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Chemical contamination of free-range eggs from Belgium

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Running Head : Chemical contamination in eggs

Abstract

The elements Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Tl, Pb and Hg and selected persistent organochlorine compounds (dioxins, marker and dioxin-like PCBs, DDT and metabolites as well as other chlorinated pesticides) were analysed in Belgian free-range

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eggs obtained from hens of private owners (PO) and of commercial farms (CF). It was found that PO eggs were more contaminated than CF eggs. The ratios of levels in PO eggs to levels in CF eggs range from 2 to 8 for the toxic contaminants Pb, Hg, Tl, dioxins, PCBs and the group of DDT. The DDT contamination was marked by substantial presence of p,p'-DDT in PO eggs in addition to p,p'-DDE and p,p'-DDD. It is postulated that environmental pollution is at the origin of the higher contamination of PO eggs. Extensive consumption of PO eggs is likely to result in TEQ intake levels exceeding the tolerable weekly intake.

Key words: free-range hens, eggs, pesticides, heavy metals, CALUX, dioxins, POPs, PCBs, environmental contamination, food safety, home-produced food

Introduction

Currently consumers increasingly prefer “free-range” eggs over battery eggs due to the general perception that these products are attributed a healthier nature and better nutritional qualities. Therefore, private owners (PO) often keep and raise hens in a free-range system. Free-range is a term that brings to mind idyllic barnyard scenes, whereby hens can run free and have access to pasture for foraging. Collecting the flock’s eggs is considered an enjoyable and profitable business. However, since the hens live in close contact with the outside environment they are exposed to contaminants. Moreover, contaminants’ levels in the eggs from free-range hens are considered good indicators of the contamination of the environment the hens live in (Chang et al., 1989). Eggs are fat rich food items and, hence, humans consuming free-range eggs are eventually exposed to the lipophilic contaminants that accumulated in these eggs. It is generally accepted

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4 that fatty food intake constitutes a predominant exposure route for polychlorinated
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6 dibenzo-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated
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8 biphenyls (PCBs), organochlorinated pesticides (OCPs) and other persistent organic
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10 pollutants (POPs). On the other hand, the general population is also exposed to heavy
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12 metals through both food intake as well as inhalation.
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18 According to the literature background total TEQ levels for dioxins and dioxin-like
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20 PCBs in commercially available eggs sampled in several European countries range from
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22 0,69 to 2.76 pg TEQ per g egg fat (Hamm et al., 2005, Eljarrat et al., 2002, Gallani et al,
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24 2004, Baars et al., 2004, Focant et al., 2002). The data do not accurately distinguish
25
26 between free-range and battery farm eggs, however, and often only very few samples
27
28 were analysed. For free-range hens that forage on soil higher levels up to 88 pg
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30 PCDD/F WHO TEQ per g fat were observed at different occasions. These elevated
31
32 values have been linked to environmental parameters, e.g. incinerator ash contaminated
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34 soils, wood shavings in chicken litter, contaminated environmental feed ingredients
35
36 such as ball clay and kaolin and the proximity of chemical plants. (Pless-Mulloli et al,
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38 2001, Diletti et al., 2005, Hayward et al., 1999, Schmid et al., 2002, Harnly et al., 2000,
39
40 Schuler et al., 1997). Alarming high values of ~120 pg PCDD/F TEQ per g fat were
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42 found in eggs sampled nearby an old municipal solid waste incinerator in France (Pirard
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44 et al., 2005).
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54 Home produced foodstuffs are generally not submitted to any compliance control
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56 although they are consumed in large quantities by their producers. Therefore, it was
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58 decided to investigate the contamination of PO free-range eggs. Additionally, the
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4 present study was triggered by an earlier concern of the Belgian Federal Agency for the
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6 Safety of the Food Chain (FASFC). In 2002 the FASFC was informed about elevated
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8 dioxin levels in eggs from free-range hens kept by PO living north of Antwerp
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10 (Belgium) (Pussemier et al., 2004). Observed levels were considerably higher than
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12 those found in free-range eggs from commercial egg production farms. In its advice
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14 (2002/35 www.favv.be) the FASFC committee emphasized the risks associated with
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16 regular consumption of such eggs as well as the need for more elaborate studies to
17
18 estimate the impact of the problem and its possible health effects.
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26 The design of this study focuses on contaminations by POPs, including dioxin-like
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28 chemicals, PCBs, OCPs, as well as heavy metals, in eggs from free-range hens, sampled
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30 in all regions of Belgium. The objectives of this study are (1) a better insight in the
31
32 occurrence patterns, (2) an evaluation of the measured levels and/or excesses, and (3) a
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34 prudent risk assessment.
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36

37 **Materials and methods**

38 *Sample collection*

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40 Egg samples (free-range) from different regions in Belgium were acquired during the
41
42 months April- July 2004 (spring 2004), both from free-range hens held by private
43
44 owners and from commercial egg production farms. Egg samples were obtained from
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46 22 private owners residing in different parts of Belgium. Additionally, samples from 19
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48 commercial egg production farms were collected with the kind help of the Belgian
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50 Federal Agency for the Safety of the Food Chain. Both organic (n = 8) and traditional
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52 farms (n = 11) were included. Each sample was made up by collecting 12 eggs from one
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54 breeding. Eggs were kept at 4 °C until analysis.
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Investigated Analytes

The investigated analytes comprehend seven marker PCBs (IUPAC numbers 28, 52, 101, 118, 138, 153, 180), polychlorinated dioxins and furans, dioxin-like PCBs and the OCPs hexachlorobenzene (HCB), α -, β - and γ - hecachlorocyclohexane (HCH) isomers, heptachlor, heptachlor epoxide, aldrin, dieldrin, α - and β - endosulfan, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT, methoxychlor and endrin. The elements analysed are Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Tl, Pb and Hg.

Analytical procedures

Elements. The elements were analysed by inductively coupled plasma-mass spectrometry (ICP-MS). Two of the 12 eggs obtained by each breeder were used for duplicate analyses of the elements (i.e. two separate analyses on two different eggs from the sample).

The external surface of eggs was washed and dried before the separation of yolk and white that were subsequently analyzed. Subsamples were weighed (0.2-0.5 g) and mineralized by acid digestion (4 mL nitric acid + 4 mL demineralised water) in pressure vessels of a X-PRESS (CEM Corporation, NC, U.S.A.) microwave digestion system. The obtained solutions were diluted (to a factor 400 which allows avoiding matrix effects) directly in tubes for ICP-MS autosampler. The ICP-MS system used was a VG PQ-ExCell (TJA, U.S.A.). A calibration curve (eight aqueous standards between 0.1 and 10 $\mu\text{g/L}$, corresponding to 40–4000 $\mu\text{g/kg}$ in the egg) was established with freshly prepared solutions of the same acidity as samples. Under standard analytical conditions,

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4 the following isotopes were measured: ^{51}V , ^{53}Cr , ^{55}Mn , ^{59}Co , ^{60}Ni , ^{65}Cu , ^{66}Zn , ^{75}As ,
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6 ^{82}Se , ^{95}Mo , ^{114}Cd , ^{121}Sb , ^{205}Tl and ^{208}Pb . Mercury was determined using a separate
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8 analytical method based on the atomic absorption of mercury vapour after combustion
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10 of the raw sample in a stream of oxygen. The released mercury was first trapped on a
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12 gold amalgamator. It was then released by heating and its vapour conducted to the
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14 absorption cell. The system used was AMA-254 (Altec, Czech Republic).
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21 *Fat extraction.* The yolks of 10 of the 12 eggs obtained from each breeding were pooled
22
23 for the analyses of organohalogenated contaminants. The pooled yolks were frozen
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25 before analysis.
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28 They were lyophilised during 48 h and then extracted with hexane (pestanal, for residue
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30 analysis grade) by pressurized liquid extraction (PLE) using a Dionex (USA) ASE
31
32 extractor. Cells of 33 mL were filled with freeze-dried samples and anhydrous Na_2SO_4 .
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34 The extraction conditions were: 125 °C, 1500 PSI, two static cycles of 5 min. The
35
36 extracts were dried afterwards under a stream of nitrogen until constant weight.
37
38 Generally around 25% of fat was obtained from the egg yolks. A part of the obtained fat
39
40 was used for the analyses of the seven marker PCBs and the organochlorinated
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42 pesticides and another part was used for the determination of dioxins and dioxin-like
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44 PCBs.
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52 *Analysis of marker PCBs.* Marker PCBs were determined by gas chromatography with
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54 electron capture detection (GC-ECD) and GC/MS/MS (for confirmation). The method
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56 is based on the prEN1528-1-1996E method (EN, 1996) and the recommendations of the
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58 Belgian Bureau of Accreditation Beltest (Beltest, 2000). 0.4 g of fat was dissolved in 2
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4 mL of hexane and 50 µl of a 1 mg/mL PCB 143 solution was added to control the
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6 purification step. The resulting solution was loaded on an acidified silicagel column
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8 composed of 6 g of acidified silica gel (60/40 silica/H₂SO₄), 1 g of Al₂O₃ containing 8
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10 % of water and on the top, 0.5 g of anhydrous Na₂SO₄. The tube containing the fat
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12 solution was rinsed twice with 2.5 mL of hexane, and the column was further eluted
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14 with 20 mL of hexane. After concentration under a gentle dried-air stream to ca 1 mL,
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16 50 µl of a 1 mg/mL mirex solution was added as internal standard and 3 µl was injected
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18 into the gas chromatography (GC) system. Analysis was performed on Trace 2000
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20 (Thermo-Finnigan, Interscience, Belgium) equipped with ECD at 300 °C, an auto
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22 sampler AS2000, a HT-8 capillary column (25 m, 0.22 mm x 0.25 µm) (SGE, Achrom,
23
24 Belgium). Argon/methane was used as make-up gas and Helium was used as carrier gas
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26 (1 mL/min). Injection was done at 220 °C in a split/splitless mode. The temperature
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28 program was set at 100 °C for 0.5 min, then increased by 25 °C/min to 270 °C and held
29
30 for 8 min. PCBs results above the MRL were confirmed by GC-MS/MS, an ion Trap
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32 Polaris-Q from Thermo-Finnigan (Interscience, Belgium) equipped with a HT-8
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34 capillary column (25 m, 0.22 mm x 0.25 µm). The flow of helium was 1 mL/min and
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36 the temperature programme was set at 90 °C for 0.5 min and then increased by 25
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38 °C/min to 220 °C, the temperature was then increased by 5 °C/min to 265 °C and held
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40 for 6 min. As a quality control, a blank solvent, a blank procedure and a control sample
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42 were run within each series. The limits of quantification are 10 ng/g of fat.
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54 *Analysis of pesticides.* OCPs were analysed by GC-ECD and GC-MS/MS (for
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56 confirmation). The method is also based on the prEN1528-1-1996E method (EN, 1996).
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59 0.2 g of fat was dissolved in 2 mL of hexane and 50 µl of a 1 mg/mL
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4 dibromooctafluorobiphenyl solution was added to control the purification step. The
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6 resulting solution was loaded on an alumina column composed of 10 g of Al_2O_3
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8 containing 8 % of water and on the top, 0.5 g of anhydrous Na_2SO_4 . The tube containing
9
10 the fat solution was rinsed twice with 1 mL of hexane, and the column was further
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12 eluted with 80 mL of hexane. After concentration under a gentle dried-air stream to ca 1
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14 mL, 50 μl of a 1 mg/mL mirex solution was added as internal standard and 1 μl was
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16 injected into the gas chromatography (GC) system. Analysis was performed on the same
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18 system as for PCBs but the Helium flow is 1.4 mL/min and the temperature of the
19
20 column was set at 80 °C for 2 min, then increased by 4 °C/min to 180 °C and finally by
21
22 6 °C/min to 270 °C and held for 10 min. The identity and concentration of all pesticides
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24 exceeding the MRL (Maximum Residue Level) (Royal Order, 2000a) were confirmed
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26 on a DB1701 capillary column (60 m x 0.32 mm x 0.25 μm) (J&W, Alltech, Belgium).
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28 The Helium flow was set at 3 mL/min and the program temperature was: 100 °C for 2
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30 min, then increased by 20 °C/min to 180 °C, then increased by 1.8 °C/min to 245 °C
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32 and finally by 20 °C/min to 265 °C and held for 5 min. The identity and concentration
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34 for unusual ratio of DDT isomers were confirmed by GC-MS, an ion Trap Polaris-Q
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36 from Thermo-Finnigan (Interscience, Belgium) equipped with a HT-8 capillary column
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38 (25 m, 0.22 mm x 0.25 μm). The flow of Helium was 1 mL/min and the temperature
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40 program was set at 80 °C for 2 min and then increased by 12 °C/min to 270 °C and held
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42 for 15 min. As a quality control, a blanco solvent, a blanco procedure and a control
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44 sample were run within each series. The limits of quantification are 10 ng/g of fat.
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56 *CALUX assay*. Dioxins and dioxin-like compounds were determined by the chemically
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58 activated luciferase gene expression (CALUX) bioassay provided by Xenobiotic
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Detection Systems Inc.(XDS) (USA). This assay does not provide information about the presence of individual PCDD/F congeners. It gives an overall response for all compounds that are able to activate the aryl hydrocarbon receptor (AhR) and AhR-dependent gene expression. The bioassay result can be considered as a TCDD toxicity or TEQ value since the calibration in this assay is performed with 2,3,7,8-TCDD. 1 g of fat (ASE extracted) was purified on an acidified silica gel column and dioxin-like compounds were isolated by passing the eluate over an activated carbon column (X-Carb from XDS). A PCB fraction containing the dioxin-like PCBs and a dioxin fraction containing the polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans were separately obtained by elution of the carbon column with a mixture of hexane/ethyl acetate/toluene (8:1:1) and with toluene, respectively. Both fractions were analysed with the CALUX assay yielding a dioxin TEQ result and a PCB TEQ result. Details of the *in vitro* assay were previously described (Van Overmeire et al. 2004). Briefly, mouse hepatoma H1L6.1 cells, grown in 96 well plates, were exposed to the purified extracts for 20 h and the produced luciferase was quantified by measuring the luminescence with a Berthold Orion luminometer after adding of luciferin. A standard curve of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (range: 0.098-50 pg /well) is included on each plate. The CALUX method has been validated for the screening of egg samples. The precision meets the requirements of the EU (EU, 2002). The mean coefficients of variation for repeatability and for intralaboratory reproducibility were 10 % and 21 %, respectively. With each series of purification a quality control sample at 3 pg TEQ/g fat and a procedural blank were included. Quality control of the plates with cells and extracts was performed as described (Windal et al. 2005a). The method has also been evaluated by comparison with GC-HRMS results during the EU

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4 DIFFERENCE project for different sample matrices among which eggs (Carbonnelle et
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6 al., 2004a).

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9 *Statistical analysis*

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11 For the statistical calculations, the concentration was considered as zero when specific
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13 compounds gave a result below the LOQ. Pearson correlation coefficients were
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15 calculated with STATISTICA version 6.0 (1998).
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19 **Results**

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21 *Heavy metals*

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23 Levels of 13 elements (mostly belonging to the group of so-called “heavy metals”) were
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25 determined in eggs obtained from private owners (PO) and in eggs from commercial
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27 farms (CF). Descriptive statistics (including medians and range) of the results are shown
28
29 in Table I (PO) and in Table II (CF). Figure 1 shows the ratios (PO:CF) of median
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31 concentrations determined in the eggs. This ratio is close to one for most substances
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33 except for Mo (ratio = 0.54) and for some toxic elements (Pb, Tl, Hg and Co) for which
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35 the ratio ranges from 2 to 6.
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43 Insert Table I and Table II and Figure 1

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45 *Organochlorinated compounds*

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47 Several pesticides, PCBs as well as dioxin-like compounds have been determined in PO
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49 eggs and in CF eggs. Descriptive statistics for the compounds for which the mean levels
50
51 were higher than the quantification limit are summarised in Table III for the eggs from
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53 hens of PO and in table IV for CF eggs. Results are shown, for the sum of seven marker
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55 PCBs, for the sum of aldrin and dieldrin and for the sum of DDT, DDE and DDD
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57 because the Belgian legislation foresees a norm level (maximal allowed level) for these
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4 sums of PCBs and pesticides (Royal Order, 2000a and 2000b). In addition, dioxin and
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6 PCB TEQ values are given.
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11 Insert Table III and Table IV
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16 *Marker PCBs.* The median of the sum of the seven marker PCBs (PCB 28, 52, 101, 118,
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18 138, 153, 180) is 32.30 ng /g fat for the PO eggs with levels ranging from 0 to 351 ng/g
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20 fat. The median PCB level in eggs from commercial farms is zero and levels range from
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22 0 to 63.20 ng/g fat. In 8 of the 22 PO egg samples none of the marker PCBs was
23
24 detected while in 95% of the CF eggs no marker PCB congeners were detected. The
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26 congener profile for the eggs from hens of PO is shown in Figure 2. The most abundant
27
28 PCB congeners are PCBs 153, 138 and 118. For all samples the mean contribution of
29
30 these three congeners to the sum of the seven marker PCBs is 82 %, of which PCB 153
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32 accounts for 49 %.
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40 Insert Figure 2
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45 *OCPs.* The values for the sum of aldrin and dieldrin are in general low but elevated
46
47 levels were found in PO eggs as compared to CF eggs. The median values for PO and
48
49 for CF eggs are 14,35 and 0 ng/g fat, respectively. The maximal values amount to
50
51 115.40 and 18.10 ng/g fat, respectively. The levels for the sum of DDT, DDE and DDD
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53 in PO eggs range from 26.40 to 5250 ng/g fat with a median value of 125.45 ng/g fat.
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55 Six samples of PO eggs exceed the norm level of 500 ng/g fat. These elevated values
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57 result in a mean level for all private owners of 770,71 ng/g fat (above the norm level).
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4 In CF eggs lower levels were found ranging from 0 to 171.90 ng/g fat (median: 10.70
5 ng/g fat). They were calculated for PO eggs, for the six PO egg samples that exceed the
6 norm value and for the CF eggs. In the farms' eggs only p,p'-DDE and p, p'-DDD
7 contribute to the sum with median values of 100 and 91 % respectively, while in the
8 eggs obtained from private owners there is, in addition to p,p'-DDE and p,p'-DDD also
9 a considerable contribution of p,p'-DDT to the sum of DDT, DDE and DDD. The
10 contribution of p,p'-DDE to the sum DDT/DDE/DDD ranges from 13 to 97% (median:
11 52 %) and the contribution of p,p'-DDT to the sum DDT/DDE/D ranges from 2 to 56 %
12 with a median of 32 %. In addition, the profile for all egg samples analysed is shown in
13 Figure 3. p,p'-DDE was found in all PO egg samples, p,p'-DDT in 91 % of the
14 samples, p,p'-DDD in 77 % of the samples and o,p'-DDT in 27 % of the samples
15 (Figure 3A). In CF egg samples p,p'DDD and p,p'DDE were detected in 63 % and 47
16 %, respectively while p,p'-DDT and o,p'-DDT were not detected in CF eggs (Figure
17 3B).

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40 Insert Figure 3

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44 *Dioxin-like chemicals.* Evaluation of the dioxin like activity revealed higher TEQ levels
45 in PO eggs than in CF eggs. The dioxin TEQ values range from 2.28 to 23 pg /g fat for
46 PO eggs (median 8.53 pg TEQ/g fat) and from 0.5 to 4.21 pg/g fat for CF eggs (median
47 1.04 pg TEQ/g fat).

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54 The dioxin like activity for the PCB fraction obtained after purification of the egg fat is
55 generally low for both types of eggs but here is a difference visible between the levels in
56 the different types of eggs. The PCB TEQ levels in PO eggs are higher than the levels in
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4 the CF eggs. The median levels for PO eggs and CF eggs are 1.73 and 0.69 pg TEQ/g
5 fat, respectively. In PO eggs the PCB TEQ values range from 0.06 to 11.84 pg/g fat and
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7 in CF eggs they range from 0.31 to 1.69 pg TEQ/g fat. The ratio of the median PCB
8
9 TEQ value for PO eggs to the median PCB TEQ value for CF eggs is 2.5.
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13 Discussion

14 *Contamination levels*

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18 *Heavy Metals.* For many elements such as Cu, Zn, Mn, Ni, As, Se, Cd and Sb the
19 median values are similar whatever the origin of the eggs (OP or CF) (see Table 1 and
20 Figure 1). Overall, it can be said that the levels determined in this study are in
21 agreement with those documented elsewhere (FSA, 2004, Leblanc et al., 2004). For Mo,
22 it appears that eggs from PO have a lower median concentration (ratio PO: CF = 0.54).
23 This can be explained by the fact that the feed used by PO cannot provide the same
24 intake level as can be obtained with commercial feed. The latter is supplemented with
25 mineral elements and, more specifically, with Mo. For Pb, Tl, Hg and Co, the ratios of
26 median concentrations range from 2 to 6, indicating much higher levels of these
27 contaminants in PO eggs. For these elements, the agreement with published data (FSA,
28 2004, Leblanc et al., 2004) is not always satisfactory but it must be added that the mode
29 of egg production (battery or free-range) and the method of determination of the very
30 low concentrations of elements are not fully documented in the above mentioned
31 studies. Our results can also be compared with the data obtained on eggs taken from
32 markets in Huelva, a Spanish city with strong industrial activity. In our study, the levels
33 determined in the eggs from the commercial farms are lower for Cd and Hg but higher
34 for As and Pb than the levels determined in Spain (Bordajandi et al., 2004). On the other
35 hand, the levels that we found for Cu and Zn are comparable with the levels published
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4 by these authors. Moreover, the Cd, Hg and Pb levels that we obtained for both egg
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6 types were clearly higher than the levels found in free-range eggs in Ireland (FSAI,
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8 2004). This suggests a higher background soil contamination with these toxic elements
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10 in Belgium as compared to Ireland.
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16 *Marker PCBs.* For the eggs from the egg production farms no significant contamination
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18 with indicator PCBs was observed. On the other hand, the norm level of 200 ng/g fat for
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20 the sum of the seven indicator PCBs (Royal Order, 2000b) was exceeded in two PO egg
21
22 samples. In PO eggs the most abundant PCB congeners were PCBs 118, 138, 153, and
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24 180. The low levels of PCBs 52 and 101 are in accordance with literature data
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26 demonstrating metabolism of these two congeners in hens (De Vos et al., 2005,
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28 Maervoet et al., 2004). The low levels of PCBs 52 and 101 in the eggs that were
29
30 analysed in our study are therefore linked to either a low intake of these congeners or to
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32 metabolism of the ingested amounts. The mean level of the sum of the seven marker
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34 PCBs for the CF eggs is in the same range as the level for indicator PCBs in Irish free-
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36 range eggs (Tlustos et al., 2004) and the level in commercial eggs from Catalonia in
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38 Spain (Eljarrat et al., 2002). The levels that we found in PO eggs are approximately
39
40 twice as high as the levels for total PCBs (13 congeners including the seven indicator
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42 PCBs that we analysed) in eggs from markets in Huelva, a Spanish city situated in an
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44 industrial area (Bordajandi et al, 2004).
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54 *OCPs.* The results for most OCPs are well below the Belgian tolerable levels. Only for
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56 the sum of DDT, DDE and DDD some high exceedings, up to 10 times the tolerated
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58 level, were observed for PO eggs. In the present study a mean of 770,71 ng/g fat for the
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4 sum of DDT, DDE and DDD was obtained for the eggs from private owners. To put the
5 data of our study in perspective they can be compared with literature data. Vieira et al.
6 performed a study on a limited number of egg samples in Brazil, where DDT has been
7 used to control leishmaniasis. These authors report average Σ DDT concentrations in
8 chicken eggs of 1983 ng/g fat (n = 3) and 730 ng/g fat (n = 6) with 82 % of p,p'-DDE. It
9 is interesting to note that these eggs were sampled in the vicinity of places where DDT
10 had been used 7 and 9 years before the sampling (Vieira et al., 2001).
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23 DDT, and more particularly, the main compound in the technical pesticide product, was
24 found in all PO eggs. Knowing that the use of DDT in Belgium has been banned more
25 than 25 years ago, the mean DDT/DDE ratio of 0.68 that was found in eggs obtained
26 from private breeders might indicate recent use of DDT (to decontaminate the breeding
27 place). This is illustrated by one case of contaminated PO eggs (4200 ng/g fat) that we
28 studied in more detail. The p,p'-DDT/p,p'-DDE ratio was unusually high in this case
29 (1,42). We investigated a dust sample taken inside the hens' house as well as a soil
30 sample from the land on which the chickens of this breeder were allowed to forage.
31 Remarkably, in the dust sample, both the Σ DDT (5942 ng/g) and the percentage of p,p'-
32 DDT (85.2%) were higher than in the soil sample (3416 ng/g and 40.9%, respectively),
33 which pointed to the dust inside the hens' house as the primary source of DDT
34 contamination in this breeding. This suggests that DDT was formerly used inside the
35 hens' house (most probably before the ban on DDT, according to the breeder) and
36 because of the limited degradation of DDT under indoor conditions a high proportion of
37 untransformed DDT has been found in this case. This phenomenon seems typical for the
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4 private holders of chickens as in eggs from commercial farms no traces of p,p'-DDT
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6 were detected.
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11 *Dioxin-like activity.* The European Community has promulgated strict maximum levels
12 for food and feed (EU, 2001a and EU, 2001b). They are expressed in TEQ units of 7
13 PCDDs and 10 PCDFs with an assigned TEF value (Van den Berg et al., 1998). It must
14 be noticed that recently new maximum levels for the sum of dioxins and dioxin-like
15 PCBs have been set (EU, 2006). For a transitional period the existing levels for dioxins
16 will continue to apply in addition to the newly set levels for the sum of dioxins and
17 dioxin-like PCBs. Typically, concentrations are determined by capillary gas
18 chromatography (GC) in combination with high-resolution mass spectroscopy (HRMS),
19 whereas the data presented in this study are based on the CALUX technology (Garrison
20 et al., 1996, Van Overmeire et al., 2001, Baeyens et al., 2004 and references herein).
21 While CALUX assay derived TEQs and GC-HRMS TEQs have been observed to be
22 relatively close for numerous sample matrices (Denison et al., 2004), they can differ too
23 and a variety of factors contribute hereto (Windal et al., 2005). Conceptually, CALUX
24 analyzes the overall biological activity or induction of gene expression caused by all
25 AhR ligands (agonists and antagonists) present in the sample extract, while a chemical
26 analysis focuses on selected numbers of compounds only. In the present study CALUX
27 dioxin TEQ values are compared to the compliance concentration for dioxins of 3 pg
28 TEQ per g fat for eggs (including free-range) (EU, 2001a, EU, 2004). An estimate of
29 the TEQ values for dioxin-like PCBs, obtained with the CALUX approach, is also
30 presented in this paper. For eggs the maximal level for the sum of dioxins and dioxin-
31 like PCBs is 6 pg WHO TEQ/g fat (EU, 2006). Over 75 % of the observed dioxin TEQ
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4 values of PO eggs exceed the current norm concentration for dioxins of 3 pg TEQ per g
5 fat. The median of the sums of CALUX dioxin TEQ and CALUX PCB TEQ values
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7 obtained in this study is 9.66 pg TEQ/g fat for PO eggs and 1.87 pg TEQ/g fat for CF
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9 eggs. Even when CALUX results are considered non-quantitative or semi-quantitative
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11 (Hoogenboom et al., 2000) the median value for CALUX dioxin TEQ of 8.53 pg TEQ
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13 per g fat definitely indicates non-compliance with the norm level. Van Overmeire et al.
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15 (2004) calculated that the decision limit or CC_{α} corresponding to the norm level of 3 pg
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17 PCDD/F TEQ per g fat for milk samples amounts to 4.53 (Van Overmeire et al., 2004).
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19 The CC_{α} concept takes into account the measurement uncertainty. Hence, assuming a
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21 similar measurement uncertainty for egg fat analyses, all results above the 1st quartile
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23 significantly exceed the EU norm value. This illustrates the extent of the dioxin
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25 contamination problem for PO eggs. Additionally, the observed values range from 2 to
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27 23 pg TEQ per g fat; a very broad range that suggests different contamination sources,
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29 presumably in combination with varying foraging regimes. In general, these
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31 measurement results compare well with previously published data. An average and
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33 maximal value of, respectively, ~10 and ~13 pg TEQ per g fat were obtained for few
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35 GC-HRMS analyses of PO eggs sampled in the vicinity of Antwerp, Belgium
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37 (Pussemier et al., 2004). Several studies carried out in neighboring countries do also
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39 confirm our data. Maximum values of ~23 pg I-TEQ per g fat and ~8 pg PCDD/F TEQ
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41 per g fat were mentioned by two German studies (Furst and Wilmers, 1993; Malisch,
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43 1998) and one Dutch study (Traag et al. 2002), respectively. The PCB TEQ values
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45 measured during the present study are low. However, one must keep in mind that these
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47 are CALUX data and that the cells used in this assay are less sensitive to dioxin-like
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49 PCBs than to PCDDs and PCDFs (Brown et al., 2001). A comparison of GC-HRMS
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4 and CALUX results published by Carbonnelle et al., (2004b) suggests a threefold
5 underestimation by the CALUX values (Carbonnelle et al., 2004b). This factor was
6 confirmed by validation results (data not shown). Assuming an underestimation by a
7 factor of three, the PCB TEQ values for PO eggs would range from 0.2 to 35 pg TEQ
8 per g fat and the maximal total sum of PCDDs, PCDFs and dioxin-like PCBs would
9 amount to ~58 pg TEQ per g fat.
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19 All together, the data obtained during this study illustrate unmistakable contamination
20 with dioxins and dioxin-like chemicals in PO eggs. The more that CF eggs revealed
21 much lower concentrations. Their median and maximum dioxin TEQ values are eight
22 and five times lower, whereas their median and maximum PCB TEQ values are three
23 and seven times lower, respectively.
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34 *Correlations between contaminants and differences between PO and CF eggs*

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37 *Environmental contaminants.* For PO breeding, the most probable contamination source
38 of the hens and their eggs with heavy metals and persistent organic compounds is the
39 environment (contaminated soil, grit, grass, earthworms, soil-dwelling insects, etc)
40 (Stephens et al., 1995, Schuler et al, 1997, Kijlstra, 2004). In PO eggs, indeed,
41 significant ($p > 0.01$) correlations were found between some typical environmental
42 contaminants such as Tl and Hg ($r = 0.76$), indicator PCBs and dioxins ($r = 0.59$), As
43 and DDT/DDE/DDD ($r = 0.53$), indicator PCBs and DDT/DDE/DDD ($r = 0.51$), Tl and
44 DDT/DDE/DDD ($r = 0.50$), and between dioxins and dioxin-like PCBs ($r = 0.48$). When
45 considering eggs from CF, it appears that the correlations between these environmental
46 contaminants are no longer significant ($p > 0.01$). This suggests that the origin of PO
47 eggs contamination is environmental and that the contamination of the environment is
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4 rather widely disseminated for a large number of contaminants. The significant
5 correlation between the levels of various contaminants in PO eggs is in agreement with
6
7 good correlations found for dioxins and the indicator PCBs in serum and plasma
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9 samples in Belgium (Fierens et al., 2003, Van Wouwe et al., 2004). Pauwels et al. also
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11 found a correlation between CALUX TEQ values (obtained with rat cell assay) and the
12
13 sum of four indicator PCBs in serum and in follicular fluid (Pauwels et al., 2000).

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15 Recent data of concentrations of heavy metals determined in cord blood in Belgium
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17 indicated that Cd levels in Flanders were enhanced, compared to levels found in other
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19 countries (Centre for environment and health, 2005). Also, a recent study (Goemans et
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21 al., 2003) has shown that eels caught in rivers from Flanders were largely contaminated
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23 by various chemicals such as PCBs, heavy metals and, to a lesser extent,
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25 organochlorinated pesticides such as DDT and metabolites. This supports the idea of a
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27 widely distributed environmental contamination of these contaminants in Belgium.
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38 *Metal elements.* On the other hand, the CF eggs are characterized by very significant (p
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40 < 0.001) correlations between some metal elements such as Cu and Zn ($r = 0.94$), Zn
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42 and Co ($r = 0.85$) and between Cu and Co (0.79). Such very significant correlations
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44 between these three metals are not found anymore in PO eggs where the correlation
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46 coefficients dropped to 0.58, 0.44 and 0.43, respectively. This suggests, once again, that
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48 hens from PO are fed in a less controlled way whilst commercial breeders use
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50 exclusively commercial feedstuffs supplemented with specific metal elements in very
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52 precise concentration ratios.
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Differences between PO and CF free-range eggs. Considering all the results together, it appears that the lower contamination observed in CF eggs (although free-range) is probably due (i) to the less extensive contact of these hens with the environment (high number of birds and, hence, less space available per hen) and (ii) to the exclusive use of (supplemented) commercial feed. It is also worth mentioning that within the commercial free-range farms it was not possible to find out any significant distinction between the organic and conventional modes of production considering either the quantitative analysis (contaminant levels determined) or the qualitative analysis (correlations found between groups of contaminants) (results not shown).

Health consequences due to the consumption of home-produced free-range eggs

The consumption of home-produced free-range eggs could lead to unusual effects due to both the elevated contamination of the eggs and their possible unusual consumption rate. To estimate the dietary intake of the examined contaminants via the consumption of eggs, we considered three scenarios: A) “normal consumption” whereby one egg per day is consumed and the contaminants’ concentration is the median value for that compound that is obtained in this study, scenario B) a case of “extreme use” whereby three eggs with the obtained median concentration are consumed daily and scenario C) the case of “extreme contamination” whereby one egg daily is consumed and the contaminants’ concentration is the maximal value for that compound that is obtained in this study. We compared these intakes to the tolerable weekly intake (TWI) for the specific contaminant when a TWI was available. For the heavy metals we only focused on Pb, Cd and Hg because for these metals the ratio between the median levels in PO eggs to the median levels in CF eggs was higher than one and we used the obtained

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4 levels for the PO eggs. In Figure 4 the fractions of the provisional tolerable weekly
5 intakes (PTWI), as established by the WHO that are reached are shown for the three
6 intake scenarios. The fractions are all below 1 %, except for Pb for which an egg intake
7 according to scenarios B and scenario C results in 3,6 % and 5,9 % of the PTWI,
8 respectively.
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23 The estimation of the exposure to dioxins and dioxin-like PCBs for the same three
24 scenarios is shown in Figure 5. The results are shown for the two types of eggs (PO and
25 CF). To be able to compare the results with the TWI the total TEQ (dioxin TEQ+ PCB
26 TEQ) values were used. The results are compared to the tolerable weekly intake of 14
27 pg WHO TEQ/kg bw for dioxins and dioxin-like PCBs as determined by the Scientific
28 Committee on Food of the EU (EU, 2001c). The contamination with dioxin-like
29 compounds is elevated in such a way that it has pronounced effects on the TEQ intake.
30 A 'normal egg consumption '(scenario A) leads to 48 % of the TWI whereas the same
31 consumption of eggs from commercial farms does not exceed 10 % of the TWI (Figure
32 5). For extreme use and extreme contamination of PO eggs (scenarios B and C) the TWI
33 is exceeded. The present results indicate that consumption of home-produced eggs leads
34 to a TEQ intake almost five times as high as the intake by the same consumption of
35 free-range eggs from commercial farms.
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Insert Figure 5

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4 It is clear that consumption of PO eggs will increase the body burden of the consumers.
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6 It must be stressed that regular consumption of such eggs (e.g. a daily consumption of
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8 one egg) increases the total TEQ intake with almost 50 % of the tolerated intake. This
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10 finding leads to concerns. Dioxin intake occurs predominantly via consumption of fatty
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12 food from animal origin, with meat, dairy products and fish being the major contributors
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14 (Focant et al., 2002). This means that, in addition to eggs, there is already considerable
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16 dioxin TEQ intake. It is thus important that consumption of such eggs is not
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18 exaggerated in order to keep the TEQ intake as low as possible. Moreover, it has
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20 already been shown that PCBs and PCDD/F concentrations in serum of people in
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22 Flanders are higher than in other countries. (Koppen et al., 2002). This suggests that
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24 Belgian people are subjected to higher background exposure to dioxin-like compounds
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26 as compared to other countries.
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32 Finally, it has already been noted that private owners of free-range hens might consume
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34 large quantities of the same food type: their own produced eggs (Stephens et al., 1995).
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36 If these food items are contaminated these individuals may have different exposures
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38 from what is considered 'background' and they should, therefore, be considered as a
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40 special sub-population (Stephens et al., 1995).
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47 **Conclusion**

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49 The most striking finding of this study is the clear distinction between the results
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51 obtained for the eggs from private owners and those for free-range eggs obtained from
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53 professional farms. The difference is the most visible for environmental contaminants
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55 like dioxins, PCBs, DDT (and metabolites) and toxic heavy metals (Tl, Pb, Hg).
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57 Apparently several types of contaminants are present in the environment and they
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4 finally accumulate in the eggs via soil-egg transfers. This is to be expected from their
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6 long residence time in the environment and their bioaccumulating potential. The threat
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8 for human health increases by the presence of a mixture of different toxic compounds at
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10 elevated levels, which leads to an increased risk for additive or synergetic effects
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12 between compounds.
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18 Consumers of free-range eggs obtained from private owners should be aware of the
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20 possible health risks associated with the use of these products especially because eggs
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22 add to the total diet and other food items also contribute to a large degree to the total
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24 intake of contaminants. Currently, we believe that the rearing conditions and the
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26 influence of the environment in which the hens live play a major role to explain the
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28 difference between levels of compounds in the two types of eggs that were investigated.
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30 The fact that home-produced eggs are significantly more contaminated with dioxins,
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32 DDT, Pb, Tl and Hg than free-range eggs from farms calls for serious attention. It is of
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34 great importance that the involved consumers are aware of the health consequences
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36 associated with excessive intake of their obtained eggs.
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44 The results obtained in this study were passed to the Ministry of Public Health and the
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46 Federal Agency of the Security of the Food Chain. The latter one installed a working
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48 group to forward suggestions. This working group is currently investigating the present
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50 and additional results.
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53 54 55 56 **Acknowledgements** 57 58 59 60

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5
6 acknowledged for its support in the sampling of commercial farms. We are also grateful
7
8 for the active participation of the private owners in this study.
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4 Figure 1: Ratio of median concentrations in eggs from private owners (PO) to median
5 concentrations in eggs from commercial farms (CF) for different elements.
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11 Figure 2: Congener profile of marker PCBs in eggs from private owners.
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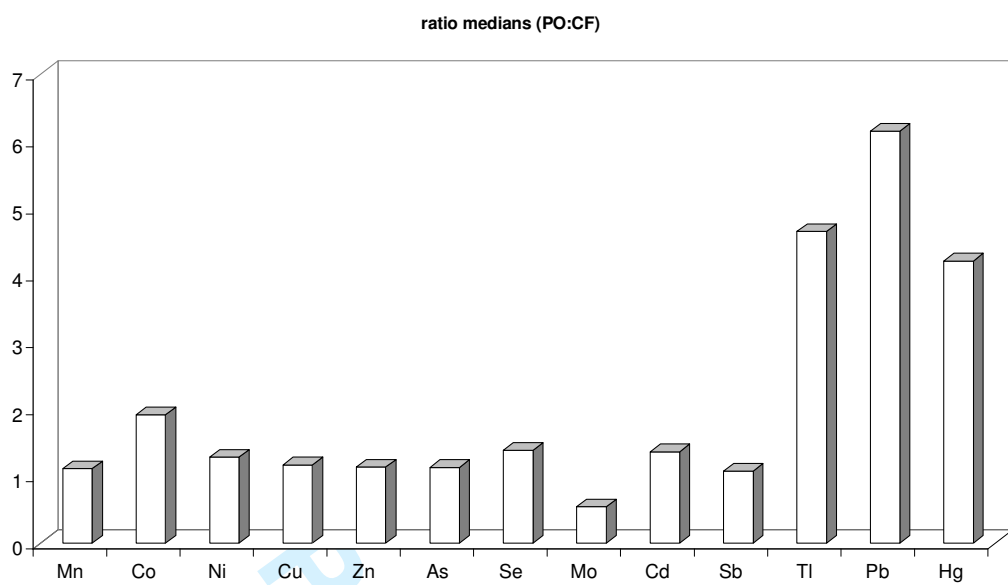
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16 Figure 3 A: Percentage contribution of different DDT forms to sum DDT/DDE/DDD in
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23 Figure 3B: Percentage contribution of different DDT forms to sum DDT/DDE/DDD in
24 eggs from commercial farms.
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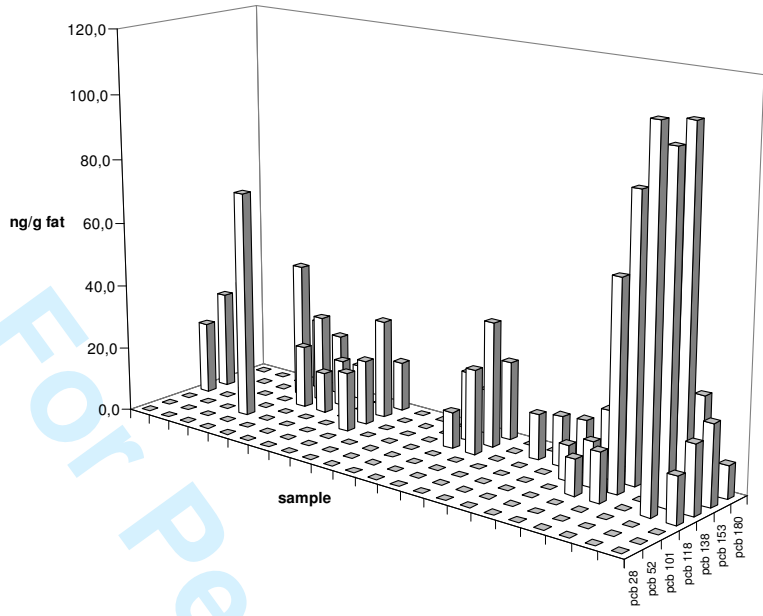
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30 Fig 4: Percentage of provisional tolerable weekly intake (PTWI) for Pb, Cd and Hg that
31 is obtained for different egg intake scenarios (normal use: intake of 1 egg daily and
32 median contamination; extreme use: intake of 3 eggs daily and median contamination;
33 extreme contamination: intake of 1 egg daily and maximal contamination) (assumed
34 levels are levels obtained for eggs from private owners).
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45 Figure 5: Percentage of tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs
46 that is obtained for different intake scenarios of eggs from private owners (PO) and eggs
47 from commercial farms (CF) (normal use: intake of 1 egg daily and median
48 contamination; extreme use: intake of 3 eggs daily and median contamination; extreme
49 contamination: intake of 1 egg daily and maximal contamination).
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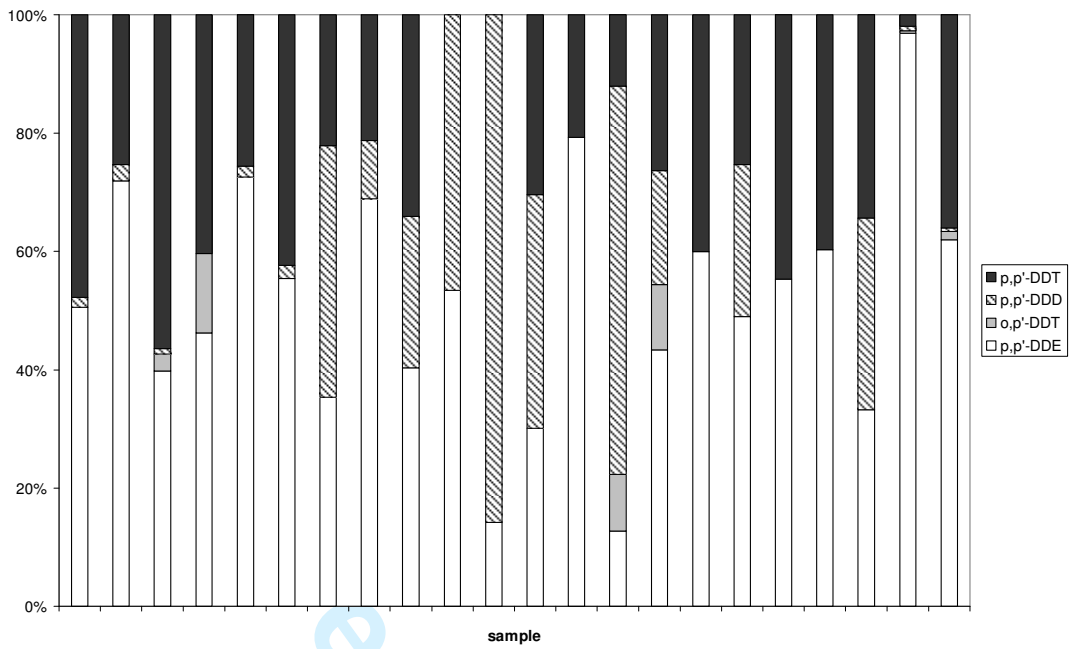
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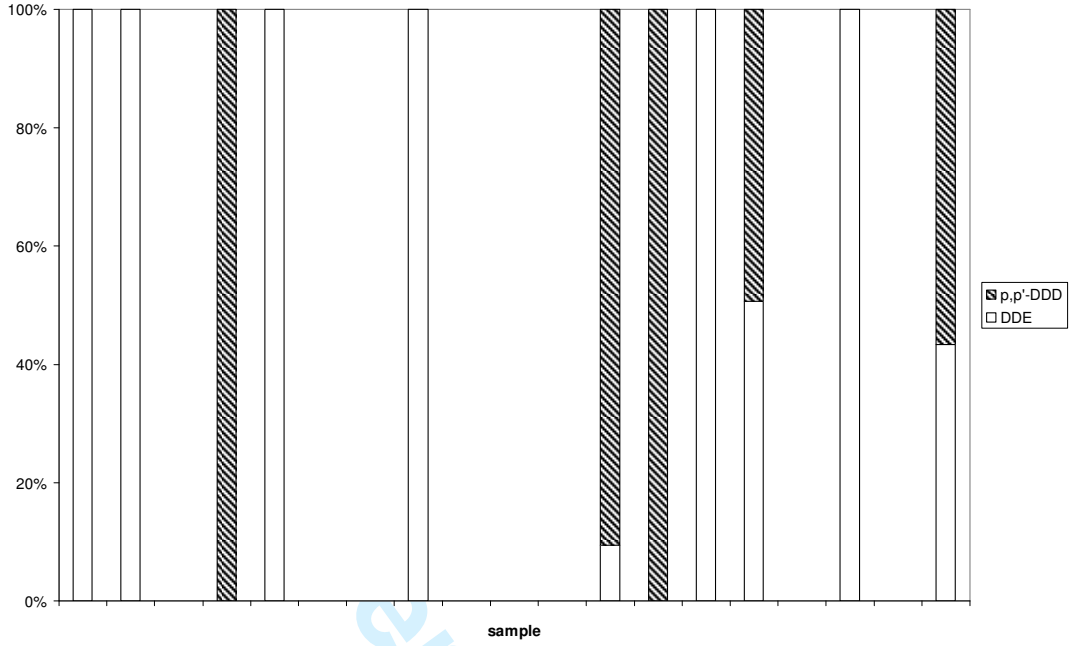


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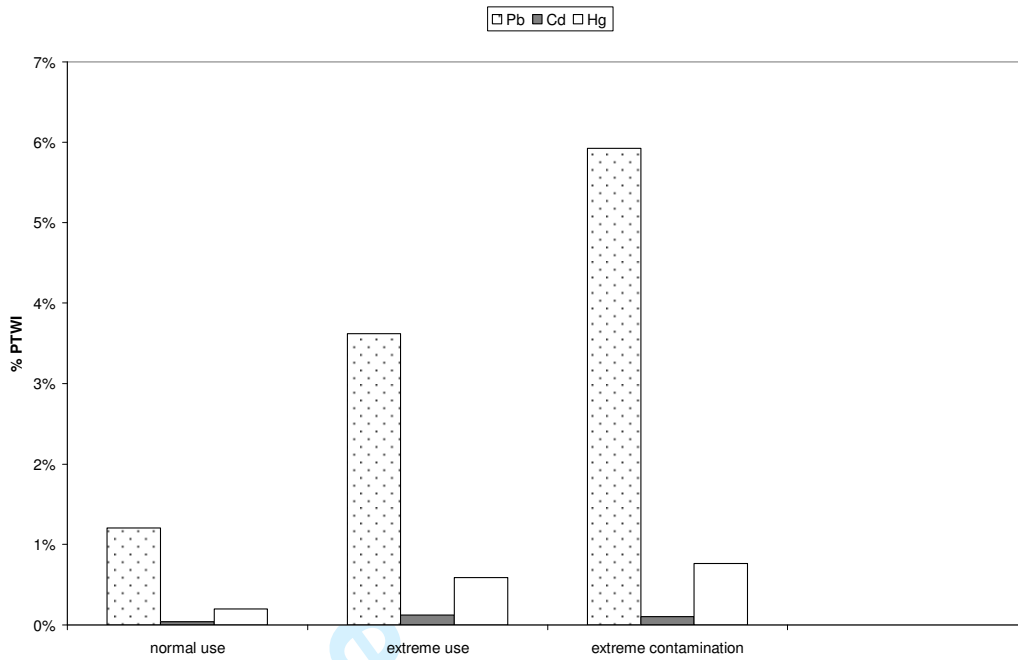


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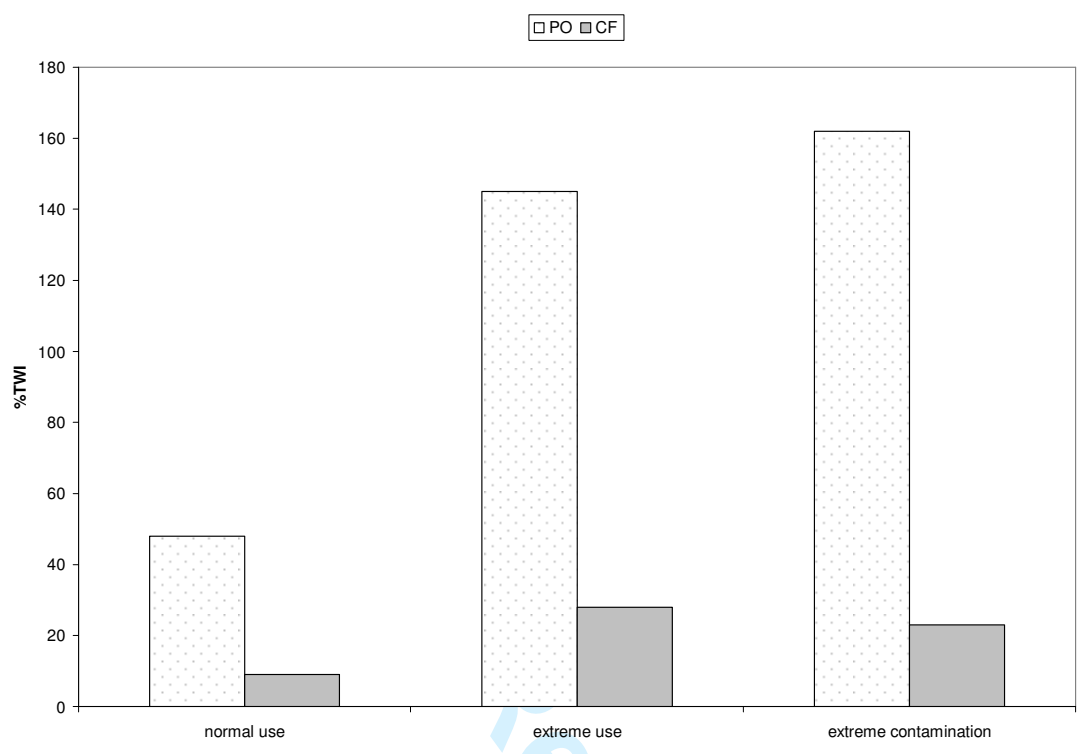
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Table I: Descriptive statistics of concentrations of elements ($\mu\text{g}/\text{kg}$ egg) in eggs from private owners (n = 22).

Element	mean	min	quartile1	median	quartile3	max
Mn	313.98	87.00	272.29	312.51	349.18	620.67
Co	5.15	1.73	3.59	4.43	5.75	12.93
Ni	36.58	13.78	26.54	31.86	44.60	85.00
Cu	603.64	430.17	554.58	597.5	646.88	829.17
Zn	11543.64	7655.00	10702.50	11237.5	12554.17	15093.33
As	15.95	8.94	12.86	16.09	17.65	24.26
Se	272.97	109.42	205.18	295.2	325.42	477.33
Mo	26.35	5.93	21.02	26.06	31.68	52.45
Cd	0.53	0.07	0.18	0.45	0.93	1.12
Sb	4.23	0.89	2.32	3.54	5.61	9.02
Tl	0.69	0.08	0.30	0.53	0.74	2.54
Pb	68.56	19.06	33.67	48.83	90.54	239.58
Hg	2.05	0.70	1.09	1.58	2.33	6.19

Table II: Descriptive statistics of concentrations of elements ($\mu\text{g}/\text{kg}$ egg) in eggs from commercial farms (n = 19).

Element	mean	min	quartile1	median	quartile3	max
Mn	280.25	159.00	198.75	282	313.00	502.00
Co	2.35	1.17	2.02	2.32	2.73	3.60
Ni	22.41	1.67	5.10	24.88	32.89	77.81
Cu	507.40	329.20	483.75	511.58	551.57	622.17
Zn	9743.95	6005.00	8957.50	9930.83	11102.90	13285.00
As	13.42	3.03	9.53	14.26	17.53.00	20.41
Se	197.1	107	172.56	213.36	228.60	270.00
Mo	49.52	9.35	28.47	48.38	58.66	158.83
Cd	0.27	0.07	0.13	0.33	0.33	0.49
Sb	2.53	0.33	1.55	3.30	3.33	4.01
Tl	0.22	0.03	0.07	0.11	0.23	1.08
Pb	8.91	1.03	2.46	7.94	12.58	32.03
Hg	0.51	0.17	0.26	0.37	0.66	1.29

Table III: descriptive statistics of levels of organochlorinated compounds in eggs from private owners (n = 22).

contaminants	mean	min	quartile 1	median	quartile 3	max	norm
Sum 7 marker							
PCBs (ng/g fat)	58,72	0	0	32,30	73,80	351	200
Sum aldrin/dieldrin (ng/g fat)	27,29	0	0	14,35	40,55	115,40	200
Sum DDT/DDE/DDD (ng/g fat)	770,71	26,40	50,25	125,45	519,53	5250	500
Dioxins (pg CALUX TEQ/g fat)	10,18	2,28	5,57	8,53	12,01	23	3
Dioxin-like PCBs (pg CALUX TEQ/g fat)	2,05	0,06	0,50	1,73	2,68	11,84	

Table IV: Descriptive statistics of levels of organochlorinated compounds in eggs from commercial farms (n = 19).

contaminants	mean	min	quartile 1	median	quartile 3	max	norm
Sum 7 marker PCBs (ng/g fat)	3,33	0	0	0	0	63,20	200
Sum aldrin/dieldrin (ng/g fat)	3,33	0	0	0	3,75	18,10	200
Sum DDT/DDE/DDD (ng/g fat)	17,30	0	0	10,70	14,15	171,90	500
Dioxins (pg CALUX TEQ/g fat)	1,44	0,50	0,62	1,04	1,94	4,21	3
Dioxin-like PCBs (pg CALUX TEQ/g fat)	0,77	0,31	0,53	0,69	0,91	1,69	

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