DIOXINS IN FREE RANGE CONSUMPTION EGGS FROM VIETNAM: LEVELS AND HEALTH RISKS

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Introduction

The two major sources of TCDD contamination from Agent Orange in the South of Vietnam are the spray missions by aircrafts and the spills or leakages that occurred at the former US military airbases where the herbicides were stored and dispensed[1]. At Bien Hoa airbase, it was documented that at least 4 spills had occurred in the 1970s including spilling of 28,000 liters of Agent Orange and 10,000 liters of Agent White[2]. Soil, sediments, fish and meat products in Bien Hoa hot spot were shown to be highly contaminated with dioxins. Consequently, people that consume these contaminated foods had higher levels of dioxins in blood and human breast milk in comparison to people in unexposed areas[3,4,5]. However, although free-range consumption eggs are important food components in local people's diets, to our current knowledge no data regarding dioxin levels in free-range eggs from Vietnam nor their contribution to human dietary intake of dioxins has been reported. The present study shows that the contamination levels in poultry eggs can be very high, especially at spill sites, leading to potential health risks for consumers of these eggs.

Materials and methods

Collection of eggs

A total number of 94 eggs from free-ranging chicken and ducks were collected from 9 different houses and small farms in three selected areas. In Bien Hoa, four houses were selected from four sites: Bien Hoa airbase, Bien Hoa airbase surrounding, the former military post and the former bombs and chemicals storage. In Sac Forest, two farms located in the forest edge and one house in the forest centre were selected for sampling. As representatives for the general background area, two houses in Vĩnh Cửu district (one in Lợi Hòa, one in Bình Lợi) were chosen. All eggs were boiled for 15 min; the yolk was separated from the white and transported to RIKILT for dioxin and PCB analysis. In total, 94 individual eggs were analyzed using the DR CALUX® assay. From those samples, 23 eggs were selected representing a broad concentration range for further GC-HRMS confirmation of the TEQ levels.

Fat extraction

The fat of the egg samples (yolk) was extracted using the ASE 350 (Dionex, USA) with hexane-acetone (1:1) as extraction solvent. The extraction program consists of 3 cycles, including heating at 100°C during 5 min at a pressure of 1500 PSI, static time of 5 min, rinse volume 100%, purge for 120 seconds. Solvent residue was evaporated and the remaining lipid was kept in the refrigerator for further analysis.

DR CALUX bioassay

An aliquot of 0.5 g fat was purified on a column containing 10 g acid silica (33% H_2SO_4). Samples were loaded onto the columns and subsequently eluted with 30 ml hexane-DEE (97:3) as described by Bovee et al.[6]. The final extracts were evaporated in the presence of 40 µl DMSO, which was added to 2 mL incubation medium. An amount of 250 µl was added in triplicate to three different wells of a 48-well plate containing p-GudLuc-transfected H4IIE cells (obtained from Wageningen University and similar to the ones sold by BDS (Amsterdam)) in 250 µl medium. After 24 h, the medium was aspirated, the cells were washed and lysed and an aliquot was used for determining the luciferase content in a Luminoskan (Thermo Labsystems). Results were expressed as bioanalytical equivalents (pg BEQ/g fat) estimated from a TCDD calibration curve (curve fitting using Graphpad Prism 5 software, Sigmoid dose-response variable slope). The term CALUX BEQ is used instead of the former TCDD-TEQ to acknowledge the fact that not all compounds present in the sample extract that produce a response in the test may obey all requirements of the TEQ-principle[7].

GC-HRMS analysis

Levels of dioxins, non-ortho and mono-ortho PCBs were determined by high resolution GC/MS, basically as described by Tuinstra et al.[8] but using a Power-PrepTM automated cleanup system (FMS, Boston, USA). Prior to clean-up, ^{13}C labeled dioxins and dioxin-like PCBs were added to the fat obtained from the samples. The samples were transferred to the Power-Prep system and purified on an acid silica column, a basic alumina column and an activated carbon/celite column. The extract fractions were evaporated under a nitrogen flow at 40°C in a turbovap system (Turbovap II, Caliper Life Science) to reach the final volume of 500 µl. Measurement of dioxins and PCBs was performed by GC-HRMS using an Agilent (Wilmington, USA) 6890 Series gas chromatograph coupled to an AutoSpecUltima high resolution mass spectrometer (Waters, Milford, USA). The GC column was a DB5 MS (60 m, 0.25 mm i.d., 0.25 µm; J&W, Folson, USA). The mass spectrometer was operated at a resolution of 10.000 in electron impact ionization mode, using selected-ion monitoring.

Results and discussion

CALUX results

The BEQs determined in the egg yolks with the DR CALUX bioassay ranged from 3 to 348 pg BEQ/g fat with highest levels found in the egg samples from the hot spot (figure 1). Big variations were found between total BEQs of individual eggs from the same location as well as between the mean BEQs of eggs from different farms. For instance, the mean BEQ of samples in the former bombs store is 20 times higher than the mean BEQ of samples collected in the former military post. In general, the mean BEQ levels in duck eggs were significantly higher than in the chicken eggs collected from the same farm (P<0.001).

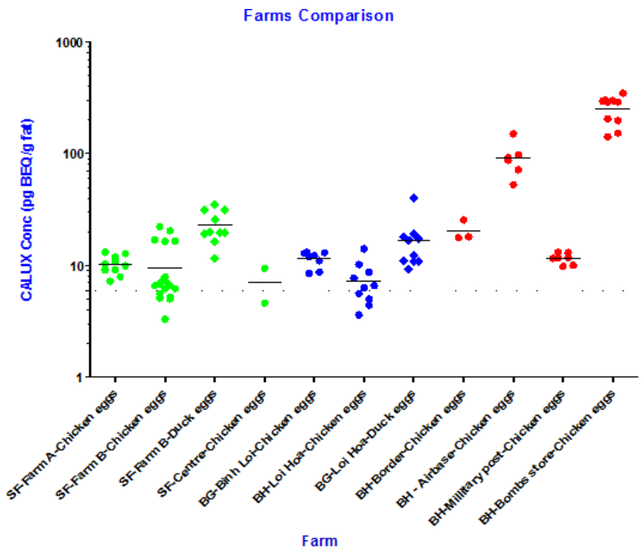

Figure 1 Bioanalytical results (pg BEQ/g fat) of chicken and duck eggs from different farms

Note: The dotted line represents the EU-ML of 6 pg TEQ/g fat for the sum of dioxins and dioxin-like PCBs

GC-HRMS results

A number of samples were selected for GC-HRMS analysis. The total TEQ/g fat for these chicken eggs ranged from 5.8 to 249.1 pg TEQ/g fat for the Bien Hoa hot spot, from 3.3 to 9.7 pg TEQ/g fat for background areas and from 3.2 to 8.2 pg TEQ/g fat for Sac Forest. The mean TEQ of the samples from the hot spot area is 15 to 19 times higher than in other areas (data not show). The dioxin concentrations in all samples from the Bien Hoa hot spot exceeded the EU maximum level (ML) for the sum of dioxins (3 pg TEQ/ g fat). The results of our research confirm the findings of previous studies about dioxin contamination in other food products from Bien Hoa air base and vicinity[3-5].

In all three areas, PCDD/Fs-TEQ contributed most to the total TEQ. More specifically, TCDD accounted for 61 to 96% of the total TEQ in eggs from the Bien Hoa hot spot (Table 1). By contrast, in eggs from background areas TCDD contributed between 13 and 16% of the total TEQ (except 1 duck egg in Loi Hoa with 70% TCDD). From Sac forest this percentage ranged between 16 to 34% in chicken eggs and 66 to 68% in duck eggs. The strongly elevated dioxin content as well as the high contribution of TCDD to the total TEQ in the egg samples from the hot spot suggests that the contamination originates from Agent Orange and also demonstrates that TCDD is very persistent in the environment and can contaminate local food products at very high levels. Besides, while 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD contributed most to the PCDD/Fs related TEQ, most non-ortho-PCBs related TEQ was attributed by PCB 126. In case of mono-ortho-PCBs, PCB 105 and PCB 118 were the most relevant congeners (data not show).

Table 1. Concentrations of PCDD/Fs, non-ortho (no), mono-ortho (mo) PCBs, TCDD (pg TEQ/g fat) in chicken (C) or duck (D) egg samples from different areas in Southern Vietnam

Sample	Egg type	CALUX BEQ	PCDD/Fs TEQ	no-PCB TEQ	mo-PCBs TEQ	Total TEQ	TCDD TEQ	TCDD (%)
Bien Hoa airbase I	C	72	54.5	4.3	0.23	59.1	43.3	73
Bien Hoa airbase II	C	92	89.0	5.7	0.37	95.0	75.9	80
Bien Hoa airbase surrounding	C	18	16.9	1.4	0.03	18.2	11.1	61
Former bombs store I	C	197	221.5	0.7	0.02	222.2	213.1	96
Former bombs store II	C	295	248.0	1.0	0.03	249.1	238.3	96
Former military post	C	10	4.8	0.9	0.03	5.8	0.7	13
LoiHoa I	C	4	2.8	0.5	0.01	3.3	0.4	13
LoiHoa II	C	14	7.8	1.6	0.01	9.4	1.3	14
LoiHoa III	D	17	6.8	1.1	0.02	7.9	0.9	12
LoiHoa IV	D	40	18.1	0.9	0.03	19.0	13.2	70
LoiHoa V	D	19	10.2	2.2	0.03	12.4	1.7	14
LoiHoa VI	C	6	4.2	1.0	0.01	5.1	0.7	14
LoiHoa VII	D	9	5.1	1.0	0.01	6.1	0.8	13
BinhLoi I	C	9	8.2	1.5	0.02	9.7	1.5	15
BinhLoi II	C	13	7.9	1.6	0.02	9.5	1.5	16
Sac Forest border Farm A	C	9	4.3	1.0	0.02	5.3	1.4	26
Sac Forest border Farm B I	C	3	2.4	0.8	0.05	3.2	1.1	34
Sac Forest border Farm B II	D	35	16.0	0.8	0.02	16.9	11.2	66
Sac Forest border Farm B III	D	16	10.4	0.5	0.01	10.9	7.4	68
Sac Forest border Farm B IV	C	22	7.6	0.6	0.02	8.2	1.3	16
Sac Forest border Farm B V	D	26	14.7	0.7	0.02	15.4	10.6	68
Sac Forest border Farm B VI	C	16	7.1	0.5	0.02	7.6	1.2	16
Sac Forest centre	C	7	3.0	0.8	0.01	3.8	1.1	29

Contribution of eggs to human TEQ body burden

The estimated weekly intakes (EWIs) of dioxin-like compounds via consumption of eggs from the three study areas are shown in table 2. The calculations are based on an average body weight assumed to be 50 kg for an adult Vietnamese and 18 kg for a 5 years old child. An average Vietnamese consumes approximate 22.1 g egg per day, in which chicken egg accounts for 17.5 g[9] and duck eggs assumed to be 4.6 g. This equals to the consumption of 3 local chicken eggs and half a duck egg per week. The mean and highest GC-HRMS determined concentrations were used to calculate the intakes in average and worst case scenarios. The EWIs calculated from our study revealed that there is a serious risk for the local residents, especially for children in the hot spot. Note that the WHO established a tolerable weekly intake (TWI) for dioxins from 7 to 28 pg TEQ/kg bw/week. There is also a tolerable monthly intake (TMI) recommended by JECFA of 70 pg /kg bw/ month which is comparable to 14 pg TEQ/kg bw/week as set by the EU Scientific Committee of Food. Based on these standards, the EWI of the children in the hotspot area in the worst case scenario is exceeding the EU-TWI and JECFA TMI up to 12 times and the WHO-TWI from 6 to 24 times. In other words, by consuming 4/5 of a local egg (contamination level of 249 pg TEQ/g fat) per week, an adult will already reach the TWI of 14 pg TEQ/kg bw. For children, a weekly diet containing 1/3 of this contaminated egg is enough to reach the mentioned TWI. In another study, Tran et al. demonstrated that extremely small amounts of local food products of animal origin in the Bien Hoa hot spot would already fill up the WHO-TDI[10]. However, the authors did not take eggs into account. As eggs are frequently consumed by Vietnamese people, it is expected that the total dietary intake of dioxins in this hot spot area is much higher if these contaminated eggs are taken into account. The dietary intakes of dioxins from eggs in the two other areas are lower than in the hot spot and comparable to those found in some EU countries with the EWIs for average and worst case scenarios being on average 2.4 and 3.3 pg TEQ/kg bw/week for adults living in Sac Forest and background area.

Table 2 Estimated weekly intake of dioxins and dioxin-like PCBs from the consumption of free range chicken and duck eggs by the residents living in three areas in Vietnam

Areas	Scenario		Dietary intake of PCDD/Fs and dl PCBs (pg TEQ/kg bw/week)		
			chicken eggs (*)	duck eggs (**)	Total eggs (***)
Bien Hoa hot spot	Average Case	Adult	26.5	N/A	
		Children	72.8	N/A	
	Worst case	Adult	61.0	N/A	
		Children	167.7	N/A	
Sac Forest	Average Case	Adult	1.4	0.9	2.3
		Children	3.8	2.5	6.3
	Worst case	Adult	2.0	1.1	3.1
		Children	5.5	3.0	8.5
Background areas	Average Case	Adult	1.8	0.7	2.5
		Children	5.0	2.0	7.0
	Worst case	Adult	2.4	1.2	3.6
		Children	6.5	3.4	9.9

Note: Dioxin weekly dietary intake with daily consumption of 17.5 g chicken egg (*), 4.6 g duck egg (**) and 22.1 g total egg (17.5 g chicken & 4.6 g duck egg) (***). N/A: calculations are not available due to lack of data on dioxin levels in duck eggs from the hot spot

Conclusion and recommendations

In the Bien Hoa hot spot and areas where soil and sediments are highly contaminated with dioxins due to Agent Orange, dioxin concentrations in local free-range chicken and duck eggs are highly elevated. Consumption of the contaminated eggs alone already causes exceedance of the TWI for dioxins as advised by the WHO in the local residents, especially children (up to 24-fold higher than the TWI). This may pose threats to human health. As other local food sources are also polluted with dioxins in the hot spot areas, it is important to educate and raise awareness of the people to consume no or less food products from home-raised animals.

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