FORENSIC INVESTIGATIONS IN COMPLEX POLLUTION CASES INVOLVING PCBS, DIOXINS AND FURANS: POTENTIAL PITFALLS AND TIPS

Jean Christophe Balouet[1], Francis Gallion[2], Jacques Martelain[3], David Megson[4,5], Gwen O'Sullivan[6]

1 Environnement International, 2 rue du Hamet, 60129 Orrouy, France
2 Legis Environnement, BP 2006, 79403 Saint Maixent l'Ecole, France
3 TERRAQUAtron, 61route de Saint Romain, 69450 St Cyr au Mont D'Or, France
4 Ryerson University, 350 Victoria Street, Toronto, ON, M5B 2K3, Canada
5 Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Toronto, ON, M9P 3V6 Canada
6 Department of Environmental Science, Mount Royal University, 4825 Mount Royal Gate SW, Calgary, AB, T3E 6K6, Canada

1 INTRODUCTION

Polychlorinated biphenyls (PCBs), dioxins (PCDDs) and furans (PCDFs) are frequently detected in grass, soil and farm animals near incineration plants, electric transformers recycling facilities, or after major fires during environmental forensic investigations. The sale of farm animals for food above established PCBs and PCDD/F limits is forbidden, and therefore are slaughtered, or removed from contaminated areas to graze on uncontaminated food, in an attempt to detoxify. This policy can result in substantial financial losses to farmers and other parties on whose property these contaminants are detected. If regulated levels are exceeded in one sample, the impacts are rarely limited to one area, or within the sampling period. It is therefore important to establish the extent of the contamination, distinguish the potential source(s) and identify the duration of the contamination event. These determinations are especially challenging to account for background noise or when multiple sources of these contaminants are present within a 10 kilometre (km) radius of the investigated area and could have contributed to the contamination.

This manuscript examine potential pitfalls in the analysis of (PCBs), dioxins (PCDDs) and furans (PCDFs) in terms of their regulated levels, sampling challenges, the analysis of samples and statistical interpretation of the test data.

2 REGULATED LEVELS

PCDD/F and PCBs are detected in the environment as complex mixtures. To assess the risks to human health and the environment, internationally agreed Toxic Equivalency Factors (TEFs) have been developed and are regularly updated by the World Health Organisation (WHO). The current TEFs are based on the findings of Van den Berg *et al.*[1] The biological effects of PCDD, PCDF and PCBs are mediated through the aryl hydrocarbon receptor (AhR) which has a high affinity for 2,3,7,8-substituted PCDD/Fs and co-planar PCBs with either one or no chlorines in the 2, 2’, 6 or 6’ positions. Table 1 lists the 17 PCDD/Fs and 12 PCBs with TEFs from Van den Burg *et al*.[1]

Threshold and alert levels have been established by WHO for different matrices, and are expressed in TEQ (Toxicity Equivalent), for PCB DL (dioxin like), dioxins (PCDD) and furans (PCDF). More recently, other threshold levels have also been established for the 7 indicator PCBs (PCBIs) (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180). Common limit values can be established as follows for samples such as cattle and grass based on WHO TEFs (Table 2). Alert levels have also been generated for grass (12% humidity) at 0,5 pg g^{-1} (picogram per gram)WHO -PCDD/F-TEQ and 0,35 pg g^{-1} WHO-PCBDL-TEQ. (e. g. European Directive 2006/13/CE)

Table 1. *2005 World Health Organization (WHO) Toxicity Equivalent Factors(TEFs)*

Dioxins	WHO TEF	Furans	WHO TEF	PCBs (Cl substitution)	WHO TEF
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	PCB-77 (34-3'4')	0.0001
1,2,3,7,8-PeCDD	1	1,2,3,7,8-PeCDF	0.03	PCB-81 (345-4')	0.0003
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PeCDF	0.3	PCB-105 (234-3'4')	0.00003
1,2,3,6,7,8--HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1	PCB-114 (2345-4')	0.00003
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1	PCB-118 (245-3'4')	0.00003
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,7,8,9-HxCDF	0.1	PCB-123 (345-2'4')	0.00003
OCDD	0.0003	2,3,4,6,7,8-HxTCDF	0.1	PCB-126 (345-3'4')	0.1
		1,2,3,4,6,7,8-HpCDD	0.01	PCB-156 (2345-3'4')	0.00003
		1,2,3,4,7,8,9-HpCDD	0.01	PCB-157 (234-3'4'5')	0.00003
		OCDD	0.0003	PCB-167 (245-3'4'5')	0.00003
				PCB-169 (345-3'4'5')	0.03
				PCB-189 (2345-3'4'5')	0.00003

Table 2. *WHO established Toxicity Equivalent (TEQs) for meat and grass, 1998 and 2005 values in picogram per gram (pg g^{-1}) and nannogram per gram (ng g^{-1})*

Meat FAT	Toxicity Factor	TEQ Σ(PCDD+PCDF+PCBDL) pg g^{-1} I TEQ WHO	PCB ind ng g^{-1}
2011	I-TEF WHO 1998	4,5 pg g^{-1} I-TEQ WHO 1998	Not applicable
2012-13-14	I-TEF WHO 2005	4 pg g^{-1} I-TEQ WHO 2005	40 ng g^{-1}
Grass	**Toxicity Factor**	**TEQ Σ(PCDD+PCDF+PCBDL) pg g^{-1} I TEQ WHO**	
2011	I-TEF WHO 1998	1,25 pg g^{-1} I-TEQ WHO 1998	
2012	I-TEF WHO 2005	1,25 pg g^{-1} I-TEQ WHO 2005	

3 SAMPLING

Numerous samples are usually gathered during the course of an environmental forensics investigation. These might be collected from air particles (on an Owen gage and from emission source), as well as gas phases, vegetation, soils and animals, leading to complex data set with the tens of thousands of data points.

There are several pitfalls when selecting appropriate samples, including the following:

- When sampling air and particulate matter to consider and monitor wind direction. The sampling strategy should be designed to include a background sample which is upwind of the suspected source(s). Most passive samplers collect dust from all directions and so need to be combined with meteorological data to establish sources. However there have been recent advances in sample collection technologies that allow 360° sampling at 15° intervals to establish sources of dust contamination.[2]
- When sampling the grass, it is essential to understand that these contaminants will be found at higher concentration in winter, during the plant's dormant season, and at lower concentrations during spring due to grass growth. Other complex mechanisms such as microbial activity, photolysis and selective evaporation are identified, which can reduce concentrations or alter chemical signature / fingerprint (i.e. less chlorinated molecules are more degraded than the more chlorinated ones).
- When sampling surface soils the samples are all made at same depth (between 0 and 5 cm maximum) as deeper soils are likely to be less contaminated. Similarly, it is important to check that soils have not been perturbed: i.e. ploughed, or disturbed by cattle feet in muddy areas, which would result in contaminants' dilution.
- When sampling animals if the goal is to compare total concentrations then it is preferable to obtain samples from the same tissue type as concentrations vary in different tissues based on the lipid content. Therefore all data should be lipid corrected to allow for a representative comparison between samples. Concentrations can vary in different tissue types however the signature appears to be very consistent, indicating that whilst collection of the same tissue type is preferable, the signature in different animals can be compared using samples obtained from different tissue types.[3]
- Impact to farmland, crops and livestock also depend on distance, wind direction, topography. In some very rare instances, when farm animals are sampled from freezers, expert needs to check that selected meat does not originate from distant areas.
- The background concentrations and signature need to be established during investigation and several samples are therefore required from several distant areas, away from source, up to 10 km (see Figure 3 for example).

In all cases, samples should be properly labelled and stored in containers appropriate for the analysis that is being undertaken. As some PCB, PCDD/F congeners are semi volatile samples should be packed tightly in glass containers to reduced headspace and stored in cool boxes at 4°C once collected, to reduced potential losses through evaporation. All samples should be transported to the laboratory as soon as possible (within 24 hrs) with appropriate chain of custody documentation.

4 ANALYSES AND STATISTICAL INTERPRETATION

4.1. Analysis

When regulatory authorities are involved, 2,3,7,8 substituted PCDD/F and WHO12 PCBs are often of forensic interest (Table 1). For these contaminants, GC-MS or GC-ECD provide a mean to quickly determine whether a sample is grossly contaminated. Many commercial laboratories provide limits of detection (LODs) in the range of 10 – 1000 µg kg^{-1}. This can be a significant limitation as background total PCB concentrations (in UK urban soils) are between 0.01 – 40 µg kg^{-1}. [4] Therefore, sample clean-up and analysis by HRMS is often required to improve limits of detection to less than 1 µg kg^{-1} by removing or filtering out many interfering compounds. Whilst the 2,3,7,8 substituted PCDD/F and WHO12 PCBs can establish risks to human health, additional congeners may be needed to identify the source. This can be a time intensive task using GCMS or GCHRMS as several runs using different column types are needed to produce a comprehensive congener specific data set, due to multiple co-elutions of PCB and PCDD/F congeners (there are over 400 PCBs and PCDD/Fs).

The development of comprehensive two-dimensional gas chromatography (GCxGC-TOFMS) provides an extra dimension of separation which significantly increases the resolving capacity. This has allowed for the identification of over 190 individual PCB congeners along with simultaneous identification of other organohalogenated contaminants. [5,6] Figure 1 displays the separation of 173 peaks from 209 PCBs achieved using a PCB specific Rtx-PCB column on GC-MS, compared to 200 peaks from 209 PCBs which was achieved using GCxGC-TOFMS (Rtx PCB and Rxi-17 columns). However, such expertise is usually at great costs, for example analytical costs for the PCBDL, PCDD / PCDF and PCBIs range in the 500 US$, and go as high as 2000 $ for documenting all PCB and PCDDF congeners.

4.2. Statistical Interpretation

In most investigations involving PCBs, PCDD/Fs the results are screened against a specific health value to establish the risks. This can be a simple comparison of individual sample concentrations against a threshold level, or the use of statistics to determine if an area (or site) is above the threshold value at a 95% degree confidence level. However, for many forensic investigations the goal is not to determine if the concentration is above a threshold, but to establish where the contamination has originated from. In these instances, absolute concentrations are often of little use and signatures based on the relative proportions of individual congeners are more useful, especially when combined with multivariate statistics.

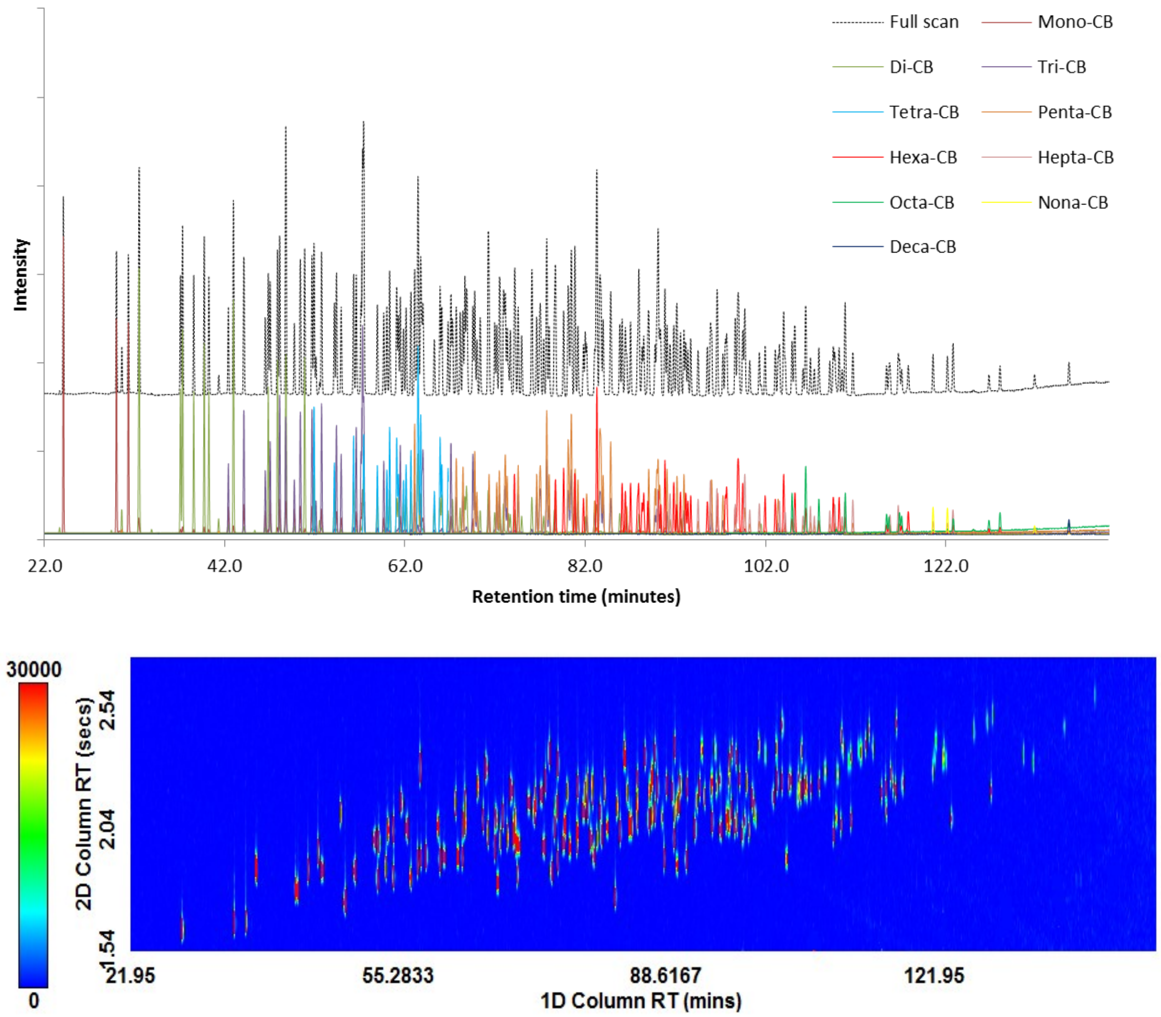

Figure 1. *Comparison of PCB separation using GC-MS (above), compared to GCxGC-TOFMS (below). Figure adapted from Megson et al. 2014.*[3]

Statistical methods are of major relevance when comparing signatures through fingerprinting: i.e. using R2 to determine the confidence interval, and multivariate analysis to compare hundreds of samples and dozens of compounds. Most statistical techniques require some prior knowledge of the contamination event. However, some systems with multiple sources and degradation pathways are too complex to establish a priori assumptions;[7] in such investigations, exploratory data analysis techniques, such as principal component analysis and polytopic vector analysis, can be used to identify the source(s) of contamination. Principal component analysis may be especially useful for identifying temporal patterns in the data. In some cases it can be important to identify if a change in the signature has occurred after a specific date (for example when a company was supposed to implement procedures to reduce its emissions to air). Figure 2 shows how principal component analysis was used to show that the absence of a distinguishing pattern in dust collected over a three year period.

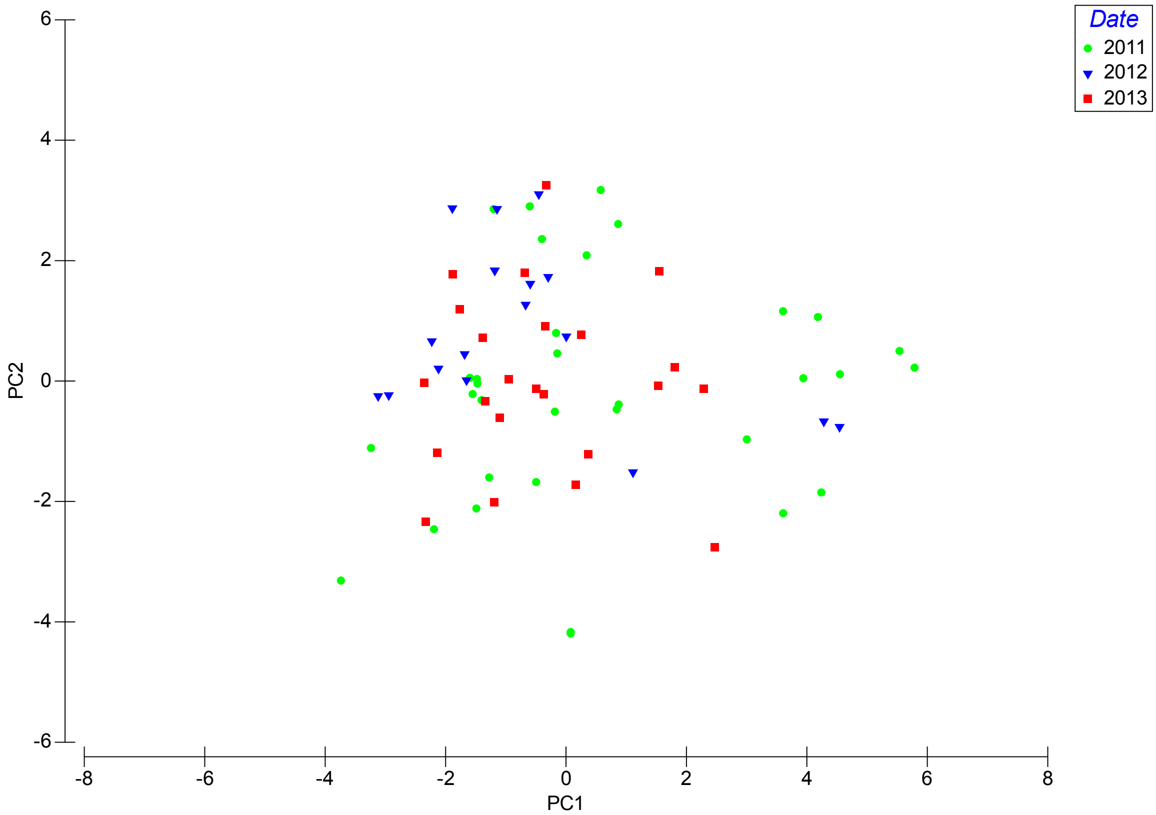

Figure 2. *PCA scores plot showing no noticeable change in dust signature over a three year monitoring period*

In these cases it is also important to encompass and distinguish other potential contributors such as domestic heating, backyard burning, slash and burn as well as the background noise: The US EPA have produced a list of potential contributors, which is useful for forensic investigations, and is accessible at http://www.epa.gov/ncea/pdfs/dioxin/2k-update/. Historical research and proper investigations are essential to characterize potential sources so as to distinguish liabilities and allocate torts as honestly as can be. It is here important to characterize the background noise: when threshold limit values are exceeded in grass and cattle, the background noise typically accounts for around 2 % of the contamination.

4.3 Modelling Data

Expertise is required to identify the source by mapping the plume impacts for the different environmental media and receptors and comparing the chemical fingerprint of contaminants sampled at the source and at a distance. The concentration maps can be centred by source, when the impact distance can exceed 6 km, as documented for PM10, [8] depending on wind conditions or height of emission source.

Documenting the exact contribution of suspected source versus other potential contributors is difficult. Some simple methods allow for the calculation of how much the supposed source has been contributing, within a given distance to source, using the following parameters and formulae:

- Annual emissions: Em_{ann};
- Distance to source: Dis_{source} used as a radius. Several models exist to estimate particle concentrations, according to their size, wind speed, and height of chimney as release point;
- Annual mass production for grass by surface –dry weight = 12% Humidity: $Prod_{mass}$ in kg/m^2 or ton/hectar (1000 kg/10 000m^2), for example 6 tons of hay per hectare;
- Concentration: Such a simulation can be operated on regulated level or on observed concentrations $Conc_{reg}$ or $Conc_{obs}$ or to estimate impacts at a given level $Conc_{theor}$. Concentrations are usually expressed in pg g^{-1} TEQ for PCBDLs, PCDDs, PCDFs, and in ng g^{-1} PCBI, and
- Impacted surface: $Surf_{imp}$.

A simple formula can be based under the assumption that the contamination results in homogenous concentrations, independent on distance or winds direction or speed.

Contaminant concentration in a given radius:

$$Conc_{theor} = Em_{ann} / Dis_{source}^{2} \times \pi \times Prod_{mass} \qquad (1)$$

With an example of Em_{ann} 0,5 g TEQ , Dis_{source} 3000 m , $Prod_{mass}$ 6 tons ha^{-1} or 0.6 kg per m^2, $Conc_{theor}$ = 0,29 pg g^{-1} TEQ

The same formula can be used to determine the theoretical radius within which a regulated level would be found, if homogenous concentrations. In the preceding example, the threshold limit of 0,35 pg g^{-1} TEQ PCBDL for grass is exceeded within a radius of 3.2 km. The meat fat in most cows, however, would contain TEQ PCBDL values exceeding this value whilst veals contamination is commonly twice higher than milking cows. These calculations are usefully complemented by congener fingerprint profiles calculated from samples. Figure 3 presents the following theoretical profile for vegetal concentrations from samples made within same wind direction, and at varying distances. The abscissa –in meters- represents the distances to source (upwind in negative numbers and downwind in positive numbers) and ordinates represent the pollutant concentrations.

Such simple graphs, derived from Excel, can be used to estimate concentrations $Conc_{theor}$ at Dis_{source}, whether upwind or downwind. Such quasi-Gaussian profile further enables the possibility to establish that:

- the higher concentrations are by source;
- impacts are slightly higher downwind;
- the background noise is found at 10 km distance, upwind and downwind, and
- the absence of secondary sources that would have caused significant / local or temporal concentration anomalies.

In this theoretical case, the background noise is too low to cause concentrations in excess of regulated thresholds, as is an almost universal condition, though depending on the possibly high concentration of industrial plants, sources that would exist nearby.

Conc	0,01	0,03	0,2	1,5	4	5	2	0,5	0,1	0,01
Distance	-10000	-5000	-2500	-1000	-500	500	1000	2500	5000	10000

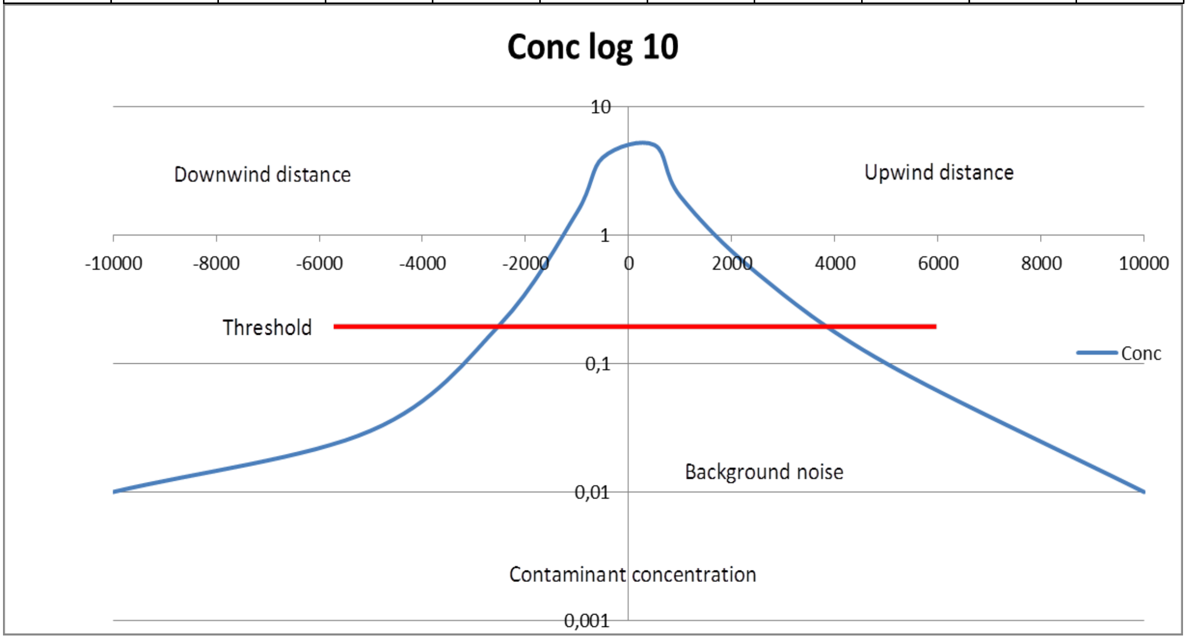

Figure 3 *Theoretical concentration profile for vegetal samples made within same wind direction, and at varying distances.*

5 DEBATES AND POTENTIAL PITFALLS

5.1 Time

Time is a critical factor because it can take years before the pollution is first documented.

To the parties and their consultants, it is primordial to focus on the situation at the time the case and the supporting evidence did build, possibly years before forensic involvement. Dendrochemical evidence gained from pine or picea needles can help document current and past contamination, typically year by year and back to 7 or 8 years before sampling time, also providing the chemical fingerprint of the 35 sought congeners.

In cases where an industrial party is continuously involved, they will do their best to abate their emissions, would it be by mechanical / physical / chemical control systems, or by reducing their activities, either on an annual basis, or within weeks or months before analytical campaigns are conducted, of which they proactively are informed. In such a situation, measured concentrations would not exceed regulated thresholds, although they may have at other times.

Furthermore, environmental forensic expertise also can address preventive considerations: this *in futurum* part of experts' tasks is to check whether proper solutions are in place or additional measures should be set. Such anticipative considerations are essential to help the judge and law enforcement authorities make sure that the pollution problem will not continue after litigation of the dispute, or would not occur in other circumstances.

With the adoption of international conventions, such as the 1989 Aarhus Convention or Sustainable development, the experts' mandates are turning more and more complex as they involve considerations to Human Rights, or environmental damage, that goes far beyond common forensic practice in the 20th Century.

5.2 Combining Datasets

Care is required when combining and comparing datasets from different sampling investigations, as analytical procedures are constantly changing. Similar challenges exist when comparing samples from different matrixes as the extraction and clean up procedures will differ depending on the matrix and possibly even the sample. This is especially important when comparing low level concentration data, in instances where the concentration is below the limit of detection (LOD) a value of the LOD or LOD/2 is often substituted. [9] If the LOD is different between the two or more datasets this can indicate a difference between the datasets which may not actually exist in the samples.

Another consideration with PCB and PCDD/F data collected over different time periods is the fact that the TEFs are regularly reviewed and updated by WHO. The most recent values were released in 2005 and so it is important to understand that any TEQs produced before 2005 may have been calculated using the 1998 WHO TEFs and any TEQs calculated before 1998 may have been calculated using the 1988 NATO I-TEQs. Depending upon which of the three TEFs are used, and on the congener mixture in the samples, it is not uncommon for the calculated TEQs to vary by over 10%. [10] Therefore care must be taken when comparing and combining datasets over different time periods and it is always useful to double check the raw data to identify which TEFs have been used in any TEQ calculation.

5.3 Evidence

In some cases local events, such as slash and burn, backyard waste burning may be inferred as temporal and local cause of exceeding threshold limits in some samples. A rule of thumb is that such events also exist in the areas where background noise is documented. Some parties may utilize selected data rather than considering the entire data set in an attempt to advocate a particular position, rather than considering all of the information and basing scientific opinions on this analysis.

The bibliographic resources available are vast, technically complex, and may be available from governmental and intergovernmental organizations or from peer-reviewed publications. Language is a significant barrier. This would require considerable resource as dozens of key references are several hundred pages, when it may be sufficient to translate the important pages only (for example the web link given in section 4.2. refers to an US EPA document several hundred pages long).

Depending on the judicial system in place in the country where the case takes place, it is more or less easy to access all pre-existing evidences. Taking just one example, when many others could be referred to, let’s consider that an industrial plant has been found to cause environmental impacts in excess of regulated level; worker’s staff, most directly exposed to industrial activity, may have been subject to biological sampling, to document their exposure to the specific pollutant. What about if such worker’s exposure has been documented by company and his exposure level is not communicated to the experts, nor attorneys and even not Court, whenever the numbers may exceed by 50 or 100 times the regulated ones? Such evidence would have proved essential to link contamination to the workplace as a source, but also to further establish toxic torts.

6 CONCLUSIONS

Environmental forensics investigations are key to Justice by honestly documenting the facts, understanding each and all of the evidences would they be served by plaintiff, defendant, or newly established as part of a litigation, whatever the data and influencing parameters can be complex. Contradictory debate is key, provided it is served with unbiased transparency and highest competent objectivity; some evidences may prove scientifically opposable, others turn as highlights when to form an honest opinion.

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