

# **Biomonitoring of Dioxins/dl-PCBs in the north of the Netherlands; eggs of backyard chickens, cow and goat milk and soil as indicators of pollution**

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## **Introduction**

In the region of Harlingen in the north of the Netherlands people have recently been 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. Using eggs of backyard chickens, cow and goat milk and soil as indicators we however set up a more adequate monitoring program. 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.

## **Materials and methods**

Harlingen is a small city (c. 16.000 inhabitants) with agricultural industry and moreover a relatively low-profiled industry that is mainly concentrated near the harbour and consists of shipyards, metal working, an oil-company, fishing industry, a plastic company and a waste incinerator. Eggs of backyard chickens were sampled and analyzed, initially at 13 and in a second stage at 18 different locations near Harlingen. The eggs have been analysed for dioxins (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) by DR CALUX<sup>®</sup> (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)). Samples of soil and samples of milk of cows and goats also have been studied by DR CALUX<sup>®</sup>.

We also found dead birds, abandoned eggnecks and thin eggshells during visual inspections of the industrial area. In addition to this reports of declining bird populations, inexplicable sudden deaths of domestic animals like goats and sheep have been given. So we decided to study the livers of hares and sheep, animals that were found dead in the terrain. The results will be published later.

DR CALUX<sup>®</sup> bioanalysis: The procedure for the BDS DR CALUX<sup>®</sup> bioassay has been described in detail previously<sup>1</sup>. Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in  $\alpha$ -MEM culture medium, supplemented with 10 % ( $v/v$ ) FCS under standard conditions (37 °C, 5 % CO<sub>2</sub>, 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 luminescence 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 ISO 17025 accredited according EC/252 guideline.

## Results and discussion

The results of the DR CALUX<sup>®</sup> bioanalyses of the eggs are shown in Figure 1.

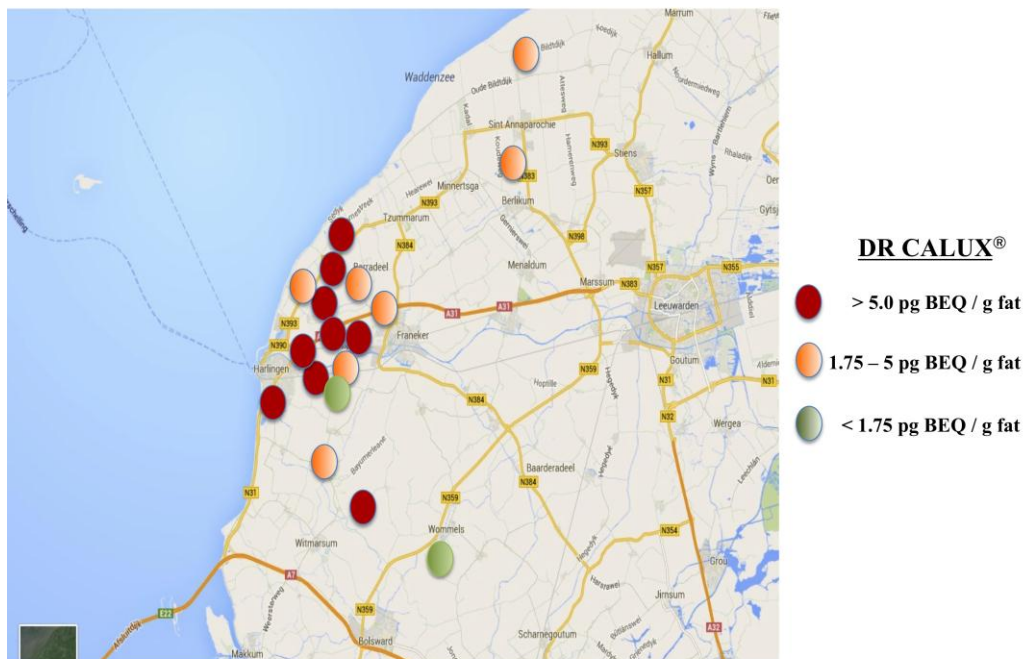


Figure 1: DR CALUX<sup>®</sup> sum dioxins/dl-PCBs in eggs of backyard chickens near Harlingen

Figure 2 gives the results of soil samples from three locations as analysed by DR CALUX<sup>®</sup>. At Midlum (HM), located at a distance of 750 metres (m) from the harbour, the level of dioxins/dl-PCBs in the soil was 4.45 pg BEQ / g product. For comparison, the level of the sum PCDD/Fs/dl-PCBs in the egg sample was 10.9 pg TEQ / g fat as measured by GC/HRMS. Historical land filling in the vicinity, backyard burning or emissions of industrial activities from the harbour could be possible sources of this pollution.

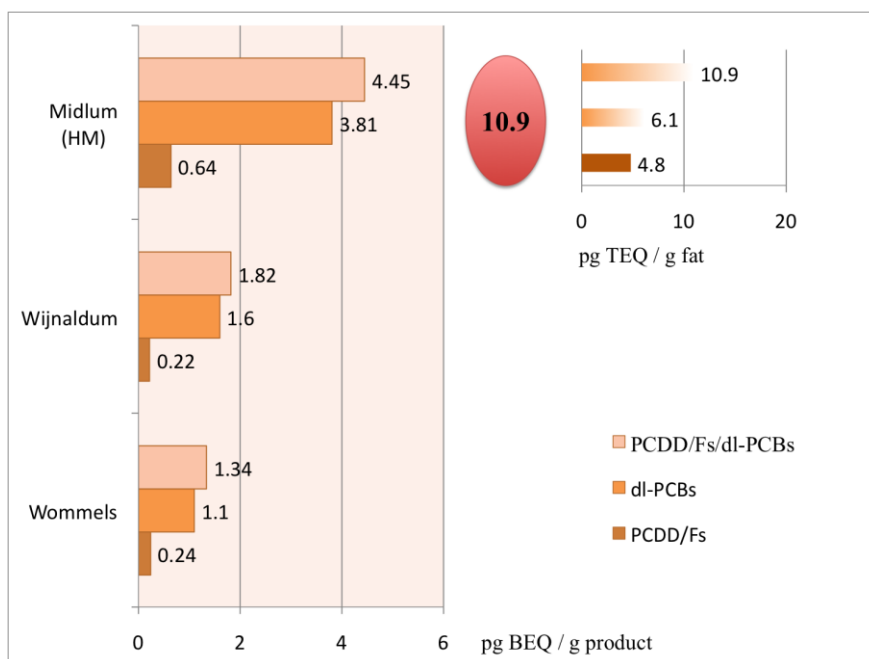


Figure 2: DR CALUX<sup>®</sup> PCDD/Fs/dl-PCBs in soil samples near Harlingen, and GC/HRMS results of Midlum (HM) egg sample

The levels of PCDD/Fs/dl-PCBs measured by DR CALUX<sup>®</sup> in goat and cow milk at three locations near Harlingen correspond with typical background levels in the Netherlands (see Figure 3).

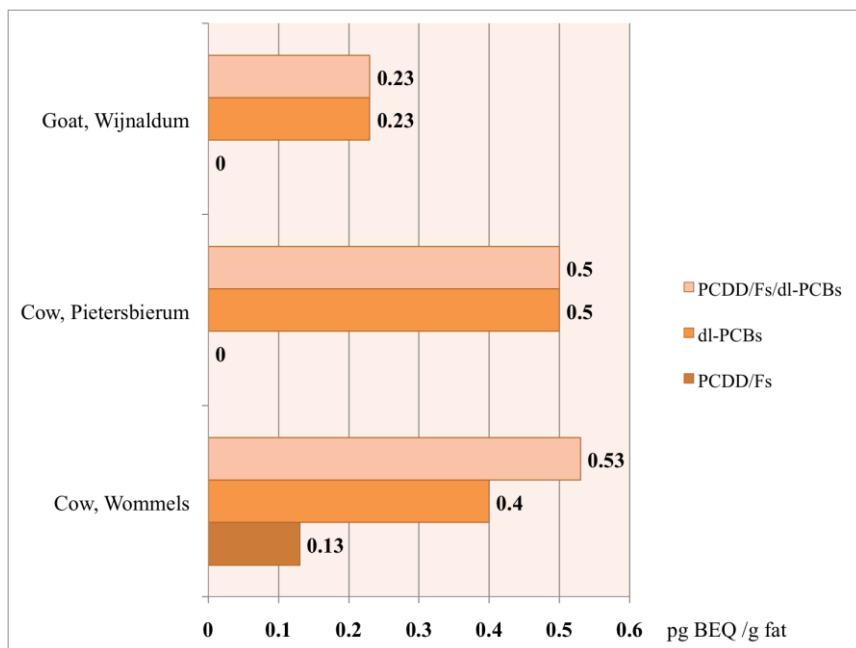


Figure 3: DR CALUX<sup>®</sup> PCDD/Fs/dl-PCBs in goat and cow milk samples near Harlingen

Figure 4 gives the levels of pollution as a function of the distance to the harbour, that is to say, the DR CALUX<sup>®</sup> and GC/HRMS analyses for the sum of PCDD/Fs/dl-PCBs in eggs of backyard chickens near the harbour of Harlingen.

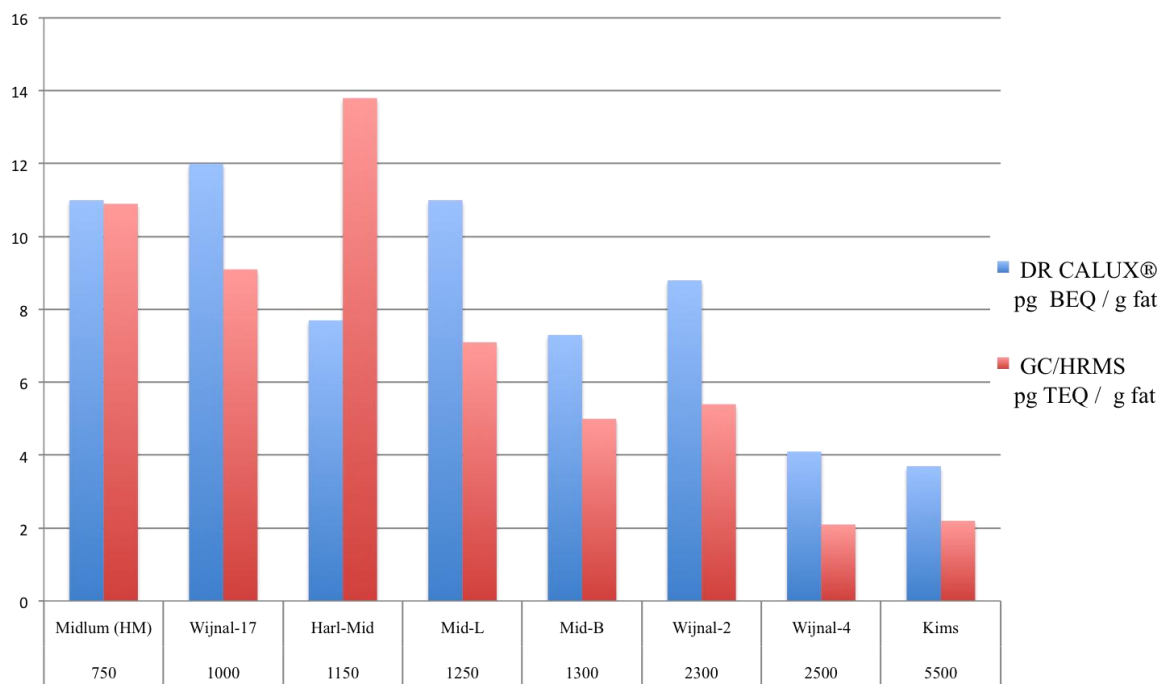


Figure 4: Levels of pollution (pg BEQ or TEQ / g fat) as a function of the distance (m) to the harbour

Figure 5 shows the levels of PCDDs, PCDFs and dl-PCBs (pg TEQ / g fat) of several 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 Midlum (HM), Wijnal-17 and Wijnal-2) and a PCDD source due to elevated PCDD levels (samples Harl-Mid, Mid-B and Mid-L).

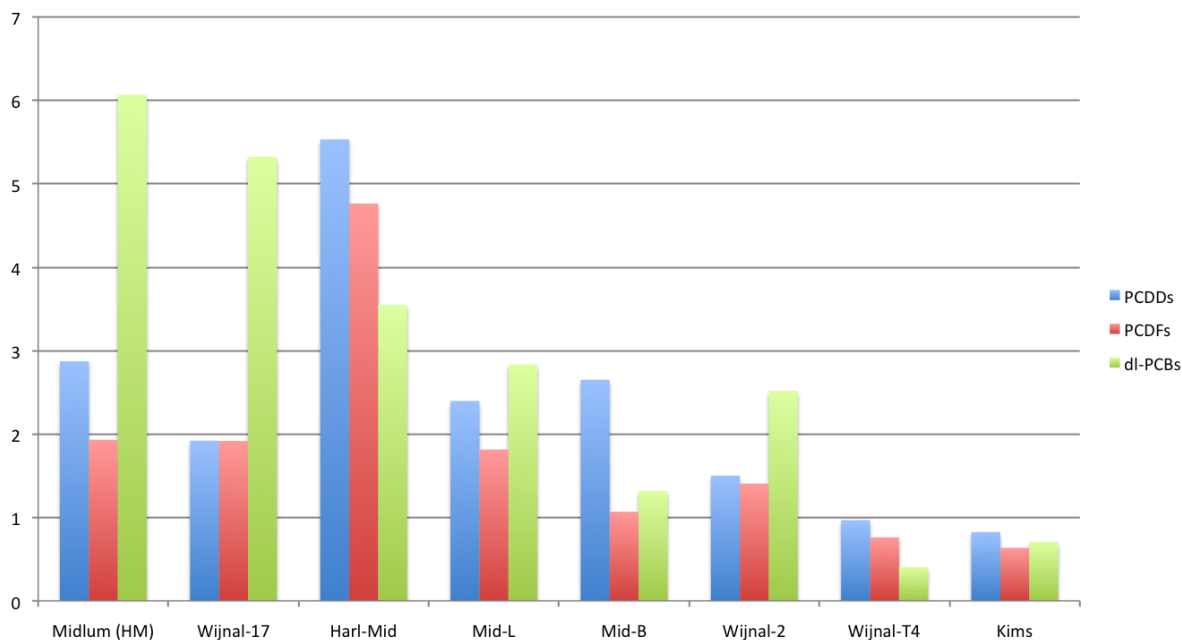


Figure 5: Levels of PCDDs, PCDFs and dl-PCBs (pg TEQ / g fat ) of egg samples near Harlingen as analysed by GC/HRMS

## Conclusions

This study shows that eggs of backyard chickens are sensitive biomonitoring parameters 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<sup>®</sup>) that are also affordable by private households, areas of public concern can be monitored in a rapid and efficient way. The correlation between DR CALUX<sup>®</sup> 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. The fact that the levels of dioxins and dioxin-like PCBs are increasing with decreasing distance to the harbour urgently calls for closer investigation in order to find the sources of the dioxin/PCB pollutions, and 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 the RIKILT Institute for sharing their DR CALUX<sup>®</sup> and GC/HRMS data of egg samples.

## References

1. Besselink, H., Jonas, A., Pijnappels, M., Swinkels, A. and Brouwer, B. (2004). *Organohalogen Compounds*, 66: 677-681.