885 for the sum TEQ. In the case of soil ingestion, we set the initial sum TEQ fraction absorbed (F_{abs_free}) at 0.5 to consider the environmental bioavailability. The congener-specific approach of the kinetic model assesses the significant difference in absorption in the case of mixture components (Notenboom et al. 2023). Roberts et al. (2019) gives a range for bioaccessible fraction from soil (%) and our calculation were based on the mean value. The free fraction from the soil showed no evidence in trend related to congener chlorine content. A summary of the absorption fraction and bioaccessibility congener-specific is given in Table S2.

Some other congeners were below the LOQ for most of the soil samples ($n = 4$, TCDD, 1,2,3,4,6,7,8-HpCDD, OCDD and OCDF) or one of the factors was not assessed in the literature ($n = 1$, the bioaccessibility from the soil for 1,2,3,7,8,9-HxCDF) (Roberts et al. 2019).

We estimated the daily soil ingestion based on the number of hens per surface area (in m^2) and the soil cover by herbage as suggested by Waegeneers et al. (2009). These specific parameters of each henhouse's outdoor run were collected during an onsite visit organized for most of the cases ($n = 19$ on 27). When an onsite visit was not possible, pictures from the site were used to assess the soil coverage and therefore to estimate the daily soil ingestion. The results from the toxicokinetic model evaluated the expected concentration in the eggs with an ingested soil mass set by visual interpretation of the field and the published literature on soil ingestion (Waegeneers et al. 2009).

In this study, the goal was to adjust the model for soil contamination and soil ingestion. After the first estimation of the model, reverse dosimetry simulations were performed to estimate the daily soil intake. The reverse dosimetry calculations were done using the model simulation from the sum TEQ concentration. First, we estimated the ingested dose to reach the concentration at the time of sampling. Then, the soil ingestion was calculated based on Equation (1). The new soil ingestion based on the reverse dosimetry was used to calculate the concentrations of the 17 congeners and summed with their respective TEF to estimate the final TEQ concentration in the eggs at a given time.

Human risk assessment due to contaminated eggs consumption

Based on the simulation from the toxicokinetic model, we evaluated the health risk of PCDD/F ingestion when eggs are consumed by human. The risk assessment was performed with the TEQ concentration. To calculate the dose ingested when eating eggs, the following parameters specific to the eggs were taken from Van Eijkeren et al. (2006): weight of an egg, fraction of yolk in an egg, and fraction of fat in the yolk. We determined at which point the recommended tolerable weekly intake from EFSA (2 pg/kg bw/week) was reached (Simon et al. 2009; Knutsen et al. 2018). The recommendations are discussed for adults (with a body weight of 70 kg) and for children of 15 and 30 kg. On average, in Switzerland these weights represent children of 3–4 years old and 9–10 years old (Eiholzer et al. 2019).

Results and discussion

Comparison between the modelling results and the in-situ measurements

Overall, the eggs were taken from laying hen having access to outdoor runs with soils contaminated with 9 to 92 ng TEQ/kg dry soil. The eggs contaminations varied from 0.5 to 34.2 pg TEQ/g lipids. The exposure times of the laying hens were 35 days to 5 years (Table S1). The highest contaminated egg (34.2 pg TEQ/g lipids) was found close to the epicentre of the contaminated area (soil concentration: 92 ng TEQ/kg dry soil). A similar correlation between soil contamination and egg contamination was found in Germany (LANUV 2019). Fifty-five percent of the egg yolks analysed had PCDD/F levels higher than the maximum limit set at 2.5 pg TEQ/g lipids by Commission Regulation (EU) n°2023/915 (repealing Regulation n°1881/2006) for PCDD/F levels in egg. All the eggs from areas above 20 ng TEQ/kg dry soil exceeded this maximum limit.

The investigation of the pattern of individual PCDD/F congener's contributions to the sum TEQ can give information on the anthropogenic sources of these contaminations. The pattern of the congeners in the eggs is relatively equivalent to the soil congeners (Figure 2 and Figure S1). There is a dominance of the congeners 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF, this predominance is even higher for the egg samples. The highest difference in occurrence of congeners in the eggs compared to soil samples is observed with higher chlorinated number (Figure S1). Higher chlorinated congeners are considered less absorbed through the digestive tract and therefore are less present in the eggs (e.g. 1,2,3,4,7,8,9-HpCDF or 1,2,3,4,6,7,8-HpCDF) (Kelly et al. 2004; Hoogenboom et al. 2006). Accordingly,

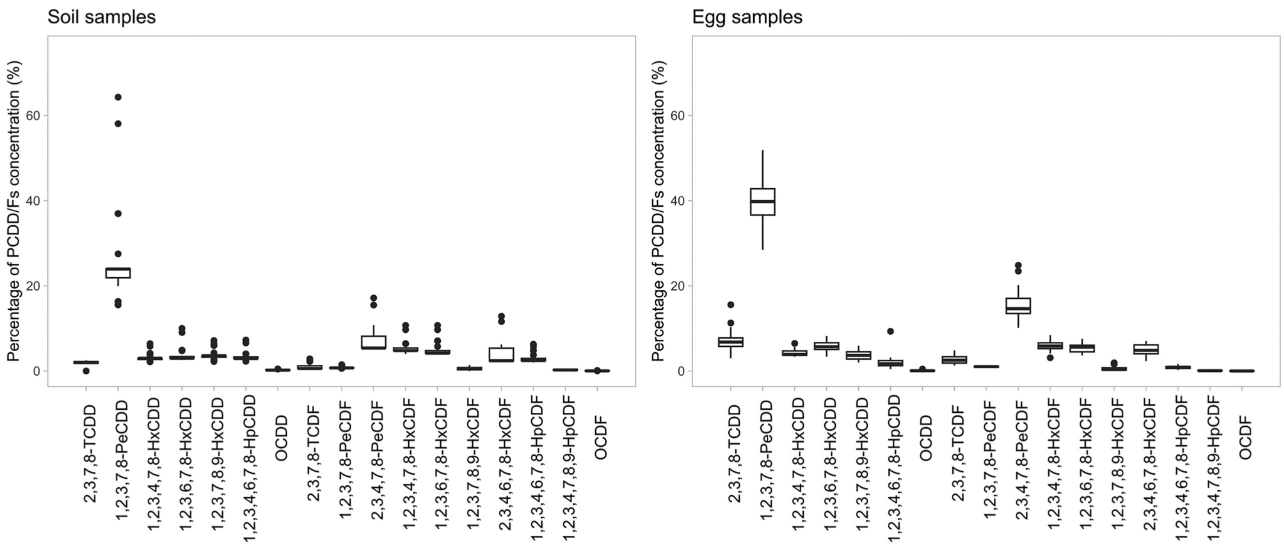

Figure 2. Congener profiles (%) and variation among the soil and egg yolk samples according to their contribution to the total concentrations in TEQ WHO-2005.

more of the higher chlorinated congeners were found in the excreta than in the feed (Pirard and De Pauw 2005; Hoogenboom et al. 2006, 2006). Other factors could influence the absorption rate of PCDD/Fs such as physico-chemical properties of the soil or the season of sampling (De Vries et al. 2006; Hoogenboom et al. 2006; Van Overmeire et al. 2009; Lake et al. 2013; Węgiel et al. 2018). The level and type of soil organic matter has been discussed in several studies, showing a reduction of the PCDD/Fs absorption with a higher soil organic content, especially condensed organic matter, for different animal species (Pu et al. 2006; Saghir et al. 2007; Delannoy et al. 2014; Lambiase et al. 2022). Finally, we observed that one single egg sample was contaminated with a higher amount of 1,2,3,4,6,7,8-HpCDD. The higher occurrence of this congener could be due to the use of a wood preservative in the corresponding henhouse.

In Figure 3a, the results from the toxicokinetic (TK) model were estimated with a soil ingestion based on the soil coverage and the area available per hen (Waegeneers et al. 2009). The simulation was done in total TEQ and for the congener-specific approach. The estimated soil ingestion varied between 5 and 30 g/day. Overall, the initial model overestimated the expected concentration in the eggs, which is preferable when discussing recommendations for egg consumption by humans, in the sense of a positive margin of error. The simulation with the total TEQ is therefore recommended.

Based on this soil ingestion estimates, the toxicokinetic model tends to overestimate the measured concentrations in the eggs when looking at the model optimized for the sum TEQ concentration. The mean absolute error is 4.4 and 3.2 pg TEQ/g lipids for the simulation in total-TEQ and congener-specific respectively (Figure 3a). The results were then compiled with the reverse dosimetry for the total TEQ concentration and for the congener-specific approach. The mean absolute error for the congener-specific approach is 3.2 pg TEQ/g lipids. Indicating that a better fitting with the soil ingestion is not sufficient to overcome the absorption rate uncertainties.

Soil ingestion

We identified one of the most sensitive and problematic parameters as the amount of soil ingested by the hens. While already discussed in several studies, this parameter remains difficult to assess (Schuler et al. 1997; De Vries et al. 2006; Ghidini et al. 2022; Lambiase et al. 2022). In the present study, we estimated the soil ingestion *via* a reverse dosimetry. The sum TEQ concentration calculated is illustrated in Figure 3a. Based on the calculated soil ingestion from the inverse dosimetry, there is a slight underestimation of the dose when looking at specific congeners (yellow triangle in Figure 3a). It may be due to some congeners having soil concentrations lower than the LOQ. We

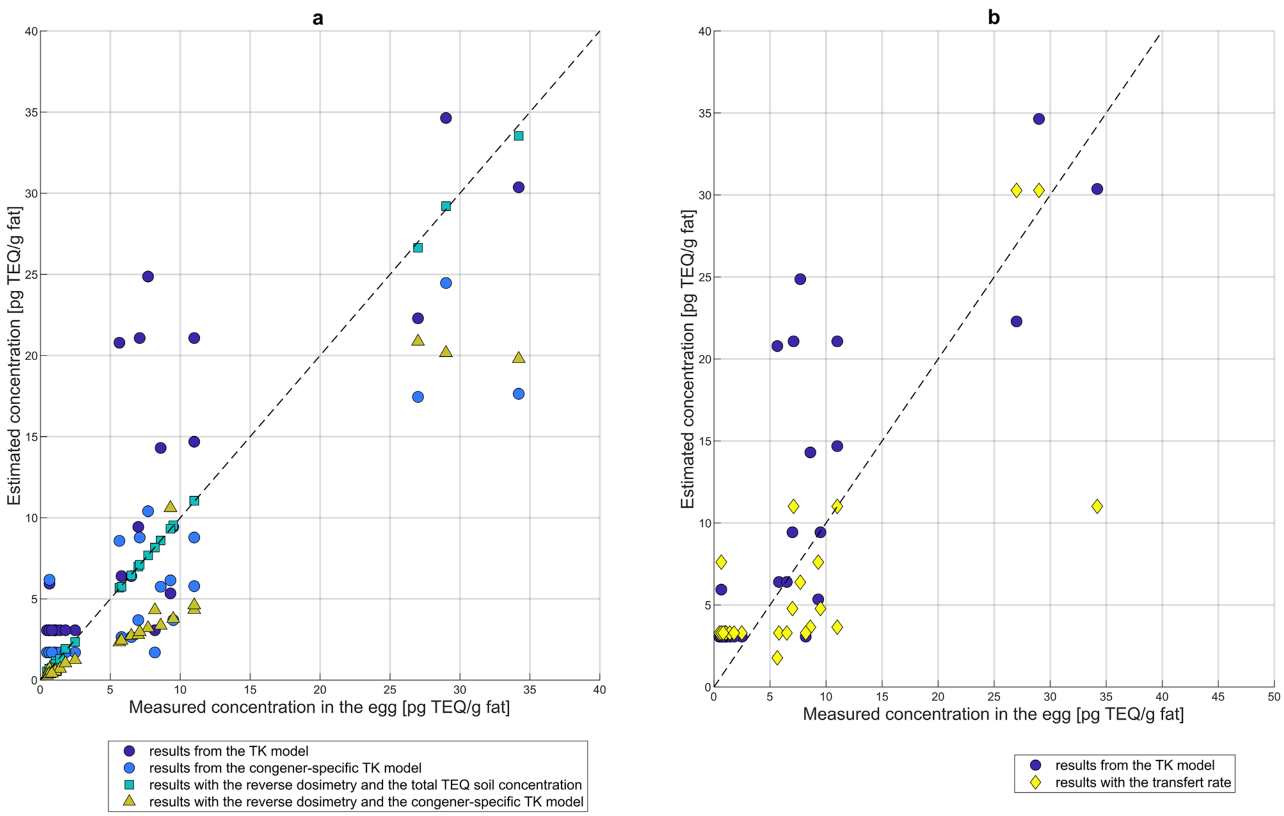

Figure 3. (a) Comparisons between estimated and measured PCDD/Fs concentrations in total TEQ in eggs for hens held on contaminated soils. The estimated concentrations are calculated with a toxicokinetic model with the total TEQ and the congener-specific approaches with a known contaminated soil. The ingested soil was computed either based on the soil coverage and the area available per hen and the methodology described by Waegeneers et al. (2009) (bullet) or with the reverse dosimetry (triangle). (b) The estimated concentration in the eggs from the toxicokinetic model are compared to an approach using transfer rate from Amutova et al. (2021) (diamond).

tested the concentration calculated with half of the limit of quantification set for those congeners in the soil samples having concentrations lower than the LOQ. The results were basically unchanged, with similar mean absolute errors.

One other explanation for the underestimation of the model with the reverse dosimetry could be due to a wrong estimation of the free fraction (F_{abs_free}) of PCDD/F when bound to soil, or an overestimation of the soil ingestion based on *in situ* observations. Roberts et al. (2019) gives a range for bioaccessible fraction from soil (%) and our calculations were based on the mean value.

The initial simulation of the model was done with estimated soil ingestion from *in situ* observations according to references from the literature (bullet in Figure 3a). Indeed, the estimated soil uptake was evaluated based on the soil coverage by vegetation and the area available per hen (Waegeneers et al. 2009). Since the flock sizes were rather small (mostly domestic-produced eggs), we did not impute a factor to reduce the soil ingestion more relevant for larger flock size (Kijlstra et al. 2007). Figure 4 illustrates the

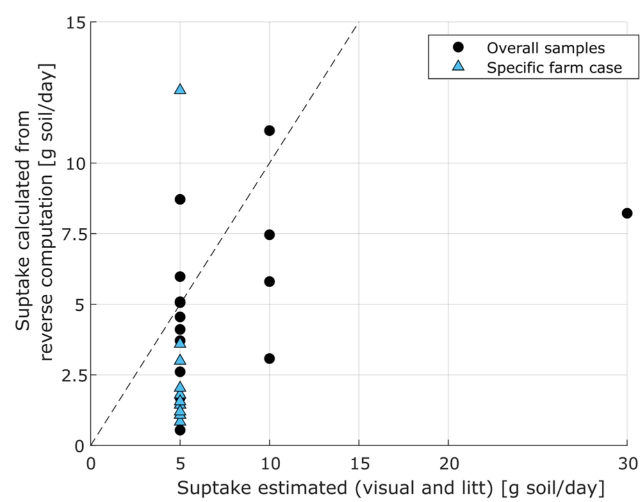

Figure 4. Ingested soil mass calculated with a toxicokinetic model-based reverse dosimetry approach, compared to the estimated soil ingestion based on *in situ* observations and the literature.

difference between the soil ingestion estimated from in-situ observations and the amount estimated with the toxicokinetic model-based reverse dosimetry. The *in-situ* estimates tended to be higher than the model-based estimates in most of the cases. Although averaging other parameters such as the ingestion of worms, insects or grubs by hens scratching in the soil are included in the estimation of the ingested soil, theses parameters can vary depending on the individual hen or the soil coverage (Schuler et al. 1997; Ghidini et al. 2022; Lambiase et al. 2022). These results suggest that the parameters usually considered – the vegetation cover and the area available – are not sufficient to predict accurately the soil ingestion. At the farm, where 12 eggs were collected simultaneously, the pecking behaviour was the dominant variable parameter since all the hens were fed and run on the same area. We can also discuss that each hen has their own metabolisation kinetic, but this is usually considered when estimating the absorption parameters. The soil coverage was high (75–90%) and the hen flock had a large amount of space available (> 50 m^2 per hen), the ingested soil according to the methodology described by Waegeneers et al. (2009) was estimated at 5 g dry soil/day. Out of 10 samples, 90% were below the estimated ingested soil mass regarding the sum TEQ concentration in the eggs (triangles in the Figure 4). However, we can see that there is an extreme value related to the egg sample with a high concentration 8.2 pg TEQ/g lipids. In comparison, the other eggs have concentration varying between 0.5 and 2.5 pg TEQ/g lipids, with a mean value of 1.1 (or 1.7 with the extreme value) pg TEQ/g lipids. When there is a sufficient area, the soil coverage does not seem to provide a fair estimate of the soil ingestion (R^2=0.26, Figure 4). The high variation in the egg concentration substantiates the conclusion that the hens' individual pecking behaviour could be the main driver of the soil ingestion level. However, this component is a difficult parameter to predict. Based on the results illustrated in Figure 4, one suitable option is to consider the soil ingestion as an interval (e.g. 0.5–12 g dry soil/day), with a high likelihood to fit in the interval (e.g. 1–5.5 g dry soil/day). It would also be possible to fit a one tailed log-normal distribution in the model instead on a single value to estimate ingested soil mass.

Intake assessment

To obtain a human egg consumption recommendation, the results of the toxicokinetic model was combined with the TWI of 2 pg TEQ/kg bw/week from EFSA (2018). Since the TWI is assessed for the total sum-TEQ, the following results are not specific to congeners. Depending on the family whose eggs were sampled, the consumption of domestic-produced eggs can vary from 2 to 14 eggs/person/week. Similar consumptions habits were recorded in Belgium (Van Overmeire et al. 2009). The result illustrated in Figure 5 is expressed in number of

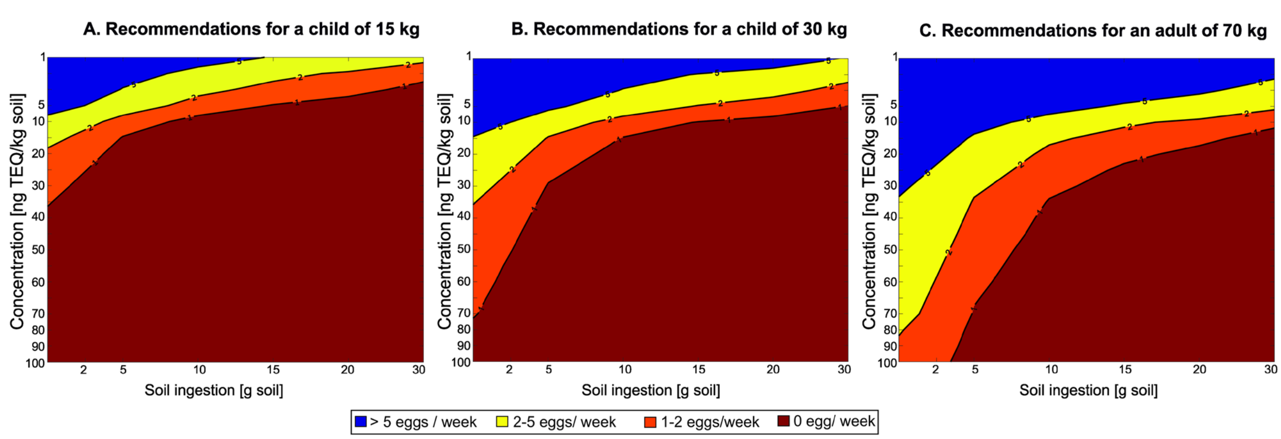

Figure 5. Recommendations of the maximum number of home-produced eggs that can be consumed until the TWI (2 pg TEQ/kg bw/week) is reached. The TWI is calculated for: a. children with body weight of 15 kg, b. children with body weight of 30 kg, and c. adults with body weight of 70 kg.

eggs that can be consumed per week until the TWI is reached. This recommendation does not take into consideration additional sources of exposure such as other food items. Figure 5 illustrates the results for a child or person with a body weight of 15, 30 or 70 kg. For adults, we would not recommend eating free-range produced eggs produced on areas with soil contamination above 90 ng TEQ/kg dry soil, because in this case human exposure lower than the TWI may be achieved only when less than two eggs are consumed per week, and less than 2 g dry soil/day is uptake by the hen, a low level of soil ingestion difficult to ensure. With a lower soil contamination (< 5 ng TEQ/kg dry soil), the consumption remains safe, although the exposure level might be higher than the EU Regulation when more than 5 eggs are consumed per week, and hen uptake more than 15 g dry soil/day. Regarding the consumption of home-produced eggs by children, we would not recommend eating eggs on areas above 40 or 20 ng TEQ/Kg soil when the child has a body weight below 30 kg or 15 kg, respectively. The threshold of 5 ng TEQ/kg soil set for adults is questionable for children. As it is difficult to guarantee soil ingestion below 5 g/day, it would be more prudent to recommend eating less than 5 eggs a week for a child of 30 kg. The recommendation for children below 15 kg (3–4 years old) should probably be extended to less than 2 eggs per week for a soil contamination below 5 ng TEQ/kg soil. The tolerable weekly intake established by the EFSA to avoid exceeding the NOAEL (Mínguez-Alarcón et al. 2017). Weber et al. (2019) already highlights that the pathway soil-chicken-eggs is one of the most sensitive exposures to PCDD/Fs for humans. Since children are the most sensitive population to chronic exposure to low doses of PCDD/Fs, recommendations and regulatory limits should be improved to protect this vulnerable population.

Conclusions

This study added substantial elements to the toxicokinetic models regarding the contamination of farmed animals with PCDD/Fs, which represents an important issue in terms of food safety. Soil could be the main source of contamination for chickens raised outdoors, mainly due to their pecking behaviour. It is important to emphasize that even at moderate PCDD/F soil concentration (around 5 to 20 ng TEQ/kg soil), it is likely that free-range eggs exceed the maximum limits fixed by EU Regulation. The most difficult parameter to predict regarding the soil to egg transfer is the soil ingestion level by a specific hen.

To adapt the current toxicokinetic model to soil contamination instead of feeding, it is necessary to add several parameters to calculate the dose entering the body compartment. These parameters are the soil concentration, the soil ingestion level, and the specific absorption rate from soil. Concerning soil ingestion, we would recommend adding an interval or a distribution since inter-individual variation is the parameter that most influences soil ingestion.

Regarding the consumption recommendations for adults, the eggs could be produced on soil more contaminated than 20 ng TEQ/kg soil if the consumers follow a weekly limit of egg consumption. However, a maximum consumption of 2 eggs per week by child should be recommended to avoid exceeding the NOAEL, resulting in potential low sperm production. Finally, to optimize the trade-off between guarantying welfare of free-range hens and ensuing eggs chemical safety, it is necessary to better understand pecking behaviour and how to limit it. Nevertheless, the soil is not the only source of contamination because worms and insects are also a pathway relating to soil ingestion.

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Ethical consideration

No spiked feed was provided to the hens, and we did not conduct any animal experimentations. The hens were raised by home-produced hens' owners, and only eggs collected along routine procedures.

Authors contributions

DV: Conceptualization; CO: Data curation; CO, MZ: Formal analysis; DV: Funding acquisition; CO, DV: Investigation; CO, MZ, DV, SL: Methodology; DV: Project administration; CO, MG, MZ, DV: Resources; CO, DV: Software; DV, AB: Supervision; CO, DV: Validation; CO: Visualization; CO: Writing – original draft; CO, MZ, SL, MG, AB, DV: Writing – review & editing.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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