Sampling, monitoring and source tracking of dioxins in the environment of an incinerator in the Netherlands

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ABSTRACT

In the north of the Netherlands, in the environment of Harlingen, an industrial waste incinerator was installed in 2011. The population in the region is concerned about adverse health effects related to possible emission of dioxins. This study compares the relative merit and reliability of four sampling approaches aimed at quantifying the possible pollution patterns surrounding the incinerator: short-term versus long-term isokinetic flue gas sampling, spatial sampling of grasses and a novel approach developed by the Toxicowatch Foundation, based on local composite sampling of ten eggs from *backyard chickens* (local area pollution load averaging by accumulating bio-entities). Based on the latter approach results of a new analytical bioassay, DR CALUX®, from 15 locations near Harlingen harbour show distinct above-threshold dioxin levels in the environs of the incinerator. Currently only short-term flue gas sampling is mandated by the authorities; based hereupon, under normal operating conditions, the incinerator appears to be compliant with emission standards. This short-term sampling scheme is seriously flawed, however, in that it only demands one continuous 12-hour sampling period *per annum* – an extreme grab sampling transgression in the time domain. In starkest possible contrast, significantly elevated dioxins emissions were measured in flue gas during events of unstable combustion conditions by continuous *long-term* measurements. The dioxin congener patterns from long-term flue gas sampling show *similar* patterns as the congeners found in backyard chicken eggs and grass, evidence that elevated dioxins in eggs is due to emissions from the incinerator. These results make it mandatory to perform long-term, continuous measurements for all sources where similar high-temperature combustion/emission processes take place. Backyard chicken eggs are a prevalent regional bio-accumulating sample type in rural and agricultural regions, which is relevant to air pollution emission monitoring. There are interesting relationships between this type of sampling bio-sensors and the special bioassay DR CALUX® features, which, although quite outside traditional contexts, never-the-less all can be well understood in the context of the Theory of Sampling. We venture to unravel the complex interactions between correct and incorrect sampling errors and the extraordinary complicated analytical issues involved in monitoring and source tracking using accumulating bio-sensors. The present contribution is but a first foray indicating the general total sample error scope and total analytical error framework needed for this approach.

INTRODUCTION

Harlingen is a small city of approximately 16 000 inhabitants, situated near the Wadden Sea, inscribed in the world heritage list of the United Nations Educational, Scientific and Cultural Organisation (UNESCO). It is an agricultural area with only smaller industries (relatively low-profile, mainly concentrated near the harbour), comprised by an oil company, shipyards, fishing industry, a plastic company and a waste incinerator, the latter built in 2011. In the area of this research are several small villages Midlum, Wijnaldum and Kimswerd, respectively 700, 100 and 600 inhabitants. In this area it is common to keep backyard chicken for private use or for small-scale marketing. The chicken are free-ranging and stay out more than eight hours a day. Below we first describe the details and specifics of the analytical procedures and analytes used in the environmental-pollution survey, many of which will be unknown or unconventional for traditional TOS audiences, followed by the specific sampling procedures developed.

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MATERIALS AND ANALYTICAL METHODS

Backyard chicken eggs were sampled at 15 different geographical locations near Harlingen and analysed for PolyChlorinated Dibenzo-p-Dioxins (PCDD), Poly-Chlorinated DibenzoFurans (PCDF) and dioxin-like PolyChlorinated Biphenyls (dl-PCB). Two analytical methods are used, a novel bioanalytical screening and a confirmatory chemical analytical reference method.

DR CALUX® is an abbreviation for a novel analytical bioassay method ('*D*ioxine *R*esponsive Chemical *A*ctivated *LU*ciferace gene e*X*pression'), which is described in detail (Besselink *et al*, 2004).

Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct were cultured in α-MEM medium supplemented with ten per cent (v/v) FCS under standard conditions (37°C, five per cent CO_2, 100 per cent humidity). Cells were exposed in triplicate on 96-well microtiter plates containing the standard 2,3,7,8-TCDD calibration range, a DMSO blank. Following an incubation period of 24 hours, cells were lysed. A solution containing luciferin (Glow Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3). DR CALUX® analysis is carried out by the laboratories of BioDetection Systems BV, Amsterdam and RIKILT, Wageningen, both certified ISO 17025.

The GC/HRMS analysis on eggs were performed by MAS (Münster, Germany), RIKILT Wageningen and Nofalab Schiedam (both in the Netherlands).

Flue gas: AMESA® continuous sampling by the Environment SA (Reinmann, Weber and Haag, 2010), analyses of the samples by Eurofins, Germany. AMESA® is a registered trademark of an EU approved long-term sampling method. These mandated analyses were done by SGS (Belgium). Grass samples by the RIVM (National Institute for Public Health and Environment in the Netherlands), analyses RIKILT, Wageningen. All labs are ISO 17025 accredited according to the European Commission (EC) 252/2012 guideline.

Methodological approach – eggs

Carefully selected biological entities used as *accumulating bio-sensors* for specific purposes have been reported in the literature, eg mussels or other bivalves for environmental monitoring of sea water calibrated with regard to oil spillage (Fitzpatrick *et al*, 1997; Dragsund *et al*, 2013), giving a relevant backdrop for the recently invoked free-ranging chicken eggs used in the present work (Arkenbout, 2014; Petrlík, 2015; Polder *et al*, 2016). For each case of such bio-sensor application, there are specific critical issues that must be honoured before conventional quantitative analysis can be carried out without ambiguity. In the present case egg yolk is used as the analytical sample medium for lipophilic substances such as dioxins, furans and dioxin-like polychlorobiphenyls (PCDD/F/dl-PCBs). The processes of biotransformation, bioaccumulation and active migration of these types of substances towards the egg (a kind of detoxification of the chicken itself) makes the egg, and especially the backyard/free range/biological egg[6], a simple but very relevant target for quantifying local pollution levels a.o. However unusual, or complex-looking from the point of view of traditional TOS audiences, in the end the outcome of analysing egg yolk for highly specific organic analytes will be like 'conventional' concentrations or grades which can be used as in standard quantitative surveys, for example delineating geographical patterns or localised temporal patterns.

The novel egg sampling procedure is highly relevant for ultra-low concentration dioxin quantification, but there are also critical success factors that need to be covered before conventional sampling parallels can be applied: the concept of using a composite sample consisting of eggs from free-ranging chickens is the logical approach for an effective accumulating analytical medium (yolk); especially the stipulation to use (at least) ten eggs (other approaches suggest 20). From a strict *composite sampling* perspective (TOS), the higher the number of eggs (each egg is an increment), the better – but tempered by local logistical and practical sampling conditions. It is clear that considerable biological knowledge is at a premium regarding the details of how chickens 'cover the ground' and what their detailed menus are. Backyard chickens eat worms (*Lumbricus terrestris*), snails, insects (*Tenebrio molitor*), plants, humus and soil particles. There's a varying relation between the dioxin contamination in worms, soil and eggs (Kijlstra, Traag and Hoogenboom, 2008) so judicious averaging is called for. Another factor contributing to the high sensibility of backyard chicken is that egg yolks from these hens contain a greater variety of fatty acids than do egg yolks from caged hens (Jones, 1968).

It would appear that the traditional lot consists of the sum-total of available free-ranging chicken eggs in a defined geographical area, to be sampled in the 2D plane. But there is more than what meets the eye. What would constitute the 'heterogeneity' of such a lot?

Organisms higher in the food chain are subject to *bioaccumulation* and *biomagnification*. Chicken accumulates dioxins because they are only very little metabolised in the lipophilic state, but instead stored in the adipose tissue; notably they are not secreted like hydrophilic material in urine or faeces. So, dioxin bioaccumulation level grows with chicken age. The process of egg-laying is a kind of detoxing process for the chicken itself (Kijlstra, Traag and Hoogenboom, 2006). The first egg after an egg-breeding stop can therefore be high in dioxins, a factor to be taken into account when *sampling* for the composite sample for analysis. Another factor is the different varieties of chickens, which can display different behaviour or metabolism. The biomagnification of the other important group of analytes persistent organic pollutants (POPs) takes place because the chicken eats organisms like worms which are contaminated with POPs.

So, an egg is not necessarily an egg (is not necessarily an egg) – it is necessary to assess the parental chicken(s) actively involved in laying eggs. A really unlucky (or distinctly ill-informed) sampling would easily willy-nilly include such over-the-top eggs; there are shades of IDE and IEE here, not to mention a significant risk of GSE, all inflating the total sampling variance. The field sampler must be professionally educated in these biological and physiological issues in order to be able to carry out the imperative CSE and ISE reduction. These issues weigh in heavily when carrying out the novel sampling approach reported on here.

Free-range chickens have a soil uptake of ten to 30 per cent of their feed, which translates to approximately 12 to 36 g of soil/day. The correlation between pollution level in soil and in chicken eggs was outlined in Arkenbout (2014), Kijlstra, Traag and Hoogenboom (2006) and van Eijkeren *et al* (2006). When chicken forage on soil with contaminant levels above 2 pg TEQ/g ds, this result in dioxin levels above

6. Clarification: backyard chicken, free-range chicken and biological chicken. In the Netherlands 'biological' means chickens that are fed with biological (controlled) feed, with the somewhat paradoxical result that a backyard chicken is not a biological chicken. The expression 'free-range' is used commercially and here means chickens that have the possibility to go outdoors (but most of the chickens stay inside). Backyard chickens have to be out more than eight hours per day.

the threshold value of 2.5 pg TEQ PCDD/F (or >5 pg TEQ PCDD/F/dl-PCBs) in eggs. Weber *et al* (2014) relates that the problematic levels in soils are 1.4 to 4.2 ng TEQ/kg for PCDD/F and 2.8 to 8.4 for sum PCDD/F+PCB. These levels of dioxins are common in many areas of industrial emissions and residential areas.

Such dioxin contributions represent a varying, or persistent background level, no doubt involved in laying the basis for official background levels and critical thresholds. Should there exists localised areas of enhanced dioxin loads the analytical result of a composite sample would also be enhanced. This effect could be viewed as a conventional GSE.

A minimum of ten eggs per location were collected, because the small size of household flocks in practice limits a delivery of more than ten simultaneous eggs. The ten-increment composite sampling is also used in other researches, but different numbers are in use; there is unfortunately no international sampling harmonisation – yet (Overmeire *et al*, 2006; Petrlík and Behnisch, 2015). In standard analysis for commercial eggs a total number of increment equalling 20 eggs is used. Special care was taken to collect a composite sample of ten eggs from *different* hens, because of the evidence of intra-sample variation for dioxins in eggs. Kijlstra, Traag and Hoogenboom (2006) explain this by different potential contact of hens with the environment. Eggs were collected from a biological chicken farm 35 km from Harlingen (well outside the incinerator area studied here) to be used as analytical reference blanks (see Figure 1).

In this study eggs were collected exclusively from backyard chicken so from relatively small flocks (<30 chickens). In the Netherlands the enforcement of commercial eggs starts at a flock size above 200 chickens (IKB, *Integrale Ketenbeheersing*). In Kijlstra, Traag and Hoogenboom (2006) a negative correlation between the level of dioxin pollution and flock size in commercialised chicken farms was observed. Also the Danish study by Sørensen *et al* (2013) shows more examples of threshold-exceeding of dioxin levels when the flock size is smaller. Kijlstra, Traag and Hoogenboom (2006) explains the correlation between flock size and lower dioxin contamination as follows: the larger the flock, the less chickens go outside and will therefore have less contact with the polluted environment.

Chickens (and their eggs) are therefore complex 'active sampling agents' (sensors) but potentially excellent indicator species for contaminated soils when all the above caveats have been properly counteracted. But there are as yet only very few systematic studies linking pollution sources and loads, related soil levels and the resultant contaminant levels in bio-accumulating bio entities used as *food* (Weber *et al*, 2014). There is a clear need for a detailed mapping of TOS' standard error types onto this fascinating area in order to reduce GSE, IDE, IEE and IPE where ever possible – and to subject the optimised sampling_plus_analysis pathway to a rigorous replication experiment (RE) characterisation of the effective reproducibility of the (10,20) egg composite sampling approach, DS 3077 (2013). These verification studies are in the pipeline at present.

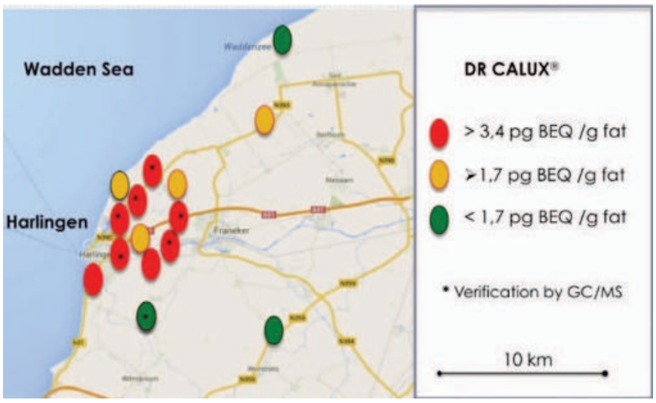

FIG 1 – DR CALUX® dioxin concentrations in eggs from backyard chickens near Harlingen, the Netherlands. Concentration values based on ten-increment composite samples, see text for details.

Methodological approach – grass

Sampling of grass was carried out by selecting two contiguous sections of 25 m² each. In each section approximately 15 evenly spaced grass tufts were identified from which grass was cut and aggregated to form a composite sample. The other section yielded an archival sample which can be used for a double-sampling RE, DS 3077 (2013).

RESULTS AND DISCUSSION

The start of the present research in the neighbourhood of Harlingen was the advent of a new analysis approach, the DR CALUX® bioassay, performed experimentally at 15 locations, Figure 1 shows the results of this bioanalytical screening of the composite samples of backyard chicken eggs. The results of this test when expressed in bioanalytical equivalents (BEQ) give an indication of the TEQ level (EU 709/2014). Extracts from the yolk are split into fractions containing PCDD/PCDFs and dioxin-like PCBs, allowing a separate indication of PCDD/PCDFs and dioxin-like PCB TEQ levels (in BEQ).

The results show a highly systematic dioxin pollution pattern in the environment of the incinerator. In the region of Harlingen *all samples* within a radius of 2 km (n = 11) were above the action limits set for the screening test of DR CALUX® of 1.7 pg BEQ/g fat PCDD/F and above the limit of 3.3 pg BEQ/g fat. The EU 709/2014 demands (in case of commercial eggs) a confirmatory analysis with a chemical method. On eight locations (n = 8) the DR CALUX® results were verified with gas chromatography/mass spectrometry analysis (GC/MS). The chemical analyses confirm the above-threshold dioxin levels in backyard chicken eggs; the levels of dioxins and dioxin-like PCBs increase towards the location of the incinerator.

But also eggs of backyard chicken in other places in the Netherlands tend to have elevated levels of dioxins, underlining the general risk of consuming backyard chicken eggs. Consumers of backyard chicken eggs must be aware that 'biological' does not necessarily mean 'less toxic'.

A subsequent comparative study in other parts of the Netherlands by Hoogenboom *et al* (2016) shows 48 per cent of the eggs of backyard chicken are compliant *below* the limit value of dioxins in food, which means that one in two eggs is below the regulatory threshold EU-value of dioxins in typical regional areas. In Harlingen *none* of the egg samples were below this limit value. A disadvantage in the study of Hoogenboom *et al* (2016) is the lack of exact sample location and corresponding analytical fingerprints. No conclusions can be drawn whether or not a relationship is with the emission of potential dioxin sources like incinerators. A request for releasing more precise data of locations and fingerprints has so far gone unanswered by the Ministry of Economic Affairs.

Grass

Figure 2 shows two maps with grass sample locations and the incinerator (see arrow) in 2014 and 2015 respectively. Grass was deliberately sampled in March after the winter period for optimal accumulation conditions, specifically to avoid the

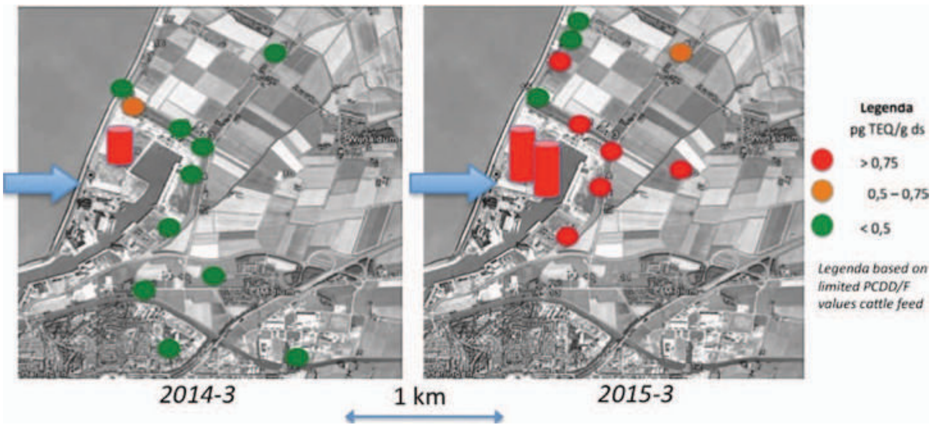

FIG 2 – Dioxin concentration levels in grass around the incinerator REC, Harlingen for the years 2014 and 2015 (arrow points to the incinerator).

influence of grazing of cattle (or sheep) and mowing activities. In 2014 an elevated dioxin deposition was measured near the incinerator of 1.2 pg TEQ/g grass. In 2015 67 per cent of the grass samples were above the 0.75 pg TEQ/g, the maximum limit for dioxins in cattle feed. Near the incinerator the dioxins are a factor 2–4 higher than the reference locations. In December 2015 all the grass samples near the incinerator were elevated with a factor 3 compared to the reference locations. This was remarkable because these sample of grass were only six weeks old (from 28 October no sheep and grass was mowed and removed). There was a huge dioxin emission event in October, but most was emitted to the Wadden Sea (North East wind). Reference locations were 1.5–11 km from the location of the incinerator. None of the reference locations were above the 0.75 pg TEQ/g. It's obvious the highest levels are near the incinerator, pointing this source as the most likely source of the found PCDD/Fs deposition.

Flue gas

The waste-to-energy incinerator in Harlingen was built 2011 and promoted as the most modern installation in the Netherlands (state-of-the-art). It was issued a dioxin emission permit threshold of 0.01 ng TEQ/Nm^3, a factor 10 *lower* than the European standard. The air pollution control devices (APCDs) are based on a dry scrubber system: an electrostatic precipitator, fabric filter, additives-injection with sodium bicarbonate and activated carbon and selective catalytic reduction. The reason for selecting a dry scrubber system (besides a lower cost) was to avoid a visible plume at the shoreline of the Wadden Sea. However, the plume of the Harlingen incinerator is very often visible and many complaints have been raised by inhabitants of Harlingen because of the 'acid or irritating plastic smell'. Although the incinerator is a modern installation, many failures, shutdown and start-ups have taken place (more than 40 times after the start in 2011). Besides the lack of efficient control of APCDs (many fall-outs), other factors like a too short chimney (44 m), windmills in the proximity and a (frequent) coastline fumigation leads to insufficient spreading and dilution of flue gas in the air.

Although today's dioxins emissions from Municipal Waste Incinerators (MWIs) are much lower than in the twentieth century, there are documented reports of high dioxin emissions during *transient operations* ie start-up and shutdown. The regulation of 0.1 ng TEQ/Nm^3 is meant for *steady state* incineration. Tejima *et al* (2007) found high dioxin emission during the start-up of the incinerator. These researchers didn't find high level of dioxins during a regular shutdown, but in the case of the REC Harlingen most often shutdowns occur due to technical failures. In the case of October 2015 there were several failures of the APCDs, resulting in high dioxin emissions. Any short-time monitoring is highly likely only to take place under the presumed *steady state* conditions, but this will not be able to further reliable estimates of the much more dangerous emission situation at transient conditions.

To better characterise the dioxin emissions of the Harlingen incinerator, a *long-term* sampling program (AMESA) was initiated and used to characterise the flue gas *directly* in the incinerator chimney. The regulatory 12 hour measurement period, which represents only 0.1 per cent of the yearly operating time, is mandated by the government as a means to determine the *annual* dioxin emissions (by linear upscaling). Figure 3 shows results of *short-term* sampling (eight measurements of six hours each in four years) as it is to be carried out according to the laws of the Netherlands. It is obvious that this short-term sampling approach does not show a consistent pattern, also because most of the critical fingerprint congeners cannot even be detected (below detection limit). These results underline the need for profound changes in the enforcement rules to eliminate unintentional dioxin production. The mandated short-term measurements are to be performed under normal operating conditions (NOC), in connection with inspections which are *announced* many months ahead – and thus specifically *not* during unstable combustion. Even under such optimal conditions (high caloric waste, no wet digestate), the incinerator could not produce consistent results of short-term sampling of PCDD/F fractions.

In contrast the results of long-term sampling (Figure 4) eight samples with long sampling periods (650–692 hours) are far more consistent in PCDD/F/dl-PCBs patterns. Also shown are the relative high contribution of the non-mandatory dl-

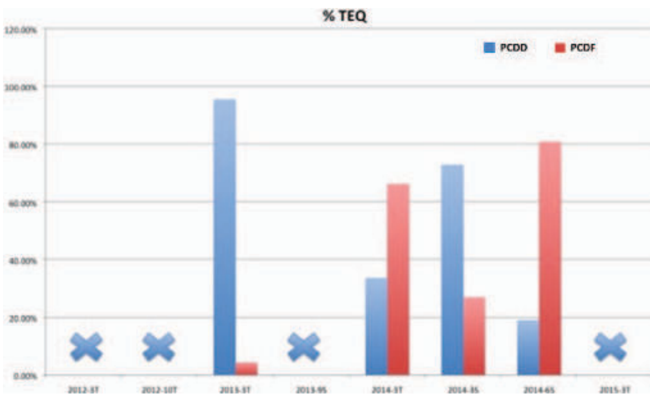

FIG 3 – Short-term sampling of the incinerator. Measurement period: March 2012– March 2015.

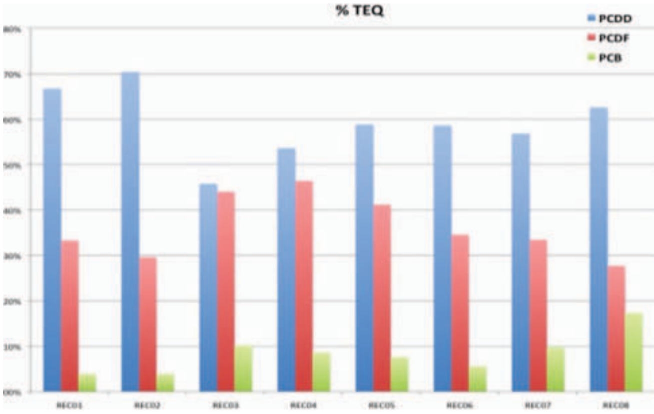

FIG 4 – Long-term sampling of the incinerator.
Measurement period: August 2015 – March 2016.

PCBs in the total TEQ. Long-term sampling give a far more realistic picture of emissions than short-term could ever give. Long-term sampling gives more realistic information about the combustion process, the functionality of APCDs during imperfect combustion, start-ups and shutdowns. Long-term sampling delivers the tools to actually meet the Stockholm Convention to reduce or minimise the dioxin emission towards zero.

Combustion failures with high risk emissions are not included in the official short-term measurements. One failure event may, in just a few hours, exceed the *annual* permitted dioxin emissions by a factor 2, expressed in TEQ, or in concentrations PCDD/F up to 1000 times higher than normal operation (Reinmann, Weber and Haag, 2010). The exclusion of failure notifications allows a wide-open loophole for non-disclosure of exceeding and harmful emissions of dioxins.

Continuous measurements also make it possible to measure POPs emissions during unstable combustion events of start-ups, shutdowns or even failures in the APCD can be detected.

In October 2015, a conglomerate of events produced a significant emission 0.17 ng TEQ/Nm^3, exceeding the general European standard of 0.1 ng TEQ/Nm^3, ie exceeding the local licensed emissions of 0.01 ng TEQ/Nm^3 by a factor 17. This threshold transgression was measured in a cassette representing 672 hours of continuous sampling (28 days). Analyses of the emission patterns show several possible sources/events of this type of high dioxin emission. The governmental enforcement relates these emissions to a 40 hour cascade of failures of the APCD. This means that the emission permit *could have* been exceeded by a factor of more than 300 in this special failure event. The incinerator plant management has promised improvement, but after several occasions of similar events, results still show threshold transgressions.

Biomarker fingerprinting

A scientific way of source tracking dioxins is by way of 'fingerprinting' a sensitive assemblage of 17 dioxin and furan congeners of selected *biomarkers* in samples from the environs and from a possible source. This set of congeners is the result of international agreement. Figure 5 shows this approach illustrating this fingerprint match-up of eggs collected from within a radius of 2 km of the incinerator (n = 6), grass sampled within in a radius of 1 km (n = 12) and flue gas sampled at the REC itself (n = 15). The fingerprints are essentially identical. Only minor details differ, and one can for example explain the abundancy of low-chlorinated congeners by processes of *biotransformation* (Fiedler, 2003). But the long-term sampling also show that the incinerator emissions are not complying with a constant fingerprint, especially not during failure

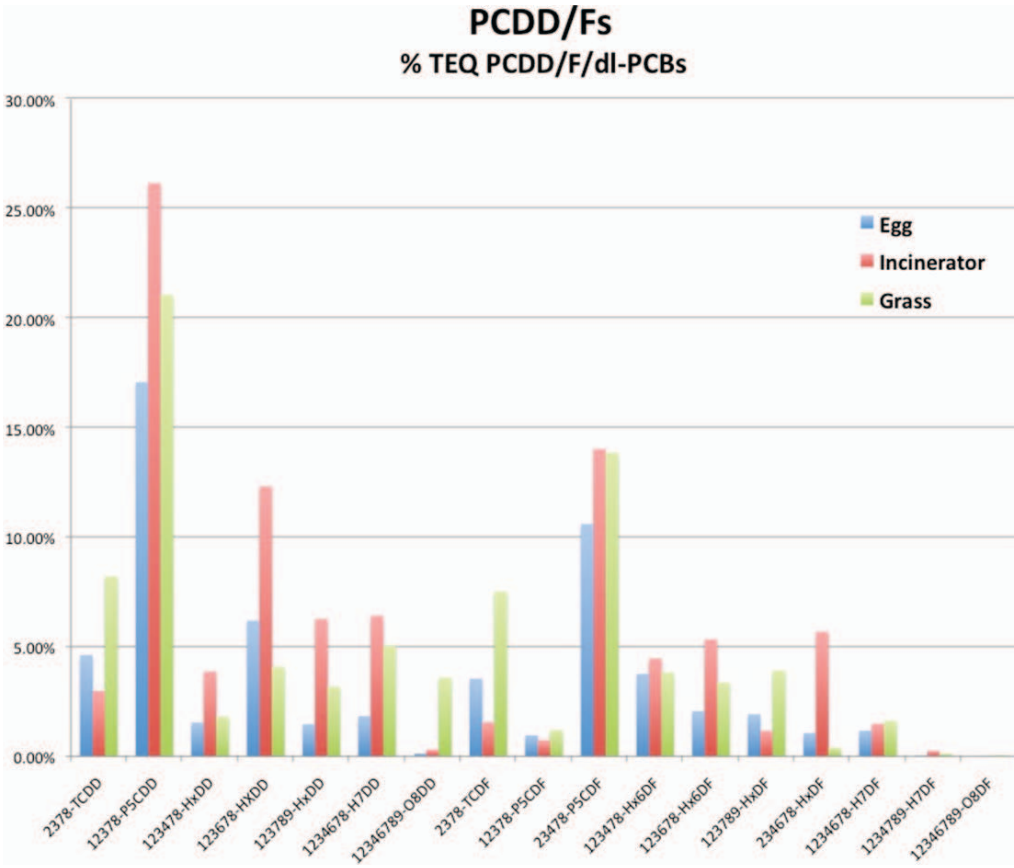

FIG 5 – Dioxin congener pattern match-up for backyard chicken eggs, grass and flue gas from the Harlingen incinerator REC, indicating a common source.

of a certain APCD, or due an *incomplete combustion* when congeners can be released to the environment with an excess factor of up to 1000 in the worst cases. These scenarios will only be exacerbated by continuously changing proportions of waste inputs and loads of industrial waste and sewage sludge.

For full verification the question arises if such closely matching fingerprints can be the result of temporary incinerator failure events – or is this an expression of a more structural process, in fact of a direct causal link? In order to answer this salient question, a further exposé of the analytical intricacies is needed.

dl-PCBs

In the literature (Weber *et al*, 2014), the contributions of dl-PCBs in the total TEQ of the flue gas is only three per cent. The REC Harlingen has a much higher percentage of dl-PCBs in the total concentration of PCDD/F/dl-PCBs in flue gas (33 per cent) and the coplanar PCB 126 accounts for an additional ten per cent of the total TEQ. The incinerator in Harlingen produces ten to 15 per cent TEQ PCBs even under normal conditions (n = 15). The relatively high concentrations of PCBs can be due to an incomplete combustion condition with too low combustion temperatures, – to a short residence time in the combustion-zone, or to a heterogeneous combustion due to waste overload on the fire grid ('cold spots'). The findings of relative high percentage of dl-PCBs may also indicate that otherwise effective combustion temperatures do in fact not achieve the regulatory demands of 850°C/1100°C for two-second periods. Verification of combustion temperature regimes is mandated by the EU regulations with both full 100 per cent, as well as 80 per cent loads measured under circumstances characteristic of the *worst* combustion conditions.

The incinerator management makes public *calculated* temperatures produced by an unverified polynomial data fitting approach, and only measured at 100 per cent load. Although dioxin-like PCBs are not included in EU regulation of dioxins, long-term sampling shows a contribution of dl-PCBs which can be considerable. It's a fundamental enforcement error, not to involve the contribution of PCB's (or even other POPs, see below) in the total TEQ accounting. This underlines the need for a revision of the regulation of POPs. Elimination of POPs in modern waste incineration monitoring turns out to be a critical issue.

Other persistent organic pollutants

A few remarks on the biotechnology of the DR CALUX® analytical method. In some papers (Hoogenboom *et al*, 2016), some results of the DR CALUX® are much higher than corresponding GC/MS analysis. This would indicate that there are several influencing factors behind DR CALUX®. An explanation can be that DR CALUX® measures the biological reaction of the cells on a *whole group of substances* with much the same reaction mechanism. Thus, while chemical analysis pinpoints and quantifies a single dioxin compound, it is likely that DR CALUX® also measure reactions of other *TEQ-related* dioxin-like compounds, eg PCBs, brominated and fluorinated compounds (see below). Conceptually, CALUX® characterises the overall biological activity or induction of gene expression caused by all AhR ligands (agonists and antagonists) present in the sample extract, while a chemical analysis focuses only on a selected number of compounds. This makes DR CALUX® a well suited bio-accumulating pollution monitor – but also emphasises that the *complete* analytical reaction framework needs to be *fully* understood. Thus, TAE needs very careful specification in the present case where several tens of analytes are in play simultaneously.

Polybrominated, or mixed halogenated dioxins and furans (PBDD/F and PXDD/F), are not yet regulated in foodstuffs, sewage sludge, soils or flue gas of an incinerator. Due to the increasing stocks of PBDD/F precursors (PBDEs and other brominated aromatic chemicals) and increasing thermal treatment (incineration) of flame-retarded waste, these should also be evaluated for possible consideration in regulatory development, because of their contribution to the total dioxin-like toxicity.

Harlingen sampling and analysis in the context of the Theory of Sampling

Backyard chickens were introduced as novel local area pollution load *averaging* bio-sensors with a time-accumulating, magnifying mechanism ending up with – *eggs*. Professional (biological, physiological) competence is needed when sampling. The above detailed exposé documents that the many biological and physiological links in this process are reasonably well known at present to be able meaningfully to direct *sampling* of sets of eggs with this provenance – *composite samples* (one egg from each individual chicken is an increment). Informed competence regarding key biological and physiological issues is critical in order to reduce or avoid significant ISE and CSE. While this 'sampling process' lie outside most traditional TOS experiences, it was shown how its elements never-the-less can be identified within the traditional TOS sampling error framework. TOS is helping to *focus* the sampling process in this application area.

A novel twist is that analyte quantification of the specific organic compounds used in dioxin, POP and DI-PCB characterisations, are only arrived at as end-products of quite complex bioassay approaches involving several tens of analytes simultaneously – different from traditional inorganic chemico-physical) 'analysis' of a singular (geo) chemical analyte. There are quite specific, novel TAE issues to be mastered here. In the end however, the end results are identical to other types of quantitative analytical data which can be used in spatial and/or time-dependent contexts for environmental monitoring and source tracking purposes.

It is obvious that the above-threshold transgressing dioxins emissions in flue gas would never have been detected without a program of continuous long-term measurements (sampling). This makes it highly necessary to perform continuous measurements for all industries where *similar* combustion processes take place (mainly incinerator plants). It is clear that short-term measurements cannot give a fair rendering of the actual dioxin emissions. The incinerator of Harlingen has had 35 start-ups since the installation in 2011, which makes it likely that emission thresholds of POPs have in reality been exceeded many times. Mandating only a period of 12 hours continuous measurement per annum can only be characterised as a flagrant grab sampling misunderstanding in the time domain; 12 hours corresponds to 1/(2 × 365) ~0.14 per cent coverage of a whole year.

The above descriptions and discussions, substantiated by recent reports (Arkenbout, 2014; Petrlík, 2015; Reinmann, Weber and Haag, 2010), underline the need for a careful, much improved sampling and analyses regimen for this type of complex, time-changing waste incineration emission monitoring. On all accounts (logical, scientific, incinerator plant performance) the current mandated protocols *must* be repealed and replaced by a more comprehensive measurement regimen, either a significantly longer contiguous period and/or a suitable time domain composite sampling scheme, the details of which can easily be set out based on the extensive existing information. This is the only way in which to be able to honour TOS' fundamental sampling principle (FSP) in the time domain.

CONCLUSIONS

The results of the present research show that composite samples made up of eggs from individual backyard chickens yield highly sensitive analytical biomarkers in the form of dioxins and PCBs. This is also confirmed in other publications of contamination in the vicinity of potential sources (harbour activity, waste incinerator, landfill). In the special case of the incinerator REC in Harlingen, grass could also be used as a biomarker sample type, a reflection of the short distance deposition of dioxins by this installation (short chimney, windmills and shoreline fumigation). The detailed congener patterns of flue gas match the patterns found in eggs and grass closely, which is 'a smoking gun' as identification of pollutants source go. The findings of highly polluted eggs of backyard chickens and matching congener patterns identify the incinerator as the most likely source.

Long-term sampling is far more efficient than short-term measurement of flue gas can ever be. Under NOC, the incinerator complies with emission standards, but numerous inadequate start-up procedures, shutdowns and failures in APCDs never-the-less make incinerators a substantial source of PCDD/F/dl-PCBs, brominated and fluorinated compounds. Unwillingness to release the actually measured critical temperature data also seems contrary to the international agreements of the Aarhus Convention (EC 2003/4/EC) and the Stockholm Convention (EC 850/2004).

This study is an appeal to the scientific world community to work together and find solutions for a serious ongoing pollution problem. Many different scientific fields are needed in careful orchestration: biology, poultry science, analytical chemistry in general, advanced analysis of dioxin in particular – as well as the Theory of Sampling (TOS). It is challenging, and satisfying, that one of the largest critical success factors in this context is amenable to analysis and remediation by invoking TOS' principles to the decidedly novel 'analytical sample' used here: composite increment sets, comprised by 10(20) eggs from individual free-ranging chickens (backyard or otherwise). The present contribution is but a first foray indicating the general total sample error (TSE) scope and complex TAE framework for this approach. Much interesting work remains not least the intricate TAE issues interacting with more traditional TOS and Measurement Uncertainty (MU) quests (Esbensen and Wagner, 2014).

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REFERENCES

Arkenbout, A, 2014. 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, *Organohalogen Compounds*, 76:1407–1410.

Besselink, H J A, Pijnappels, M, Swinkels, A and Brouwer, B, 2004. Validation of extraction, clean-up and DR CALUX® bioanalysis, part II: foodstuff, *Organohalogen Compounds*, 66:677–681.

Dragsund, I, Kompen, M, Holmslet, E, Sønneland, E and Christie, O, 2013. The Biota Guard marine oil leak monitoring system – novel sampling application of bivalve PAT biosensors, *TOS Forum*, 1(1):15–18 (ed: K Esbensen), doi: 10.1255/tosf.6. Available from: <http://www.impublications.com/subs/tosf/v13/S01_0015.pdf>.

DS 3077, 2013. Danish Standards (DS) 3077, Representative sampling – horizontal standard.

Esbensen, K H and Wagner, C, 2014. Theory of Sampling (TOS) versus measurement uncertainty (MU) – a call for integration, *Trends in Analytical Chemistry*, 57:93–106.

European Commission, 2003. EC 2003/4/EC, Aarhus Convention, on public access to environmental information and repealing Council Directive 90/313/EEC, *Official Journal of the European Union*, 14 February 2003.

European Commission, 2004. EC 850/2004, Stockholm Convention, on persistent organic pollutants and amending Directive 79/117/EEC, *Official Journal of the European Union*, L 158/ 7.

European Commission, 2012. EC 252/2012, Laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006, *Official Journal of the European Union*, L84/1.

European Commission, 2014. EC 709/2014, amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls, *Official Journal of the European Union*, L 188/1.

Fiedler, H, 2003. Dioxins and furans (PCDD/PCDF), in *Persistent Organic Pollutants*, volume 30, The Handbook of Environmental Chemistry, pp 123–201.

Fitzpatrick, P J, O'Halloran, J, Sheehan, D and Walsh, A, 1997. Assessment of a glutathione S-transferase and related proteins in the gill and digestive gland of *Mytilus edulis (L.)*, as potential organic pollution biomarkers, *Biomarkers*, 2:51–56.

Hoogenboom, R L, Ten Dam, G, van Bruggen, M, Jeurissen, S M, van Leeuwen, S P, Theelen, R M and Zeilmaker, M J, 2016. Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and biphenyls (PCBs) in home-produced eggs, *Chemosphere*, 150:311–319.

Jones, D, 1968. Point of distinction between battery and free range eggs, *Nature*, 220(5170):921.

Kijlstra, A, Traag, W A and Hoogenboom, L A P, 2008. Controlling egg dioxin levels from laying hens with outdoor run, Animal Sciences Group – Wageningen UR, report 123, ISSN 1570–8616.

Petrlík, J, 2015. Persistent organic pollutants (POPs) in chicken eggs from hot spots in China, Arnika research.

Petrlík, J and Behnisch, P, 2015. Persistent organic pollutants (POPs) in free range chicken eggs from Western Balkan countries, Bosnia and Herzegovina, Montenegro and Serbia, 2015, in *Toxics and Waste Programme*, Prague, ISBN: 978-80-87651-17-9.

Polder, A, Müller, M B, Brynildsrud, O B, de Boer, J, Hamers, T, Kamstra, J H, Lie, E, Mdegela, R H, Moberg, H, Nonga, H E, Sandvik, M, Skaare, J U and Lyche, J l, 2016. Dioxins, PCBs, chlorinated pesticides and brominated flame retardants in free-range chicken eggs from peri-urban areas in Arusha, Tanzania: Levels and implications for human health, *Science of the Total Environment*, pp 551–552, 656–667.

Reinmann, J, Weber, R and Haag, R, 2010. Long-term monitoring of PCDD/PCDF and other unintentionally produced POPs – concepts and case studies from Europe, *Science China Chemistry*, 53(5):1017–1024.

Sørensen, S, Krüger, L, Bossi, R, Cederberg, T L and Lund, K H, 2013. Dioxins and PCBs in hen eggs from conventional and free range farms from the Danish control program in 2012–2013, in *Proceedings 34th International Symposium on Halogenated Persistent Organic Pollutants* (São Paulo).

Tejima, H, Nishigaki, M, Fujita, Y, Matsumoto, A, Takeda, N and Takaoka, M, 2007. Characteristics of dioxin emission at startup and shutdown of MSW incinerators, *Chemosphere*, 66:1123–1130.

van Eijkeren, J, Zeilmaker, M, Kan, C, Traag, W and Hoogenboom, L, 2006. A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs: part A, *Food Additives and Contaminants*, 23(5):509–517.

Van Overmeire, I, Pussemier, V, Hanot, L, De Temmerman, Hoenig, M and Goeyens, L, 2006. Chemical contamination of free-range eggs from Belgium, *Food Additives and Contaminants*, 23(11):1109–1122.

Weber, R, Watson, A, Petrlík, J, Winski, A, Schwedler, O, Baitinger, C and Behnisch P, 2014. High levels of PCDD/F, PBDD/F and PCB in eggs around pollution sources demonstrates the need to review soil standards, *Organohalogen Compounds*, 76:815–818.