FRONT OFFICE FOOD AND PRODUCT SAFETY

ASSESSMENT OF LEVELS OF DIOXINS AND DL-PCBS IN MEAT OF LAYING HENS CORRESPONDING TO EU MAXIMUM LEVELS IN HEN EGGS

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Nederlandse samenvatting

In Nederland worden legkippen na de legperiode geslacht. In het geval van een dioxine-contaminatie kan het echter zijn dat de gehalten aan dioxinen en dioxine-achtige polychloorbifenylen (dioxin-like PCB’s; DL-PCB’s) zowel in het ei als het vlees van de kip verhoogd zijn en boven de geldende wettelijke maximumgehalten uitkomen. Echter worden van leghennen alleen de eieren routinematig gecontroleerd of ze aan de geldende maximumgehalten voldoen. Daarom wil de NVWA graag weten wat de relatie is tussen de gehalten van dioxinen/DL-PCB’s in kippeneieren en in vlees. En daarnaast ook hoe waarschijnlijk het is dat als het maximumgehalte in ei wordt overschreden, dan ook het maximumgehalte in vlees wordt overschreden. Om dit te beoordelen, is gebruik gemaakt van een bestaand overdrachtsmodel voor dioxinen en DL-PCB’s in de leghen. Het model is ontwikkeld op basis van een overdrachtstudie met leghennen. Het model bestaat uit twee compartimenten, namelijk lichaamsvet en een niet-vet (centraal) compartiment. Het model rekent met uitscheiding (excretie) van dioxinen en DL-PCB’s via eidooiervet. Hiermee kan de overdracht van dioxinen en DL-PCB’s naar de verschillende compartimenten als totale groep of afzonderlijk per dioxine of DL-PCB (congeneerspecifiek) berekend worden. Het model is gebruikt met de standaard instellingen met als aanname dat alle dioxinen en DL-PCB’s in het vet opslaan en dat concentraties in het vleesvet en lichaamsvet gelijk zijn. Ter bevestiging is ook nog naar de meetgegevens gekeken van het dierexperiment dat als basis diende voor het overdrachtsmodel. De resultaten laten zien dat bij een concentratie van dioxinen en DL-PCB’s gelijk aan het maximumgehalte in eieren, het zeer waarschijnlijk is dat ook het maximumgehalte in vlees en vleesproducten overschreden wordt.

Subject

Following a case of dioxin contamination in the area of a free range laying hen farm, the Netherlands Food and Consumer Product Safety Authority (NVWA) would like to assess the relationship between dioxin/DL-PCB concentrations in eggs and dioxin/DL-PCB concentrations in meat of laying hens.

Questions
How likely is it that the maximum levels (MLs) for dioxins and dioxin-like PCBs (DL-PCBs) in poultry meat, meat products and fat (Regulation (EC) No. 1881/2006) will be exceeded if the maximum levels for dioxins and DL-PCBs are exceeded in eggs?

Conclusions

The calculated concentration of the sum of dioxins and DL-PCBs, i.e. the sum-TEQ, in body fat that corresponds to the ML for sum-TEQ of 5.0 pg /g fat for hen eggs, is 5.0 pg sum-TEQ/g body fat.
This is higher than the ML of 3.0 pg sum-TEQ/g fat for meat, meat products (excluding edible offal) and fat of poultry in Regulation (EC) No. 1881/2006.

Similarly, the calculated concentration of dioxins, i.e. the dioxins-TEQ, in body fat that corresponds to the ML of 2.5 dioxins-TEQ/g fat for hen eggs, is 2.5 pg dioxins-TEQ/g body fat.
This is higher than the ML of 1.75 pg dioxins-TEQ/g fat for meat, meat products (excluding edible offal) and fat of poultry in Regulation (EC) No. 1881/2006.

Therefore, it can be concluded that it is very likely that the MLs in meat, meat products (excluding edible offal) and fat in poultry will be exceeded, when the MLs for dioxins (dioxins-TEQ) or for the sum of dioxins and DL-PCBs (sum-TEQ) in hen eggs are exceeded.

Introduction

Dioxins and DL-PCBs
Dioxins and DL-PCBs can enter the human food chain from the environment. For the average consumer, food is the most important route of exposure.
Dioxins[1] consist of a mixture of 17 chemically related compounds (polychlorodibenzo-p-dioxins and -dibenzofurans). DL-PCBs (dioxin-like polychlorobiphenyls) consist of 12 congeners and show the same toxicity as dioxins. The degree of toxicity of the different compounds varies widely. Because of these differences, factors are used to compare their potencies, so-called Toxic Equivalence Factors (TEF). The total (dioxin-like) potency of a mixture of these substances in a food is estimated using dose addition. In this procedure the contribution of any component in the mixture is expressed as an equivalent amount of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, the most potent representative of the group) by multiplication of its concentration with the substance specific TEF. The results of these calculations are called substance specific Toxic EQuivalents (substance-specific TEQ), which are then added up to give a total TEQ for the particular food.

Dioxins and DL-PCBs dissolve easily in fat (dietary fat; body fat) and are therefore difficult to excrete once ingested. Through transfer from animal feed, via the target animal (dairy cattle, pigs, laying hens, farmed fish) dioxins and DL-PCBs end up in animal products (meat, milk, egg). This transfer is determined on the one hand by the chemical properties of dioxins and DL-PCBs, and on the other hand by the biological properties of the target animal. By using mathematical models, all these properties can be combined. This makes this methodology ideally suited to estimate the transfer of these compounds in farm animals by means of computer simulations. For this reason, RIVM and RIKILT have carried out various animal studies in the past years and, based on the results, developed transfer models for dioxins and DL-PCBs in farm animals, among which laying

[1] Note that throughout the text the word "dioxins" includes the true (polychlorinated) dioxins as well as the (polychlorinated) dibenzofurans, unless specified otherwise.

hens. The latter model (Van Eijkeren et al., 2006) is used to answer the current question to the Front Office.

Egg production

In the Netherlands, around 10 billion chicken eggs are produced for human consumption every year. The production of these eggs has a considerable number of phases, starting with the breeding companies (parent animals). The eggs produced by these animals go to the hatchery to be hatched. The chicks then leave for the so-called rearing farms. The rearing farms raise the hen chicks into young laying hens. These animals go to the laying farms at around the age of 18 weeks, where they stay for the rest of their lives and start producing the eggs for consumption. Egg production starts around the age of 20 weeks. The laying hens stay on the laying farm until the age of approximately 20 months. At the end of the laying period, the laying hens are slaughtered (NVWA information).

Legal limits

Legal limits (i.e. maximum levels or MLs) for dioxins and DL-PCBs have been established at the EU level according to Regulation (EC) Nr. 1881/2006[2]. The relevant MLs for this question to the Front Office (FO) are indicated in Table 1. MLs are established for polychlorinated dioxins (dioxins+furans; dioxins-TEQ) and the sum of dioxins and DL-PCBs (sum-TEQ).

Table 1: Maximum levels (MLs) in poultry and poultry products from Regulation (EC) Nr. 1881/2006[3]. Only one footnote has been cited from the legislation as the other ones are not relevant for this FO assessment.

Food	**Sum-TEQ** (= sum of dioxins and DL-PCBs; WHO-PCDD/F-DL-PCB-TEQ) in pg/g fat	**Dioxins-TEQ** (= sum of dioxins; WHO-PCDD/F-TEQ) in pg/g fat
Meat and meat products (excluding edible offal) of poultry (a)	3.0	1.75
Hen eggs and egg products (a)	5.0	2.5
Fat of poultry (a)	3.0	1.75

(a) Foodstuffs listed in this category as defined in Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin (OJ L 226, 25.6.2004, p. 22).

Applied model for estimating concentrations

For calculating the dioxins-TEQ and sum-TEQ concentrations in meat, meat products and fat of poultry that correspond to the MLs in eggs, a 2-compartment transfer model, i.e. consisting of a central (non-fat) and a fat compartment, using egg yolk fat as excretion route, as described in Van Eijkeren et al. (2006) was used. The model was based on a transfer experiment described in Hoogenboom et al. (2006, see below under 'Measured concentrations from transfer experiment'), using WHO_{1998}-TEF values. The model was re-calibrated in Bokkers et al. (2014) on the basis of TEF 2005 values (see footnote 2).

[2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[3] 'Sum of dioxins' means the sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), expressed as World Health Organisation (WHO) toxic equivalent using the WHO-toxic equivalency factors (WHO-TEFs);'Sum of DL-PCBs' means the sum of polychlorinated biphenyls (PCBs), expressed as WHO toxic equivalent using the WHO-TEFs; 'WHO-TEFs' means the WHO toxic equivalence factors for human risk assessment based on the conclusions of the WHO — International Programme on Chemical Safety (IPCS) expert meeting which was held in Geneva in June 2005 (van den Berg et al., The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences 93(2), 223–241 (2006)).

Among others, the model uses the amount of body fat, egg laying frequency, egg weight, feed consumption, feed intake and sum-TEQ concentration in feed as input parameters. In the current assessment, for most parameters the default values as described in Van Eijkeren et al. (2006) were used.

The re-calibrated model calculates sum-TEQ and dioxins-TEQ concentrations resulting from transfer from feed to body fat of the laying hen as well as the corresponding concentration in egg yolk fat, either as TEQ from individual congeners or as sum-TEQ.
It is assumed that the TEQ concentrations in the fat component of meat and meat products (and other body tissues) are identical to those in the body fat. This is supported by data from a study by Pirard and de Pauw (2006), showing similar lipid-based levels for dioxins-TEQ in abdominal fat and breast tissue of laying hens. Since MLs for sum-TEQ and dioxins –TEQ for meat and meat products are also expressed as maximum amount of TEQ per gram of fat, the model-calculated concentrations in body fat can be compared to the MLs for meat and meat products.

For the sum of dioxins and DL-PCBs, the model was used with calibrated parameters for sum-TEQ (Van Eijkeren et al., 2006). As shown in Van Eijkeren et al. (2006, Figures 3 and 5) this calibration also holds for the dioxins-TEQ.
From this model, the steady state algorithm is used to calculate the body fat concentrations corresponding to the MLs for eggs for both dioxins- and sum-TEQ. Subsequently, the ratio between the egg yolk fat and the body fat was calculated. This is then used to compare the MLs in eggs with those in meat and meat products.

Applied input parameters and assumptions

(see Annex I).

<u>Body weight, absorption and body fat</u>
As in Van Eijkeren et al. (2006), the re-calibrated model applies a body fat mass of 260 g (range: 230 – 290 g). This corresponds with a body fat percentage around 13% in 45-61 week old Bovans Gold line laying hens, i.e. the hens used in Hoogenboom et al. (2006, estimated body weight: 1975 g). As no further information was available about the variation in body fat content of laying hens, for the model it was assumed that body fat percentage remained constant over time, i.e. held at its default value.

<u>Egg laying frequency and egg weight</u>
The egg laying frequency (or hen-day egg percentage) is the percentage of hens in a flock that lay an egg on any particular day, and an egg laying frequency of 100% represents one egg per hen per day. With higher egg laying frequency, more dioxins can be excreted from the body. The model uses 90% as standard egg laying frequency. In the transfer model, the egg weight (without shell) used is 60 grams, containing 5.8 g egg yolk fat.
For this FO assessment, it is assumed that the used chicken breed and the parameters from the experiment used for development of the transfer model are representative for all animals involved in the current egg production in The Netherlands.

Estimation of concentrations in meat and meat products corresponding to MLs in eggs
As already mentioned, the MLs for eggs are expressed per gram of fat, with the corresponding concentrations in meat/meat products being calculated on a fat basis, i.e. body fat compartment. For the transfer calculation, it was assumed that dioxins and DL-PCB concentrations had reached a steady state in all model compartments, i.e. the body fat, the central compartment and the egg yolk fat (excretion route). The results of this calculation, which are shown in Table 2, indicate that in steady state, the sum-TEQ

respectively dioxins-TEQ concentrations in body fat and in egg yolk fat are expected to be equal (see Annex I)

This means that, whether based on the sum-TEQ or the dioxins-TEQ, the model predicts that when the ML for egg yolk fat is exceeded, also the ML for meat and body fat will be exceeded.

Table 2a and 2b. Calculated concentrations of the sum of dioxins and DL-PCBs (sum-TEQ, 2a) and sum of dioxins (dioxins-TEQ, 2b) in meat that correspond to the MLs in hen eggs after prolonged exposure (steady state).

Table 2a

Sum of dioxins and DL-PCBs (SUM-TEQ[1]) in pg/g fat				
	Legal limit in hen eggs and egg products	Corresponding calculated concentration in body fat	Legal limit in meat and meat products (excluding edible offal) of poultry	Legal limit in fat of poultry
ML	5.0	5.0	3.0	3.0

Table 2b

Sum of dioxins (dioxins-TEQ[1]) in pg/g fat				
	Legal limit in hen eggs and egg products	Corresponding calculated concentration in body fat	Legal limit in meat and meat products (excluding edible offal) of poultry	Legal limit in fat of poultry
ML	2.5	2.5	1.75	1.75

[1] These TEQs are calculated on the basis of the WHO (2005) TEF values.

Measured concentrations from transfer experiment

For verification of/to support the above model calculations, the measured concentrations from the animal experiment (Hoogenboom et al., 2006) were evaluated in this assessment. In this experimental study, six groups of laying hens were fed with feed containing different levels of dioxins and DL-PCBs for a period of 56 days. Sum-TEQ levels in feed varied between 0.04 (blank) and 3.95 ng TEQ/kg. When focussing on day 56 and the four highest dose levels (to avoid non-detects), the dioxins-TEQ ratios for egg fat to body fat for these four dose levels were 1.2/1.1/1.5/1.1 (average 1.2) and for the sum-TEQ to 1.1/1.1/1.4/1.1 (average 1.2). It should be noted that after 56 days no steady state was obtained. At this moment in time, the egg fat contained higher levels than body fat. However, with elongation of the exposure period, the differences became smaller and it is expected that levels will eventually be similar.
The data from the transfer study confirm that egg and body fat levels are almost similar after prolonged exposure and that non-compliant egg levels imply also non-compliant levels in meat and fat of the laying hens.

Conclusions

MLs

The calculated concentration of the sum of dioxins and DL-PCBs, i.e. the sum-TEQ, in body fat that corresponds to the ML for sum-TEQ of 5.0 pg /g fat for hen eggs, is 5.0 pg sum-TEQ/g body fat.
This is higher than the ML of 3.0 pg sum-TEQ/g fat for meat, meat products (excluding edible offal) and fat of poultry.

Similarly, the calculated concentration of dioxins, i.e. the dioxins-TEQ, in body fat that corresponds to the ML of 2.5 dioxins-TEQ/g fat for hen eggs, is 2.5 pg dioxins-TEQ/g body fat.
This is higher than the ML of 1.75 pg dioxins-TEQ/g fat for meat, meat products (excluding edible offal) and fat of poultry.

Measured concentrations
The ratios of the dioxins and DL-PCB concentrations in eggs and meat measured in a transfer experiment in laying hens support the model calculations.

Conclusion
Overall, it can be concluded that it is very likely that the MLs in meat, meat products (excluding edible offal) and fat in poultry will be exceeded, when the MLs for dioxins (dioxins-TEQ) or for the sum of dioxins and DL-PCBs (sum-TEQ) in hen eggs are exceeded.

Uncertainties

- The relationship between steady state levels of the sum-TEQ or dioxins-TEQ levels in egg yolk fat and body fat, i.e. meat, was assessed using a transfer study in which laying hens were fed with a specific mixture of dioxins and DL-PCBs. The calculated steady state sum-TEQ ratio (egg yolk fat/body fat) therefore reflects the composition of this mixture. Transfer conclusions based on this ratio may be erroneous in cases where the congener mixture in feed deviates significantly from the congener mixture in the mentioned transfer study.
- The model was used with the default parameters. In reality, these parameters vary with breed and age of the laying hen. However, in the Annex it is shown that possible variation in the combined input parameters does not influence the distribution of dioxins and DL-PCBs between body fat and egg yolk fat in steady state to a major extent.
- The presented calculations are based on the assumption that dioxin/DL-PCB levels are at steady state, i.e. at a stable situation reached after prolonged exposure. It should be noted that the distribution between body fat and egg yolk fat may be different in the period before a steady state is reached yet. Regarding this, Hoogenboom et al. (2006) and Van Eijkeren et al. (2006) showed for the sum-TEQ that during the first weeks of exposure the ratio of the levels in egg yolk fat to body fat are actually higher than the ratios calculated for a steady state situation. This means that the assumption of a steady state may result in an overestimation of the concentration in body fat during the first weeks of exposure.

References

Bokkers BGH, van Eijkeren JCH, Hoogenboom LAP, Traag WA and Zeilmaker MJ. (2014) Carry-over of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) from feed to eggs of laying hens: A congener specific approach (Deliverable to the EU Project QSAFFE).

Hoogenboom LAP, Kan CA, Zeilmaker MJ, Van Eijkeren J and Traag, WA (2006). Carry-over of dioxins and PCBs from feed and soil to eggs at low contamination levels – influence of mycotoxin binders on the carry-over from feed to eggs. Food Additives and Contaminants, May, 2006; 23(5): 518–527.

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Van Eijkeren JCH, Zeilmaker MJ, Kan CA, Traag WA and Hoogenboom LAP (2006) A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. Food Additives and Contaminants, May, 23(5): 509-517.

Annex I

Sum-TEQ ratio: Steady State analysis in egg yolk fat and body fat in laying hens.

Context

Hoogenboom *et al.* (2006) describe an experiment to study the transfer of a mixture of PCDD/Fs and DL-PCBs, i.e. sum-TEQ, from feed to body fat and egg yolk fat in highly productive laying hens (laying efficiency: 0.9). The results of this experiment were used to develop a mathematical model (Van Eijkeren *et al.*, 2006). This model consists of two compartments, i.e. a central compartment and a body fat compartment, with uptake from a feed matrix as route of entrance, exchange between the central and the body fat compartment, and removal from the central compartment by means of excretion to egg yolk fat and an otherwise undefined clearance.

The model contains six unknown parameters, i.e. the fraction absorbed from the administered feed matrix (F_{abs}), the rate constant for the exchange from the central compartment to the body fat compartment (q_c, day^{-1}), the rate constant for the exchange from the body fat compartment to the central compartment (q_f, day^{-1}), the rate constant for the transport from the central compartment to egg yolk fat (y, day^{-1}) , the rate constant for the clearance from the central compartment (k) and the size of the body fat compartment (W_f).

Of these parameters q_c and q_f could be identified on the available data. The remaining parameters F_{abs}, k, y and W_f were conditionally identifiable, i.e. they were identified as a range.

As described in Van Eijkeren et al. (2006) the long-term exposure, i.e. around 200 days, to the sum-TEQ ultimately leads to a steady state situation in body fat and egg yolk fat. Furthermore, it appeared that the calibration on the sum-TEQ also applied to the dioxins-TEQ, i.e. the TEQ based on the sum of the PCDDs and PCDFs.

The sum-TEQ model calibration described in Van Eijkeren *et al.* (2006) was based on TEF values derived by WHO in 1998. In an update procedure, Bokkers *et al.* (2014) re-calibrated the model on the TEF values as derived by WHO in 2005.

In steady state the model prescribes the ratio between the sum-TEQ in egg yolk fat and body fat to be:

$$\frac{C_{y,f,ss}}{C_{f,ss}} = \frac{y \cdot W_f \cdot q_f}{W_{y,f} \cdot q_c} \qquad (1)$$

with:

$C_{y,f,ss}$	steady state concentration in egg yolk fat (pg sum-TEQ/g egg yolk fat)
$C_{f,ss}$	steady state concentration in body fat (pg sum-TEQ/g body fat)
y	sum-TEQ excretion rate constant from the central compartment to egg yolk fat (day^{-1}, calibrated)
W_f	body fat weight in laying hen (g, calibrated)
q_c	rate constant for the transport of the sum-TEQ from the central compartment to body fat (day^{-1}, calibrated)
q_f	rate constant for the transport of the sum TEQ from body fat to the central compartment (dag^{-1}, calibrated)
$W_{y,f}$	egg yolk fat (g, known)

Note that the ratio shown is independent of the administered dose rate, the fraction absorbed from the administered feed matrix (sum-TEQ: $0.78 < F_{abs} < 1$ for a matrix mainly consisting of wheat, corn and extracted soybeans, Hoogenboom *et al.*, 2006) and

the rate constant for the clearance of the central compartment (sum-TEQ*:* $0 < k <$ 0.013).
Furthermore note that the amount of egg yolk fat $W_{y,f}$ is a known parameter, i.e. experimentally determined at 5.8 g per egg.

From equation (1) the (absolute) maximum is:

$$\text{Ratio}_{\text{max}} = \left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{\text{max}} = \frac{y_{\text{max}} \cdot W_{f,\text{max}} \cdot q_f}{W_{y,f} \cdot q_c} \quad (2)$$

with absolute minimum:

$$\text{Ratio}_{\text{min}} = \left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{\text{min}} = \left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{\text{max}} \cdot \frac{y_{\text{min}} \cdot W_{f,\text{min}}}{y_{\text{max}} \cdot W_{f,\text{max}}} \quad (3)$$

For the sum-TEQ Bokkers *et al*. (2014) provides: q_c = 0.14/day, q_f = 0.060/day, 230 < W_f < 290 g and 0.044 < y < 0.057/day.

Substituting in equations (2) and (3) then gives:

$$\text{Ratio}_{\text{max}} = \left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{\text{max}} = \frac{0.057 \cdot 290 \cdot 0.060}{5.8 \cdot 0.14} \approx 1.22$$

$$\text{Ratio}_{\text{min}} = \left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{\text{min}} = 1.22 \cdot \left(\frac{230 \cdot 0.044}{290 \cdot 0.057} \right) \approx 0.75$$

and for the average of these two, based on the central estimates for W_f (which equals $\frac{W_{f,\text{min}} + W_{f,\text{max}}}{2}$) and y (which equals $\frac{y_{\text{min}} + y_{\text{max}}}{2}$):

$$\left(\frac{C_{y,f,ss}}{C_{f,ss}} \right)_{Average} = \frac{0.051 \cdot 260 \cdot 0.060}{5.8 \cdot 0.14} \approx 0.98, \text{rounded } 1$$