BIOMONITORING AND SOURCE TRACKING OF DIOXINS/DL-PCBS IN THE NORTH OF THE NETHERLANDS

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Introduction

In the region of Harlingen in the north of the Netherlands people have recently become very concerned about adverse health effects related to possible emissions of dioxins and other POPs by a recently installed waste incinerator. To evaluate these effects the local authorities make use of a very limited monitoring program. We however set up a more adequate monitoring program, using eggs of backyard chickens. Because the Dutch authorities do not routinely check the eggs from small farms (or households) – with less than 200 chickens per farm – the risk of pollution with dioxins and dl-PCBs is underestimated. After finding several egg samples that did not comply with the Dutch regulation IKB for the commercial use of eggs (in case the level exceeds 1.75 pg BEQ/g fat one is compelled to take further action) we started a more extended monitoring program, with cow and goat milk, here and sheep liver, human serum, soil and poultry feed.

Materials and methods

Harlingen is a small city (c. 16,000 inhabitants) with agricultural industry and moreover a relatively low-profile industry that is mainly concentrated near the harbour and consists of shipyards, metal working, an oil-company, fishing industry, a plastic company and a recent build waste incinerator. The incinerator burns household waste (20%), industrial waste, sewage sludge and waste from other countries. Eggs of backyard chickens were sampled at 15 different locations near Harlingen and analyzed. The eggs have been analysed for dioxins (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) by DR CALUX® (BioDetection Systems, Amsterdam and RIKILT, Wageningen (both in the Netherlands) and by chemical GC/HRMS analysis (MAS, Münster, Germany, Nofalab, Schiedam (the Netherlands) and RIKILT, Wageningen (the Netherlands)). We also studied samples of soil (n=2) and poultry feed (n=2), samples of cow milk and goats (n=3), livers of here (n=6) and sheep (n=3) and human serum (n=6) by the DR CALUX®.

DR CALUX® bioassay: The procedure for the BDS DR CALUX® bioassay has been described in detail previously[1]. Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in α-MEM culture medium, supplemented with 10 % ($^{v}/_{v}$) FCS under standard conditions (37 ^{o}C, 5 % CO_2, 100 % humidity). Cells were exposed in triplicate on 96-well microtiterplates containing the standard 2,3,7,8-TCDD calibration range, a DMSO blank. Following an 24 hour incubation period cells were lysed. A luciferine containing solution (Glow Mix) was added and the luminescense was measured using a luminometer (Berthold Centro XS3).

GC/HRMS analysis: all three labs, MAS (Münster, Germany), RIKILT Wageningen and Nofalab Schiedam (both the Netherlands) are ISO17025 accredited according EC/252 guideline.

Results and discussion

A comparative study by the Dutch authorities (RIVM[2], n=63) showed that eggs of backyard chicken in the Netherlands are 48 % compliant for the levels of dioxins. In Harlingen (North of the Netherlands) within a distance of 2 km from the harbour none the GC/MS analyses of backyard eggs (n=6) could be found compliant (Figure 1).

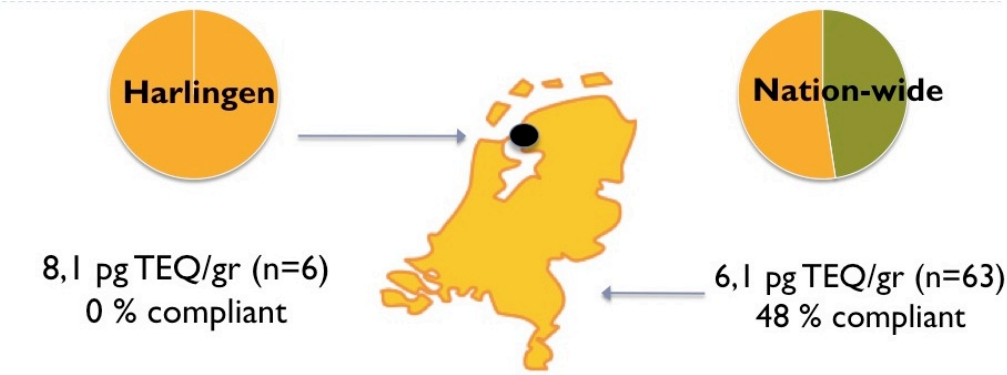


Figure 1: dioxins/dl-PCBs in eggs of backyard chickens in the Netherlands (RIVM, 2014)

The results of the DR CALUX® bioaassay on 15 locations of backyard chicken eggs, nearby the Harlingen harbour are shown in Figure 2. Six locations were analysed in May and in November. All results showed elevated levels of dioxins in November (average 215% increase), possibly induced by decreased breed-frequency in autumn or due to an active source of dioxins.

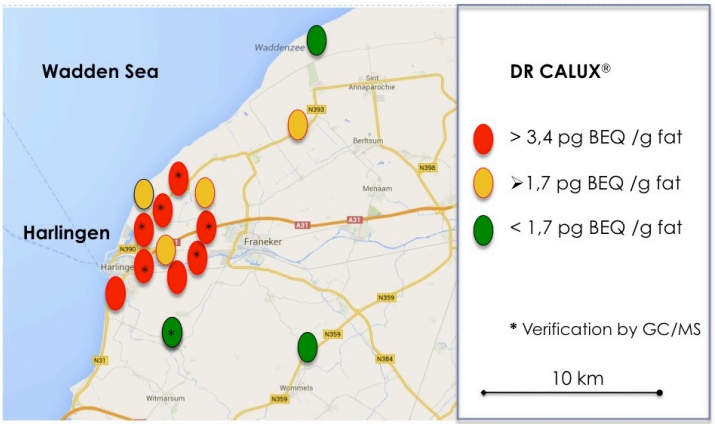


Figure 2: DR CALUX® sum dioxins/dl-PCBs in eggs of backyard chickens near Harlingen

Figure 3 shows the levels of PCDD/Fs and dl-PCBs (pg TEQ/g fat) of egg samples near Harlingen as analysed by GC/HRMS. At least two different kinds of pollution source can be derived from the figure, a PCB source due to elevated dl-PCB and PCDF levels (samples Mid-West, Wijnal-17 and Wijnal-2) and a PCDD source due to elevated PCDD levels (samples Harl-Mid, Mid-B and Mid-L).

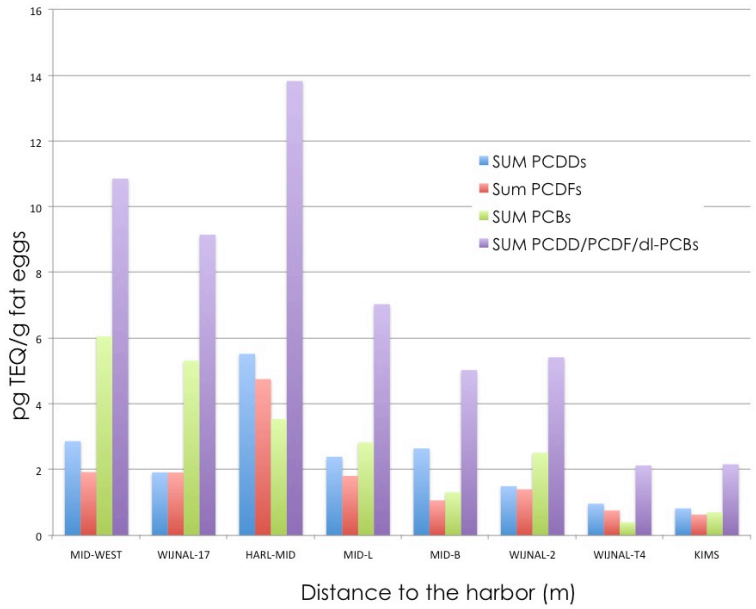


Figure 3: Levels of PCDDs, PCDFs and dl-PCBs (pg TEQ / g fat) of egg samples near Harlingen as analysed by GC/HRMS

The most toxic dl-PCB, PCB 126, shows an increasing level towards the harbour, suggesting a source in the harbor of Harlingen.

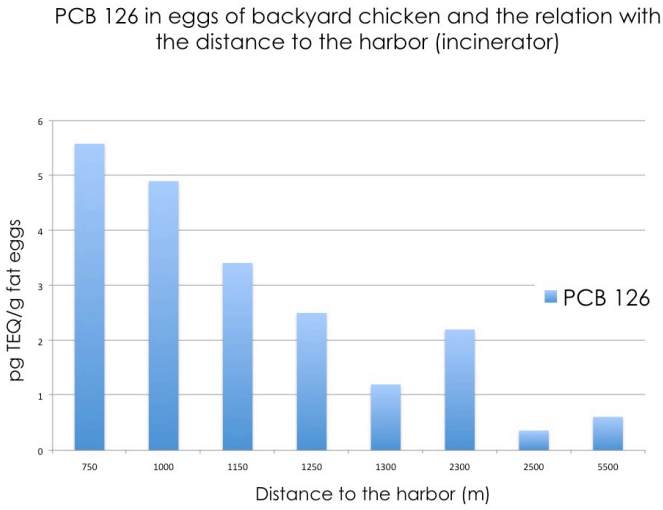
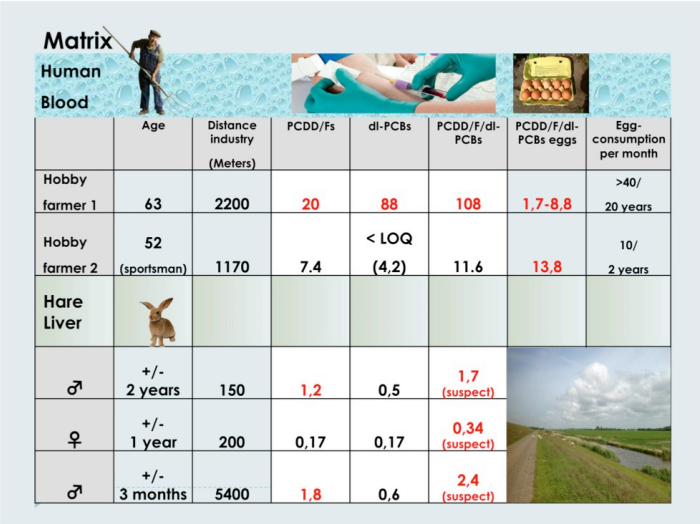


Figure 4: Levels of PCB 126 (pg TEQ / g fat) in eggs of backyard chicken as a function of the distance (m) to the harbour

Field studies near the incinerator showed declining bird population, thin egg-scales in abandoned bird nests, unexpected deaths of sheep's, pets and birds. The livers of wildlife here showed all elevated dioxin-levels above the EU-norm of 0.3 pg TEQ/g (n=9). Sheep liver (n=2) were above the EU action level of 1.25 pg TEQ/g weight. Several human serum samples (n=6) were analysed by DR CALUX®. A hobby farmer, about 60 year of age, consuming more then 40 eggs a month (from chickens with a maximal age of 18 months and eggs with 7.8 pg TEQ/g fat) had elevated serum levels of dl-PCBs (PCDD/F: 22 pg BEQ/g fat, dl-PCB-BEQ: 88 pg BEQ/g fat) as analysed by DR CALUX®. Serum from four other people living in Harlingen measured by DR CALUX ranged from 10 to 28 pg TEQ /g fat (Ten Tusschen et al. 2014).



	Age	Distance industry (Meters)	PCDD/Fs	dl-PCBs	PCDD/F/dl-PCBs	PCDD/F/dl-PCBs eggs	Egg-consumption per month
Hobby farmer 1	63	2200	20	88	108	1,7-8,8	>40/ 20 years
Hobby farmer 2	52 (sportsman)	1170	7.4	< LOQ (4,2)	11.6	13,8	10/ 2 years
Hare Liver							
♂	+/- 2 years	150	1,2	0,5	1,7 (suspect)		
♀	+/- 1 year	200	0,17	0,17	0,34 (suspect)		
♂	+/- 3 months	5400	1,8	0,6	2,4 (suspect)		

Figure 5: Analyses of human serum and here liver in the environment of Harlingen.

As result of these findings one year continuous sampling of the dioxin emissions in the flue gas of the incinerator will be performed including shutdown and start-up periods, in order to determine the source of the dioxin contamination of the environment. The program of analyses will be the 17 mandatory dioxin-congeners extended with measurements of dl-PCBs, PBDDs, PBDFs, PBBs, PBDEs, PFOS and PFOA. Addition of the brominated and fluorinated congeners can provide more data that can assist in determining the source. In parallel, an air quality-monitoring program will be started up for measurements of NO_x, SO_2, CO, PM_{10}, Cr, Cu, PB, Cd, Hg, C_6H_6, Toluene, Ethylbenzene, Xylene, HCl and HF.

Conclusions

This study showed eggs of backyard chicken to be sensitive biomarkers for dioxin/PCB contamination in the vicinity of potential sources (harbour activity, waste incinerator, landfill). By using cost-efficient screening analysis tools (such as DR CALUX®), areas of public concern can be monitored in a rapid and efficient way. The correlation between DR CALUX® and GC/HRMS proved to be very satisfactory; there were no false positive or false negative results.

The results of this study point to at least two types of source of dioxin emission near Harlingen; a PCB and a PCDD source. Results show levels of dioxins and dioxin-like PCBs are increasing with decreasing distance to the harbour. In order to investigate the contribution of dioxins and dl-dioxins from the incinerator to the environment, continuous sampling of the dioxin emission in the flue gas of the incinerator, including the start-up and shut-down periods, will be necessary. Also an adequate air quality monitoring program need to be performed to prevent adverse health impacts for domestic animals, wildlife and human beings.

Acknowledgements

This project from the NGO Toxicowatch Foundation has been funded by citizens which are concerned about industrial pollution in their environment. The Toxicowatch Foundation likes to thank RIKILT, Wageningen, The Netherlandsfor sharing their DR CALUX® and GC/HRMS data of egg samples. The continous sampling of the incinerator and the air quality program is funded by the government of the Netherlands.

References

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