Effect of Flock Size on Dioxin Levels in Eggs from Chickens Kept Outside

A. Kijlstra,*[1] W. A. Traag,† and L. A. P. Hoogenboom†

*Animal Sciences Group, Wageningen University and Research Centre, 3219 PH Lelystad, the Netherlands; and †RIKILT-Institute of Food Safety, 6708 PD Wageningen University and Research Centre, Wageningen, the Netherlands

ABSTRACT To decrease dioxin uptake by the general population the European Union (EU) has set limits to the dioxin content of many foodstuffs including eggs. Eggs from free foraging chickens are known to have a higher dioxin content compared with confined laying hens, and the question is whether these eggs can adhere to current EU regulations. The aim of the study was to investigate parameters that are involved in the contamination of eggs from chickens raised under organic conditions. Samples from 34 organic farms including soil and earthworm samples were collected between September and December of the year 2003. Dioxin levels were assayed by gas chromatography-mass spectrometry. Various parameters were collected by on farm interviews. Egg dioxin content varied between 0.4 and 8.1 pg of toxic equivalents (TEQ)/g of egg fat with a mean of 2.2 pg of TEQ/g of egg fat. Nine out of 34 farms exceeded the EU limit of 3 pg of TEQ/g of egg fat. In addition, dioxin-like polychlorinated biphenyls (DL-PCB) were measured, and 8 samples exceeded the limit for the sum of dioxins and DL-PCB. Overall, egg samples from 10 farms were noncompliant with the dioxin or total TEQ limits. No statistically significant relation could be observed between egg dioxin levels and the concentration observed in soil or earthworms. A statistically significant association was observed between flock size and egg dioxin and DL-PCB content. This effect is most likely attributable to the fact that flock size is related to the time chickens spend outside. Restricting outdoor run use on one of the farms resulted in a decrease of the egg dioxin content to a level that was within the EU limits. This demonstrates that the most likely contamination source is the soil or soil organisms but that the behavior of the hens determines the extent of the contamination. Following the completion of this study, a dioxin monitoring protocol has been set up in the Netherlands to prevent marketing of eggs with raised dioxin levels.

Key words: dioxin, egg, laying hen, organic farming, soil, outdoor run

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INTRODUCTION

Dioxins are considered to belong to the most toxic substances known and present a serious threat to the food chain. Dioxins cover a large group of dibenzo-p-dioxin and dibenzofuran congeners (Huwe, 2002). They are formed during natural and industrial combustion processes. Airborne particles from municipal waste incinerators and other (natural) fires are considered to be one of the most important sources of dioxin contamination of the environment. In addition dioxins are known to be present as contaminants in polychlorinated biphenyl (**PCB**) mixtures, pentachlorophenol, and certain herbicides like 2,4,5-T. Dioxins are degraded very slowly, and due to their persistence they are ubiquitously present in the environment of many industrialized countries. Uptake of contaminated grass or soil by farm animals results in the introduction of dioxins in our food chain (Roeder et al., 1998). Dioxins are lipophilic and tend to accumulate in the body fat of humans and animals. Human exposure to dioxins is mainly via the ingestion of animal products, whereby in countries like the Netherlands and Spain eggs have been shown to contribute for approximately 4% to the daily dioxin intake (Llobet et al., 2003; Baars et al., 2004). In addition to dioxins a number of PCB have been shown to possess dioxin-like properties. These dioxin-like (**DL**) PCB are present in small amounts in PCB mixtures and are also widely distributed throughout the food chain.

In view of the toxic nature of dioxins, international and national governmental agencies have implemented measures to reduce human exposure to dioxins and DL-PCB via the food supply. The WHO has proposed a tolerable daily intake of between 1 to 4 pg of toxic equivalents (**TEQ**) per kg of BW per d (WHO, 1998). In the year 2001, the Scientific Committee on Food of the European Union (**EU**) fixed a provisional tolerable weekly intake at 14 pg of TEQ/kg of BW/wk. The EU subsequently has set upper limits for the allowable dioxin content in many foodstuffs (EU regulation 2375/2001) and has recently extended these limits to the DL-PCB. For dioxins in eggs, an action

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[1]Corresponding author: aize.kijlstra@wur.nl

Table 1. Laying hen flock size of participating farms

Hens per farm (n)	Farms (n)
<50	1
50 to 249	10
250 to 999	2
1,000 to 4,999	11
5,000 to 10,000	6
>10,000	4

level has been fixed at 2 pg of TEQ/g of egg fat and a maximum limit forbidding the entry of eggs onto the market when their dioxin level exceeds 3 pg of TEQ/g of egg fat. For DL-PCB a similar action limit of 2 pg of TEQ/g of fat has been set and a tolerance limit for the sum of dioxins and DL-PCB of 6 pg of TEQ/g of fat. These latter limits became active on the fourth of November 2006, but the older action and tolerance limits for dioxins were maintained as well. Levels exceeding the action limit require follow-up actions to establish the source of the contamination.

Earlier studies have shown that the dioxin content of eggs from free foraging chickens is much higher than that observed in chickens kept in wire cages (Schoeters and Hoogenboom, 2006). It is assumed that uptake of soil, insects, and worms leads to bioaccumulation of dioxins in egg fat often leading to levels exceeding the EU maximal limit of 3 pg of TEQ/g. To further investigate the factors that are involved in determining the dioxin levels in eggs from chickens kept outside, we performed a survey on a large number of organic farms and investigated the correlation with a large number of different parameters. The strongest association was observed with the flock size; small flocks tending to have egg dioxin levels exceeding the limits set out by the EU.

MATERIALS AND METHODS

Participating Farms

A cross-sectional study was carried out between September and December of the year 2003. Farms were recruited on a voluntary basis by telephone. A total number of 34 organic farms with free foraging laying hens participated in the study. Farms were visited and a questionnaire was filled in with the farmer. A combined egg sample consisting of 10 to 15 eggs was obtained on the farm. Furthermore, a combined soil sample (10 different samples from the top 5 cm at various locations of the outdoor run) and a sample of 20 to 30 earthworms were taken. On one of the farms an insufficient amount of worms was obtained.

Organic Farming

Organic animal food production is regulated throughout the EU via the EU-regulation (EEC) no. 2092/91. This regulation specifies housing of hens, the feed, preventive and curative medical treatments, and interventions such as beak trimming. Indoor runs should contain no more than 6 hens per square meter, and the outdoor run should be at least 4 m^2 per laying hen. Maximum flock size per birdhouse is 3,000 chickens. There is no maximum to the number of birdhouses per farm. Organic laying hens have access to an outdoor run for a minimum of one-third of their lifetime. In practice this means that hens are allowed outdoor access for a minimal time period of 8 h per d. Feed for the hens should be of organic origin. Preventive treatments are not allowed and curative treatments are limited to one treatment with regular medicines per year (with the exception of antiparasitic treatments). Beak trimming is not allowed. In the Netherlands, compliance to these rules has been assigned by the ministry of agriculture to a separate organization named Skal. The EU regulations on organic farming do not include aspects concerning the control of food safety.

Dioxin and DL-PCB Levels

Dioxin content in eggs and other samples were performed at RIKILT (National Reference Laboratory according to EU guideline 96/23/EC) as described earlier (Hoogenboom et al., 2006). Homogenized egg samples (3 g) were spiked with ^{13}C labeled standards and taken up in

Table 2. Analysis of dioxin and dioxin-like polychlorinated biphenyl (DL-PCB) content in eggs, soil, and worms on organic laying hen farms[1]

Item	Farms (n)	Minimum	Maximum	Mean	Median
Dioxins					
Eggs	34	0.4	8.1	2.2	1.6
Soil	34	0.9	5.9	2.2	2.1
Worms	33	0.3	1.9	0.6	0.5
DL-PCB					
Eggs	34	0.0	6.0	1.5	0.9
Soil	34	0.0	0.8	0.2	0.2
Worms	33	0.0	2.7	0.3	0.1
Sum dioxins and DL-PCB					
Eggs	34	0.7	13.0	3.9	2.1
Soil	34	1.0	6.3	2.5	2.5
Worms	33	0.3	4.1	0.9	0.6

[1]Dioxin and DL-PCB content in eggs is represented as picograms of toxic equivalents (TEQ) per gram of fat; dioxin and DL-PCB content in worms and soil is represented as picograms of TEQ per gram of product.

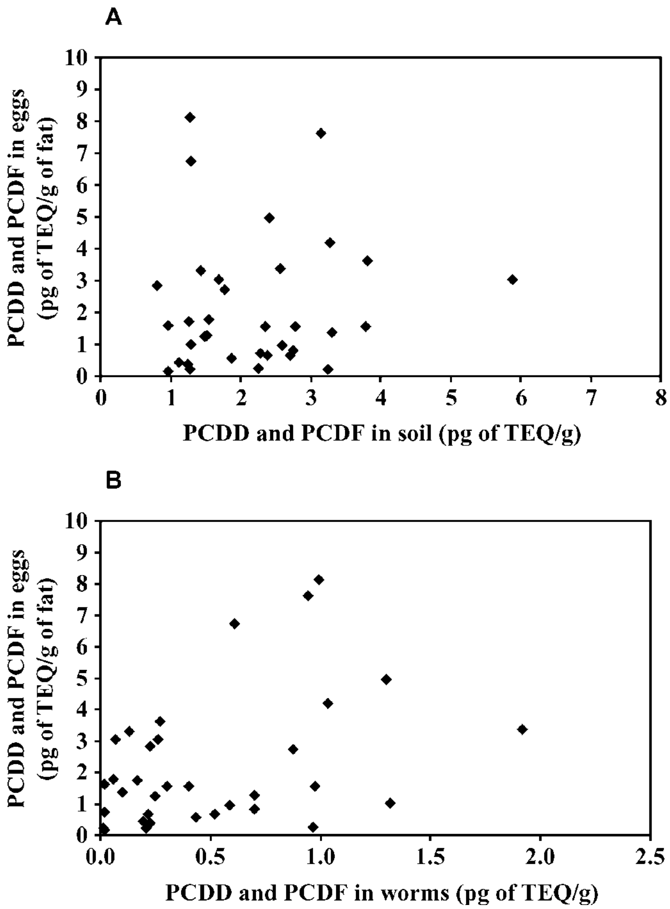

Figure 1. A) Relation between dioxin levels in soil and eggs (R^2 = 0.01). B) Relation between dioxin levels in earthworms and eggs (R^2 = 0.16). TEQ = toxic equivalents; PCDD = polychlorinated dibenzodioxins; PCDF = polychlorinated dibenzofurans.

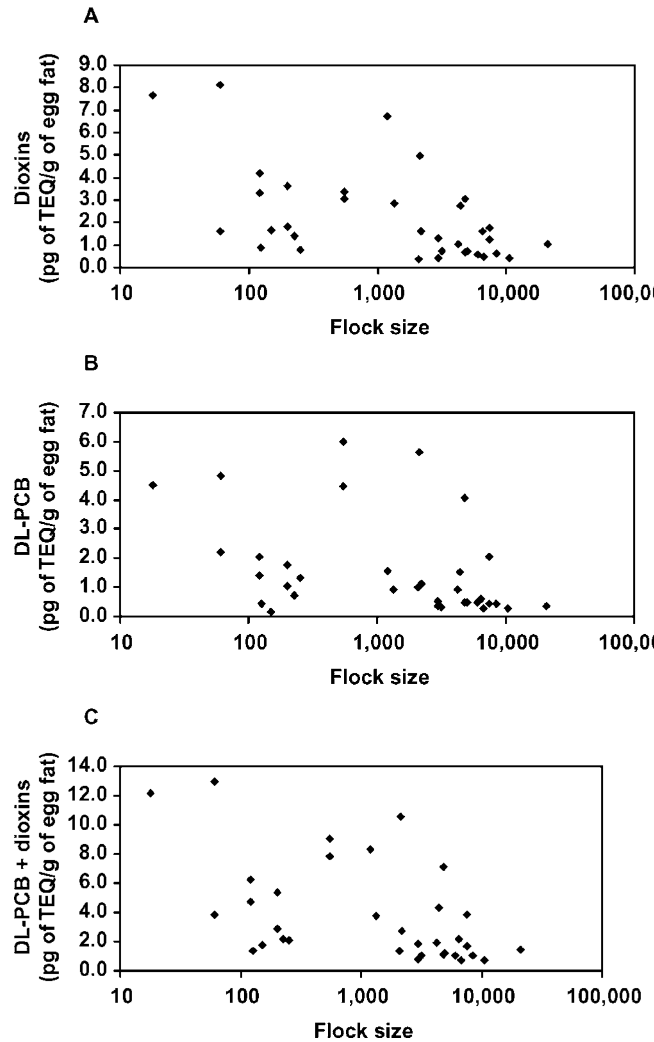

Figure 2. Effect of flock size on A) dioxin, B) dioxin-like polychlorinated biphenyls (DL-PCB), and C) sum of dioxin plus DL-PCB content of eggs. TEQ = toxic equivalents.

15 mL of ethylacetate/cyclohexane (1:1 vol/vol). Dioxins and PCB were further purified by gel permeation chromatography and an $Al_2 O_3$ column. Dioxins and nonortho-PCB were separated from other PCB via a porous carbon column. The fraction containing dioxins and nonortho-DL-PCB was dried and taken up in 10 μL of toluene. An aliquot of 2 μL was tested by gas chromatography coupled to a high-resolution mass spectrometer. Dioxin and PCB congeners were identified on the basis of retention times and ion ratios. The same procedure was applied for the monoortho DL-PCB.

The individual congeners were quantified and each congener level was multiplied with its toxic equivalency factor (Van den Berg et al., 1998). This factor denotes a given dioxin congener's toxicity relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin, which has been assigned the maximum toxicity of 1. All weighted levels were subsequently summed to derive a TEQ level for dioxins, DL-PCB, or both. If the level of a certain congener was below the level of detection, a level equal to the detection level was taken (upperbound principle).

Questionnaire

Farmers were interviewed with a questionnaire containing questions about general farm characteristics, types of laying hens, burning places on the farm, polluting industries in the farm vicinity, feeding regimen, medical treatments and condition of the outdoor run.

Flock Size and Outdoor Run Use

The literature concerning flock size and outdoor run use was studied and all available data was compiled. Part of the literature was accessed via the Organic Eprints Archive (http://www.orgprints.org/ Accessed January 15, 2007).

Analysis of Data

Data from the questionnaire and all data concerning dioxin levels were entered into a Microsoft Access (Microsoft, Amsterdam, the Netherlands) databank. The Student *t*-test was performed to assess the significance of a relation between farms exceeding the 3 pg of dioxin limit with a specific farm parameter. Regression analysis was performed using Genstat (VSN International, Hemel Hempstead, UK).

Table 3. Relation between farm egg dioxin level and various farm parameters[1]

Parameter	Egg dioxin ≤3	Egg dioxin >3
Farms (n)	25	9
Age of chickens (wk)	52 (24)	56 (25)
Laying percentage	75 (19)	76 (15)
Farmer's estimate of percentage outdoor run area used	79 (32)	91 (23)
Farmer's estimate of percentage chickens using outdoor run	78 (20)	95 (7)
Estimated percentage bare area outdoor run	48 (36)	28 (34)

[1]Numbers in parentheses indicate the standard deviation.

RESULTS

Thirty-four organic laying hen farms participated in the study. The number of hens per farm ranged between 18 and 20,850 (Table 1). Most farms (11) had Bovans Goldline chickens. Other types included Hyline (n = 4), Nera (n = 4), Lohman (n = 3), and Isabrown (n = 2). The remaining 10 farms had a mixed breed of strains.

Eggs were collected from these farms and fat isolated from the egg samples was tested for dioxin content. Dioxin content in the eggs ranged between 0.4 and 8.1 with a mean dioxin content of 2.2 pg of TEQ/g of egg fat (Table 2). Eggs from 9 farms exceeded the 3-pg limit set by the EU.

Since November 4, 2006, the EU limits also include the 12 DL-PCB, which show similar effects as dioxins, although often at much higher levels. It was decided to have tolerance limits for dioxins and the sum of dioxins and DL-PCB, of, respectively, 3 and 6 pg of TEQ/g. There is no separate tolerance limit for DL-PCB, but instead it was decided to apply action limits for both dioxins and DL-PCB, but not the sum. Action limits for eggs are both 2 pg of TEQ/g of fat. The DL-PCB levels ranged between 0.1 and 6.0 pg of TEQ/g of fat with an average value of 1.6 and a median of 1.0 pg of TEQ/g of fat. Eggs from 8 farms exceeded the action limit for DL-PCB, of which 6 also exceeded the action limit for dioxins. Total TEQ levels ranged between 0.7 and 13.0 pg of TEQ/g of fat with an average value of 3.9 and a median of 2.1 pg of TEQ/g of fat. Eggs from 8 farms exceeded the tolerance limit for total TEQ, but these included only 7 of the 9 farms exceeding the dioxin limit. As a result, samples from 10 farms out of 34 (26%) exceeded the tolerance limit for dioxins (9), total TEQ (8), or both (10).

The gas chromatography-mass spectrometry dioxin levels in the soil on the 34 farms ranged between 0.9 to 5.9 pg of TEQ/g with an average of 2.2 and a median of 2.1 pg of TEQ/g. No significant correlation was found between dioxin levels in eggs and soil (Figure 1A). Overall, gas chromatography-mass spectrometry dioxin levels in worms were much lower than those in soil ranging between 0.3 to 1.9 with an average of 0.6 and a median of 0.5 pg of TEQ/g. No significant correlation was found between worm dioxin and soil dioxin levels (data not shown), or levels in worms and eggs (Figure 1B). On a number of farms samples of feed and supplements were collected and analyzed, but these did not contain elevated dioxin or DL-PCB levels (data not shown).

Using Student's *t*-test we analyzed the egg dioxin status of the farms with various farm parameters. Farms with a dioxin content above 3 pg/g of fat were considered positive, and those with a level below 3 were considered negative. Nine farms were considered positive and 25 negative. No significant association was found among age of the chickens, laying percentage, the type of chickens, or the percentage bare area of the outdoor run (Table 3).

A significant association was found between the total number of chickens on a farm and the egg dioxin level ($P < 0.05$; Table 4; Figure 2A). The mean number of chickens on the farms negative for dioxins was 4,350, whereas the flock size on the positive farms was 1,684. For DL-PCB the relation was not as obvious as for the dioxins (Figure 2B). There was a significant relation between the log-transformed dioxin, the log-transformed DL-PCB, and log-transformed sum of dioxin plus DL-PCB levels in eggs and the log-transformed number of hens per farm ($P < 0.001$, $P = 0.01$, and $P < 0.001$, respectively).

The relation between flock size and use of the outdoor run was not studied on the farms that had entered this study, and we did not want to rely on a possibly biased answer concerning this issue from the interviewed farmers. To analyze this question we studied the literature and compiled data from various papers that provided data concerning the number of laying hens that used the outdoor run. The data (Table 5 and Figure 3) show a marked relation between flock size and outdoor run use whereby flocks with more than 10,000 laying hens spend less than 10% of the available time outdoors, whereas more than 50% of the birds in small flocks will be outside.

The farm with the highest egg dioxin content (8.1 pg of TEQ/g of egg fat) was asked to participate in an intervention study. Before starting the interventions, the eggs

Table 4. Flock size and egg dioxin status of a farm

Chickens (n)	Dioxin level ≤3	Dioxin level >3
<1,500	8	7[1]
≥1,500	17	2
Mean flock size	4,350	1,684

[1]Dioxin level was significantly higher in flocks with less than 1,500 chickens ($P < 0.05$).

Table 5. Analysis of literature data on flock size and use of the outdoor run

Flock size	Animals outside (%)	Reference
50	41.2	Hirt et al., 2000
256	38	Harlander-Matauschek et al., 2006
256	31	Harlander-Matauschek et al., 2006
490	42.1	Bubier and Bradshaw, 1998
500	29.5	Hirt et al., 2000
500	21.5	Zeltner and Hirt, 2003
500	22.5	Zeltner and Hirt, 2003
500	35	Harlander-Matauschek, 2001
500	31.3	Mußlick et al., 2004
1,000	22	Harlander-Matauschek, 2001
1,432	11.1	Bubier and Bradshaw, 1998
1,451	9.2	Bubier and Bradshaw, 1998
2,450	5.1	Bubier and Bradshaw, 1998
3,000	19.5	Hirt et al., 2000
4,500	9.8	Elbe et al., 2005
7,000	15.5	Mußlick et al., 2004
16,000	4.0	Mußlick et al., 2004
20,000	6.8	Mußlick et al., 2004

were assayed again on 2 separate occasions early in the year 2005. The dioxin content of the eggs was still very high, which confirmed our earlier observations. The interventions included the following measures: opening of pop holes at a later time point in the morning resulting in a restricted outdoor use of 8 h per day and only providing feed and water inside the barn and no more feeding of vegetable leaves and scraps outdoors. These interventions were associated with a marked drop in egg dioxin content (Figure 4). At the end of August 2005 the threat of aviary influenza resulted in government legislation whereby all chickens in the Netherlands had to be confined. This resulted in a further drop of the egg dioxin content on this farm to a level below 1 pg of TEQ/g of egg fat.

DISCUSSION

In this paper we show that dioxin content of eggs from chickens having access to an outdoor run is related to flock size. An analysis of the literature indicates that this is most probably related to the fact that flock size influences the social behavior of the laying hens. The larger the flock, the higher the tendency of the laying hen to remain inside (Hegelund et al., 2005). Reasons for this behavior are not yet known.

Flock size not only influences the behavior of the chickens, but flock size is also associated with farming practice. Larger flocks belonged to professional organic farmers, whereas the smaller flocks were kept by organic farmers in a hobby-like fashion. This played an important role concerning the time period that flocks were allowed to use the outdoor run. Many of the organic farmers with small flocks had a permanent opening to the outdoor area resulting in chickens being outside from early dawn until dusk. In the Netherlands, total daylight time varies between 8 h in winter to 15 h in summer. Organic poultry farmers with large flocks use hatches to keep chickens inside overnight, and access to the outdoor run is given after the chickens have laid their eggs in the morning (around 1000 to 1200 h). In a follow-up study we observed that small flocks that exceeded the egg dioxin limits set out by the EU were able to control their dioxin levels by taking measures that restricted outdoor use by the laying

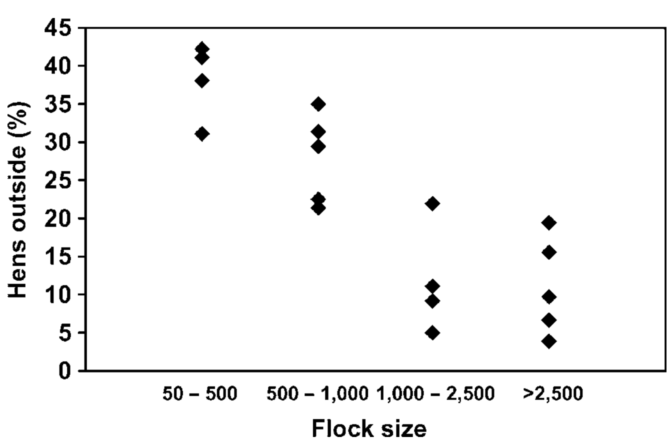

Figure 3. Effect of flock size on the use of the outdoor run (for references see Table 5).

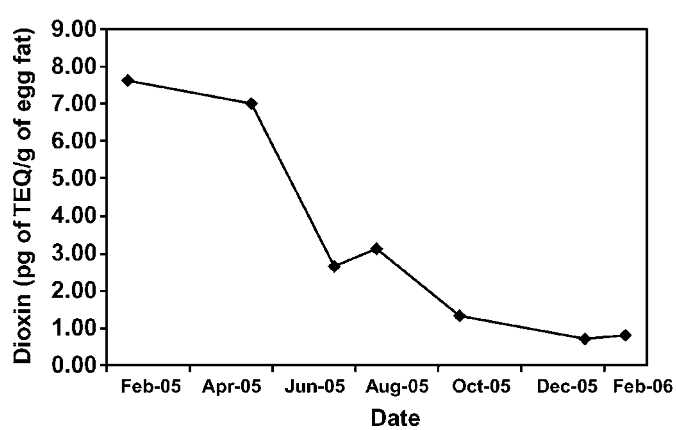

Figure 4. Effect of interventions on egg dioxin levels on a small organic laying hen farm. TEQ = toxic equivalents.

hens (our unpublished data). In extreme cases, farmers were advised to replace the contaminated outdoor run with clean soil.

It is not clear which factors determine the degree of soil uptake by outdoor hens. Soil uptake has been considered as a means of compensating for shortages in vitamins or minerals and feed (Symes, et al., 2006). Larger farms were observed to more often use vitamin supplements than smaller farms. This latter effect combined with a lower use of the outdoor run may limit the total amount of soil taken up by hens from large flocks.

Earlier publications have already shown that dioxin content of eggs originating from chickens with an outdoor run are higher than values observed for chickens kept inside (Schoeters and Hoogenboom, 2006). Most studies have addressed the presence of elevated dioxin levels in eggs from chickens raised in areas with excessive pollution due to incidents with neighboring factories (Harnly et al., 2000). Our study did not reveal major local incidents as the cause of elevated egg dioxin levels, and the raised values found probably reflect a certain background of historical dioxin pollution of the environment. On 1 farm, a fence that had been treated with waste oil could have been the source of dioxin contamination. This farm had a flock of 2,140 hens and an egg dioxin level of 5 pg of TEQ/g of egg fat. Of interest was the observation that the DL-PCB were also quite high (5.6 pg of TEQ/g of egg fat), which supports a local contaminating source of dioxins and dioxin-like PCB.

Sources of dioxins in the outdoor run are diverse and range from soil uptake to the uptake of worms, insects, or herbs. Allowing chickens to be outside for a large part of the day results in the uptake of these various dioxin sources, which are very efficiently transferred to the egg yolk (Stephens et al., 1995; Schuler et al., 1997). Another factor that is of importance with relation to organic laying hens is the fact that their beaks are not allowed to be trimmed, which enables these chickens to very efficiently pick up small organisms residing in the soil, but which may already have accumulated dioxins. Due to the fact that the Netherlands is a densely populated country, it is not surprising that the environment contains a certain degree of historical dioxin pollution caused among other reasons by unfiltered municipal waste burning until the mid-1990s, but potentially also the burning of waste by the farmers, the spreading of ash onto the soil, the presence of pentachlorophenol-treated wood, the use of PCB-containing coatings, and the leakage of PCB-oil from machinery and vehicles. Most of these sources are expected to be outside the barn, but some could actually be inside, possibly underlying the somewhat less clear relation between flock size and DL-PCB in eggs (Figure 2B). Many sources of dioxin and PCB pollution have been taken care of during the past decades, but due to the long half-life of dioxins it will probably take many years before environmental dioxin and DL-PCB levels have reached a level whereby unrestricted outdoor access will be associated with egg dioxin levels that are within the current limits set by the EU.

In organic farming, outdoor access is one of the important welfare attributes of this type of farming. Restrictions in the use of the outdoor run are therefore a contradiction to organic principles. On the other hand organic food is also considered to have health benefits for the consumer due to the fact that animals are fed organic feed, which is free of pesticides and synthetic additives (Schlatterer and Breithaupt, 2006). The possible presence of environmental contaminants in organic eggs is therefore a sensitive issue and poses a serious dilemma between food safety and animal welfare. As indicated by our study, the natural behavior of large flocks may lead to a usage of the outdoor run that limits the transfer of environmental contaminants to the eggs. Consumers should be aware of the fact that eggs from commercial small flocks but also from private owners with unrestricted outdoor access may have high dioxin levels (Pussemier et al., 2004; Van Overmeire et al., 2006). Frequent dioxin monitoring of eggs from chickens with outdoor access (not only organic) is currently implemented in the Netherlands and will thereby play an important role in improving animal welfare and maintaining the goal to reduce dioxin intake by the general population.

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