On the quality of dioxin analysis. Review of a decade of improvement.

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1 Introduction

At present, dioxin analyses are among the most reliable of existing assays of chemical substances. Unexpectedly good within and between laboratory comparibilities were achieved in the recent past when related to the very low levels in which dioxins were present in the various samples [1]. A great deal of this achievement can be ascribed to the great efforts that have been put worldwide in the improvement of techniques and methods, not least by the development of suitable gas chromatographic columns for their isomer specific separation and the availability of highly sensitive high resolution mass spectrometry instruments. On the other hand, some characteristic physico-chemical properties of dioxins permit the beneficial use of such technologies.
From a regulatory point of view, there is a significant need for accurate and reliable data on levels in the environment and the food chain. Owing to their suspicious ecotoxicological and human health risks, regulatory measures have been put in force concerning maximum allowable levels in food products and municipal and hazardous waste incinerator emissions.
Several international bodies like the WHO, BCR and CEN have contributed to the present high standard by means of organizing comparitive studies among laboratories aiming at the improvement of methods and the spread of experience and skills to the participating laboratories or the production of reference materials (fly ash, milk powder).
In this contribution, factors that play a major role in the ongoing process of improvement of quality and reliability of dioxin data will be discussed.

2 Analytical requirements

Experience acquired over the last few years shows that the delivery of good quality data requires appropriate methods and techniques as well as a set of Quality Assurance (QA) measures involving:
1. Sample pretreatment: well suited sample extraction and clean-up methods.
2. Quantification: well defined set of carbon-13 labelled surrogate standards, preferably for all seventeen 2,3,7,8-chlorine substituted congeners.
3. Analysis: a sensitive and accurately calibrated GC/MS system, preferably using a high resolution MS instrument and a GC system that enables isomer specific separation.
4. Stringent Quality Control (QC) protocols concerning first to third level quality control measures.

Most of these requirements are quite obvious. Some of them are related to each other. For instance the minimum mass resolution required will be strongly dependent on the presence, both the level and diversity of chemical substances, of other compounds that may have passed the clean-up system, *particularly (i) when they elute in the dioxin GC retention window and (ii) when they possess ions*

(molecular or fragment ions) that coincide with the measured masses for dioxins. In addition, increased mass resolution analysis may considerably decrease the lower detection limit (LOD) owing to the resulting improvement of the signal to *chemical* noise ratios. Fig. 1 shows the analysis of a cow's milk sample at 900 and 3000 RP, respectively. Prominent suspicious interferences can be expected from PCBs. Their concentration in the original sample may be several orders of magnitude higher than for dioxins and they may behave quite similar in the clean-up proces leading to significant levels in the final extract. In addition, they overlap with dioxins in both retention times and masses. To avoid interfering responses a resolving power of more than 10,000 is necessary.

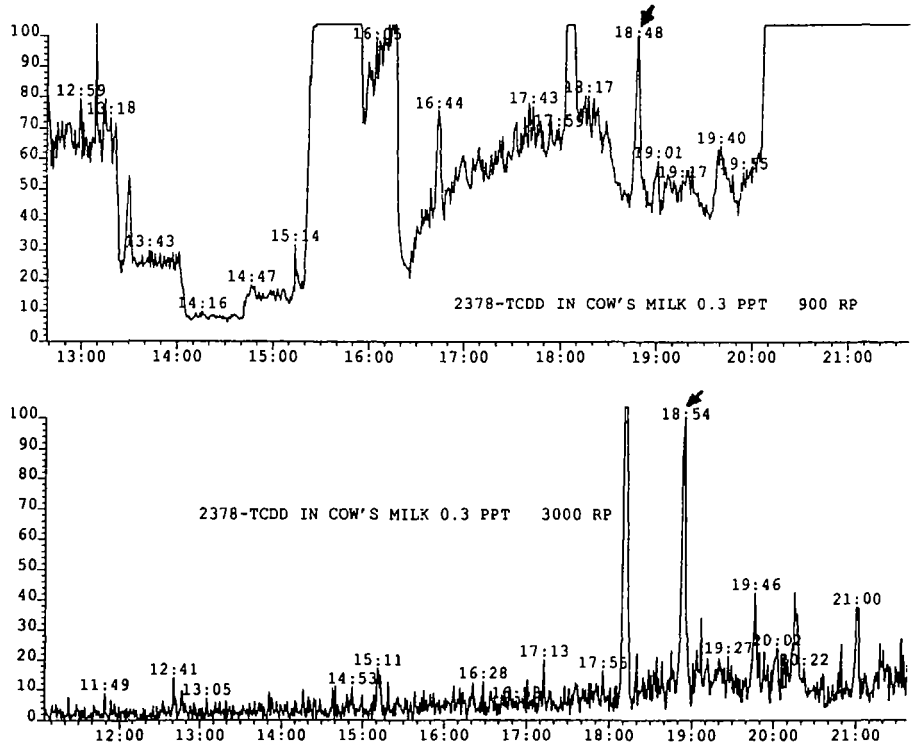

Figure 1.
Selected ion recordings showing the GC/MS analysis of 2,3,7,8-TCDD at a level of 0.3 pg/g fat in a sample of cow's milk at a mass resolution of 900 (upper trace) and 3,000 (lower trace) respectively.

Requirement 4 includes a set of criteria providing information on the quality of individual data. The first level QC measures should at least include: (i) criteria for positive identification of responses (e.g., correct GC retention time, isotope ratios and a certain minimum S/N); (ii) sufficiently low procedural and instrumental blanks (cross contamination); (iii) sufficient recovery.
The second QC level includes for example a parallel analysis of a quality control sample. Analysis of such a sample in each series of measurements provides insight in the actual performance of the method, whereas the inter-assay variability provides insight in the effects of different steps in the procedure and on the long term comparability of the analysis. In our laboratory, quality control samples of cow's milk and human milk have been used to evaluate the analytical contribution to all factors determining levels and trends of PCDDs, PCDFs and PCBs in biological samples including foodstuffs and human milk. The usefull information as provided by these QC samples is illustrated in Figure 2.

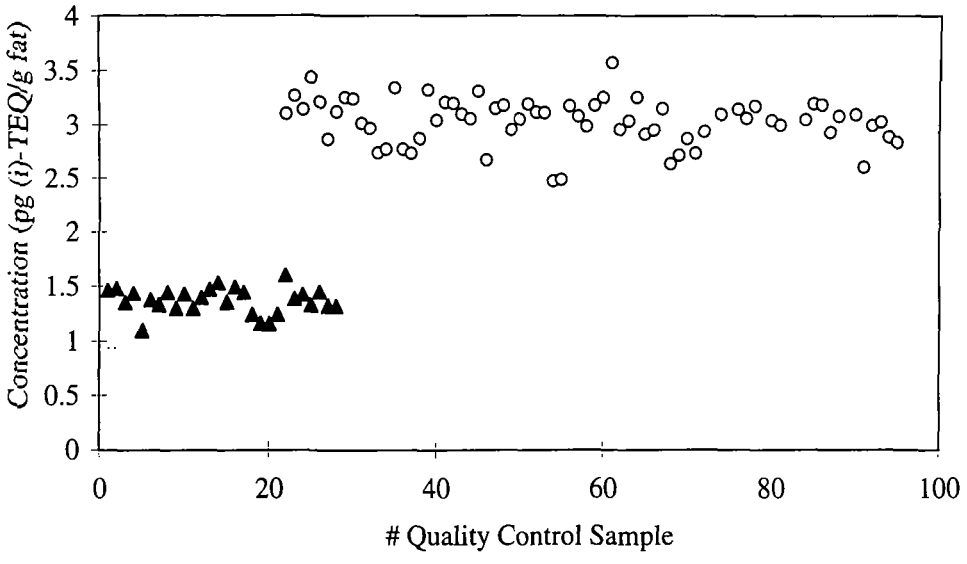

Figure 2.
Results from congener-specific determinations of PCDDs and PCDFs (levels expressed in pg (i)-TEQ/g fat) in two consecutive Quality Control samples of cow's milk showing the long-term within-lab reproducibility at the low ppt-level. Analyses were performed in the period 1989-1995. Mean concentrations were found of 1.36 (RSD of 8.6%, n=28) and of 3.03 pg (i)-TEQ/g fat (RSD of 7.0%, n=68), respectively.

Third level QC measures include participation in interlaboratory studies to compare the own performance with that of other laboratories. This gives the opportunity to detect the existence of systematic errors or otherwise weak points in the own procedure. These may range from e.g. the quality (accuracy) of the calibration standards used, unability of the clean-up method to eliminate certain (classes of) compounds or the use of an improper GC column.
Both biological and environmental analysis have their specific difficulties to produce good quality results. For biological samples, the availability of small sample quantities is often the major limiting factor. For environmental samples, the difficulty to perform isomer specific analyses of the toxic fraction (2,3,7,8-substituted congeners) may be a rather limiting factor. At present there is no single GC column known that can resolve all 2,3,7,8-substituted ones from all others [2,3]. In the case the analytical task is to determine the TEQ quantity (i.e. not the total sum of all) repeated analysis on more than one column should be part of the protocol.

3 Recent interlaboratory studies

Results of interlaboratory tests provide information on the comparability of the results produced by different laboratories.The objective of such tests is mostly emphasized on the evaluation of the reliability of a proposed analytical method. The results are usually compared using relative standard deviations (RSD(R)) or standard errors (s_R) providing quantitative information on *between-laboratory* or *interlaboratory reproducibility*. It is generally difficult to evaluate the *accuracy* in addition to the interlaboratory reproducibility, because the determination methods are sometimes conventional and in the case of *spiked samples* compounds added may behave differently to those naturally integrated into matrices. Moreover, most studies do not provide any information on problems related to sampling, which may substantially affect the results of analyses [4]. To assess the current state-of-the-art in the analysis of PCBs, PCDDs and PCDFs, results from a selected number of interlaboratory tests have been studied in more detail. Results from selected studies are presented in Table 1.

At a first look the results compared favourable when the target levels (normally ppt levels for dioxins) are compared to those of other methods for environmental contaminants. A closer examination of the results revealed, however, that particularly for dioxins in biological samples a quite poor between-laboratory comparability can be observed. When a set of samples is send to a certain laboratory, resulting data may be highly reproducible, even on a long term. However, problems may arise when a second set of samples is send to another laboratory. Only for the most abundant congeners and the least disturbed ones (no co-eluting compounds), acceptable agreement can be expected. But even in these cases, between-laboratory differences up to a factor of 2 should be taken into account. Observed between-laboratory differences are mostly associated with the factors mentioned above. Other commonly reported sources of error include the preparation and storage of analyte solutions used for calibration [5,6], errors due to the restricted *linear range* of the electron capture detector frequently used in PCB analysis, and sudden changes, such as the installation of new equipment, or other analysts taking over [7]. Programmes performed in 1988-1994 within the framework of the Marine Chemistry Working Group of the International Council for the Exploration of the Sea (ICES-MCWG) showed that only after a considerable learning process featuring optimizing GC conditions, and improving preparation and storage of standards, laboratories were able to produce comparable results within a reasonable degree of comparability [6,7,8]. Surprisingly, sources of error are not solely associated with *understandable* reasons such as limitations in separation efficiency of stationary phases, but also with trivial basic requirements of Good Analytical Practice like those regarding the preparation and storage of standards.

It is not reasonable to blame analytical chemists only. In some studies, the final result (means, RSD etc.) included all submitted results. In these cases no data could be detected as statistical outliers due to the wide spread in results. A direct consequence of this might be that the study mean does not properly represent the true value. In case this leads to a significant overestimate of the true value, results with good precision and accuracy in the study will be 'punished' by a large deviation from the statistical mean. It is obvious that results from these studies should be disregarded since it gives a false impression of the actual state-of-the-art as well as the performance of the participating laboratories in particular. To prevent frustration among participating analytical chemists, results from round-robin studies should always be evaluated on the basis of analytical-chemical performance and not on the use of statistics only. The reports from the ICES Marine Chemistry Working Group show that there are still some good examples how it should be.

4 References

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Table 1. Results from selected interlaboratory comparison studies.

Matrix	N	Analytes	Concentration (measured)		RSD(r) (%) (s_r)[a]	RSD(R) (%) (s_R)[a]	Reference
Standard solution	9	17 PCDD/Fs	12-54	µg/mL		3.7-15.1	[5]
Standard solution	39	10 PCBs	39-85	pg/µL	(1.04-1.08)	(1.16-1.33)	[6]
Standard solution	37	10 PCBs	21-76	pg/µL		(1.11-1.22)	[7]
Standard solution	31	10 PCBs	20-77	pg/µL		(1.12-1.19)	[7]
Standard solution	27	9 PCBs	72-84	pg/µL	(1.06-1.08)	(1.10-1.12)	[8]
Standard solution	14	6 PCBs				3.1-12.7	[9]
Seal blubber cleaned extract	35	8 PCBs	7.7-247	pg/µL	(1.05-1.17)	(1.24-1.62)	[6]
Sediment cleaned extract	28	6 PCBs	0.78-2.4	pg/µL	(1.11-1.18)	(1.31-1.56)	[6]
Seal blubber uncl. extract	15	10 PCBs	0.56-114	pg/µL		(1.09-5.30)	[7]
Seal blubber oil	23	10 PCBs	12-1996	ng/g		(1.12-4.81)	[7]
Sediment uncl. extract	15	10 PCBs	0.33-2.66	pg/µL		(1.20-1.72)	[7]
Sediment dried	17	10 PCBs	0.49-4.97	µg/g		(1.15-1.33)	[7]
Eel fat extract	14	6 PCBs	0.04-0.3	mg/kg		11-24	[10]
Aroclor 1242/54 (3:7)	14	12 PCBs	2.2-193	pg/µL	2.0-18.7	5.8-86.3	[11]
Aroclor 1242/54 (8:2)	14	12 PCBs	3.3-492	pg/µL	1.9-137.3	9.0-170.9	[11]
Human milk extract	14	12 PCBs	1.2-18	pg/µL	2.7-116.8	4.9-165.6	[11]
Human milk (pool 1)	11	17 PCDD/Fs	25	pg/g[b]		25-243	[12]
Human milk (pool B)	16	17 PCDD/Fs	12.6	pg/g[b]		17-200	[13]
Human milk	10	6 PCBs	0.08-4.1	ng/g		28-240	[13]
Human blood (pool C)	15	17 PCDD/Fs	48.6	pg/g[b]		35-267	[13]
Human blood	6	6 PCBs	0.01-1.4	ng/g		21-153	[13]
Cow's milk	6	17 PCDD/Fs	2.8-10.4	pg/g[b]	2-16	10-17	[14]

[a] s_r and s_R denote standard deviation of the repeatability and reproducibility, respectively, defined according to De Boer et al. [6]. For small values of s_r and s_R (<1.25), the values s_r-1 and s_R-1 may be assumed to be equal to RSD(r) or RSD(R), respectively, within 20% [7].

[b] Level(s) expressed in (i)-TEQ on fat basis.