Cod: 2.1013

CONGENER PATTERNS OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS AS A USEFUL AID TO SOURCE IDENTIFICATION DURING FOOD CONTAMINATION INCIDENTS

R. Hoogenboom[1], R. Malisch[2], G. Ten Dam[1], S. Van Leeuwen[1], H. Hove[3], A. Fernandes[4], A. Schächtele[2], M. Rose[4]

[1]*RIKILT Wageningen University and Research,Akkermaalsbos 2, Wageningen, The Netherlands*

[2]*EU-RL for Dioxins and PCBs, Freiburg, Germany*

[3]*NIFES, Bergen, Norway*

[4]*FERA, York, United Kingdom*

Introduction

According to EFSA, polychlorinated dibenzo-p-dioxins and furans (PCDD/FS), as well as dioxin-like polychlorinated biphenyls (dl-PCBs), are the most important class of contaminants in meat products[1]. Levels in food are still high enough to cause part of the population to exceed the tolerable weekly intake (TWI) of 14 pg TEQ/kg bw. Therefore, strict control by food producers and authorities is needed to ensure that levels continue to decrease by eliminating sources and avoiding introduction of new ones. Examples of the latter are the use of contaminated clay materials or contaminated chemicals in food and feed production, the application of contaminated building materials on chicken farms, or the use of inappropriate fuels for drying of feed and food materials[2].

Once a contamination in food and feed is discovered, it is essential to trace back the origin and discover the source in order to identify potentially contaminated farms, factories/production processes and food products. It has been shown that congener patterns may be very helpful in discovering the source of the contamination[3,4]. Therefore it is necessary to collate representative patterns and make them available to laboratories involved in feed and food control. The EU Reference Laboratory (EU-RL) for dioxins and PCBs established a working group of members of National Reference Laboratories (NRLs) to examine the best way to collect these patterns and to provide a useful tool for comparison and interpretation. As a start, it was decided to write a scientific paper, supported by an Excel file with various types of patterns in primary feed sources.

Materials and methods

The collation of congener patterns was started by several NRLs involved in a number of incidents. Most of these incidents were described in the literature, but often on an individual basis. Patterns in feed or feed materials were preferred above those observed in animal derived products, since such patterns are likely to be changed due to kinetics and metabolism. Such patterns will be dealt with at a second stage. Patterns clearly reflecting a mixed source were not included, since this would not be helpful in the identification of sources in a new incident. Also a number of important incidents in the literature were reviewed and their associated patterns included when available. Eventually, the patterns were collected in an Excel file, including the option to compare a new analytical result with these collected patterns.

Results and discussion

Presentation of PCDD/F patterns for comparison

When comparing patterns of PCDD/Fs it seems obvious to do this based on the contribution of the individual congeners to the sum of the measured levels. An alternative, often described in literature, is the relative contribution to the TEQ level. The latter approach has some advantages. If the pattern is dominated by the octachlorinated congeners, the other congeners will not really be visible. Furthermore, in the case of elevated levels in food of animal origin, a modified profile is obtained as result of kinetics and metabolism. In these cases, a direct comparison between that pattern and patterns from primary sources may not easily lead to a good fit. This is shown in Figure 1 for congener patterns obtained for milk and feed material during the citrus pulp incident in 1998[5]. The pattern based on TEQ contribution clearly gives a much better fit, due to the fact that the higher chlorinated congeners show rather low transfer rates to milk. This can also be overcome by applying transfer rates in the calculations, as will be examined in the future. However, since there is a reasonable correlation between transfer rates and TEFs, a comparison based on TEQ contribution may be an alternative. It should, however, be realized that some congeners with relatively high TEFs may be metabolized by certain species, as is e.g. the case for TCDF and 1,2,3,7,8-PeCDF in pigs and cows.

Figure 1 here

Figure 1. Congener patterns for PCDD/Fs (based on contribution to A: absolute level and B: PCDD/F-TEQ) in citrus pulp, feed prepared with citrus pulp and milk from cows fed with this feed[5].

Presentation of PCB patterns for comparison

In the case of PCBs, the issue is somewhat different. In most cases PCB 126 is toxicologically the most dominant congener and TEQ-based patterns will not be very informative. Furthermore, many laboratories will nowadays include the non-dioxin-like PCBs (no TEF) in the analysis, allowing a wider pattern of 12 dl- and 6 ndl-PCBs. In incidents, the presence of PCBs at significant levels, rather than the pattern, will be helpful to point out PCB oil as the likely source of the PCDFs. An exception may be the use of the PCB pattern to prove that the incident was actually caused by a particular material, as e.g. in a court case or for insurance issues. Again, for food of animal origin the application of transfer factors may be helpful, as not all PCBs are metabolized to the same extent.

Selection of significant PCDD/F congeners

For tracing sources in feed and food, first, it has to be checked how many congeners are elevated in comparison to usual background levels. In most cases, from 17 PCDD/Fs with 2,3,7,8-substitution routinely determined in feed and food, only a limited number is significantly elevated and can be used for source identification. However, also the absence of specific congeners offers important information for identification of the source. The combination may be a suitable starting point for the development of a decision support system that can assist I pointing out the potential source. In addition, also other non-2,3,7,8 substituted congeners may be helpful, but these are not routinely identified and quantified.

PCDD/F patterns collected

The working group thus far collected patterns from the following sources and incidents2:

1. PCBs (Belgian and Irish incidents)
2. Burning of waste materials (including citrus pulp)
3. Chlorophenols (sawdust, guar gum, fatty acids Germany)
4. Clay materials (ball clay, kaolinite, mabele)
5. Minerals (sequestered minerals, zinc oxide)
6. Others, like in the incident with gelatine

Figure 2 shows a flow chart to discriminate the various patterns, based on TEQ contribution.

Figure 2 here

Figure 2. Flow chart for identification of sources based on the congener pattern (based on TEQ contribution). Examples from PCBs in feed, zinc oxide in feed and kaolinic clay.

1. If primarily PCDFs, the most likely source is PCBs. The pattern may vary depending on the chlorination grade of the original PCB mixture, with high contribution of TCDF in case of a low chlorinated mixture, and a shift to PeCDFs in case of higher chlorination. Since most laboratories routinely analyze for PCBs, it will be easy to check whether this is the source. It is important that information on PCBs is provided in the analytical reports provided e.g. to the EU in case of rapid alerts.

2. If primarily PCDDs, there are roughly two options. One is the use of contaminated clays, with TEQ based patterns that are in general dominated by PeCDD and to a lesser extent TCDD, HxCDDs (in particular 1,2,3,7,8,9 HxCDD) and HpCDD. On an absolute basis, OCDD is by far the most important congener. However, also incidents with chlorophenols show only PCDDs. Depending on the chlorination grade there is a shift from primarily TCDD, as in the Seveso incident and agent Orange (trichlorophenol, 2,4,5-Trichlorophenoxyacetic acid), to primarily HpCDD, as occurred with materials contaminated with pentachlorophenol. In the German incident in 2010/2011, an intermediate pattern with high contribution of 1,2,3,7,8,9-HxCDD was observed, but also the presence of relative high levels of tetrachlorophenol.

Checking for chlorophenols may be an alternative to decide between clay materials and a pattern based on tetrachlorophenols, although the patterns can be distinguished.

3. A mixed PCDD/PCDF pattern can be caused by different sources. The pattern dominated by the PeCDD/Fs and to a lesser extent TCDD/Fs points in the direction of burning of chlorinated plastics (PVC). Depending on the materials involved there may be a higher dominance of either the PCDDs or PCDFs. A mixed pattern is also observed in the case of certain minerals, such as recycled zinc oxide and the so-called sequestered minerals, that were produced by heating minerals in the presence of kelp. These mineral based patterns in general show much higher contributions of the HxCDFs and can as such be distinguished from the patterns caused by burning.

4. A fourth category involves profiles containing only one, or maybe two 2,3,7,8-substituted congeners, as in the gelatin incident in 2006, but also in a more recent incident in Chile (2013). Such patterns are probably related to the production of specific chlorinated chemicals, but these chemicals were never identified. However, it also applies to trichlorophenol and 2,4,5-T, which show only TCDD in the congener pattern. It is important to analyze such products with untargeted techniques like TOFMS.

The currently collected patterns are a first start to develop a database for assisting authorities and food producers in tracing the source of a contamination. These patterns are collected in an Excel file that allows comparison of a new test result based on the congener profile of both absolute as TEQ based levels. Future activities will be directed towards the inclusion of transfer factors (carry-over factors) in relevant animal species to further improve the database. In addition, the possibility to develop a decision support system will be investigated.

Acknowledgements
The authors would like to acknowledge the contribution of the various NRL laboratories within the EU.

References
1. EFSA (2012) EFSA Journal, 10(6), 2741.
2. Hoogenboom, L.A.P., Traag, W.A., Fernandes, A., Rose, M. (2015) Food Control, 50, 670-683.
3. Malisch, R., and Kotz, A. (2014) Science of the Total Environment, 491-491, 2-10.
4. Hoogenboom, R., Zeilmaker, M., Eijkeren, J. van, Kan, K., Mengelers, M., Luykx, D., Traag, W. (2010) Chemosphere, 78, 99-105.
5. Malisch, R. (2000) Chemosphere, 40, 1041-1053.

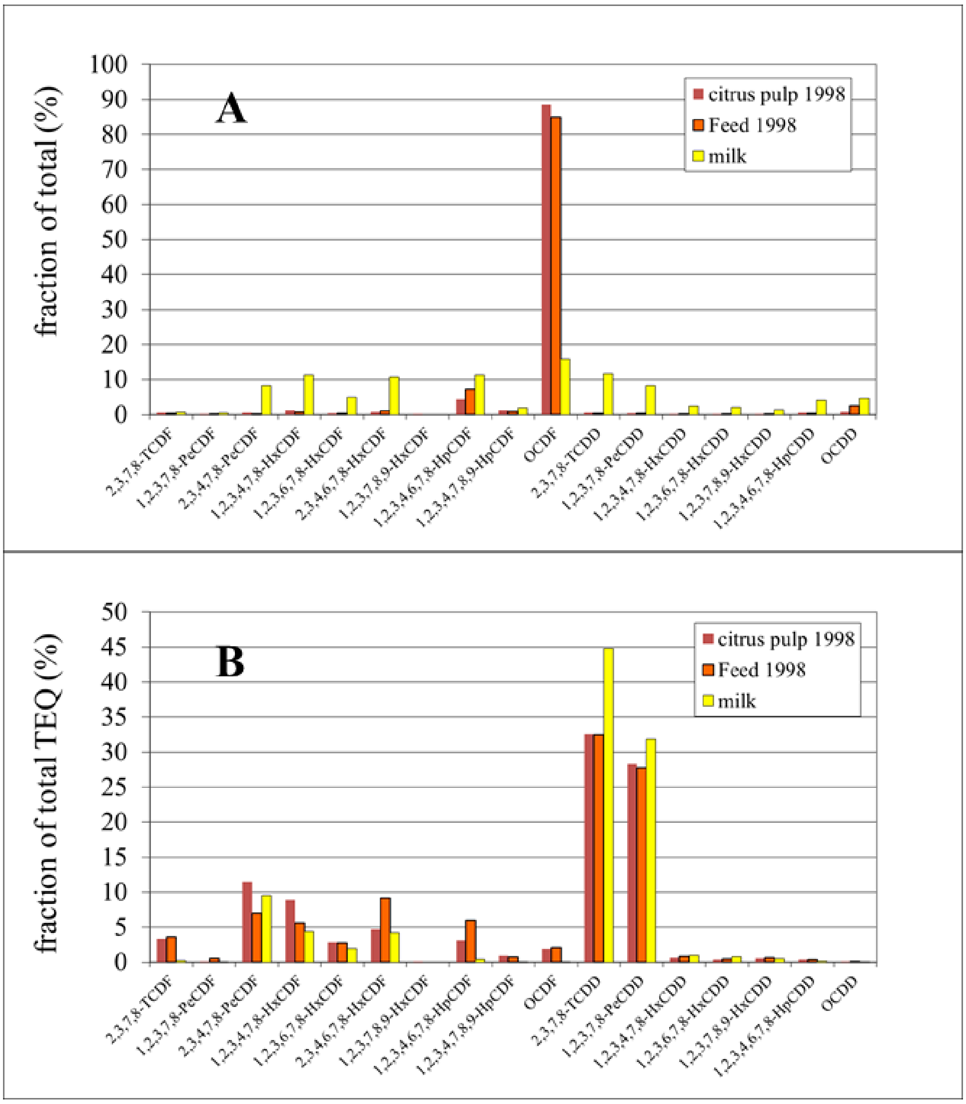
A
fraction of total (%)
citrus pulp 1998
Feed 1998
milk
100
90
80
70
60
50
40
30
20
10
0
2,3,7,8-TCDF
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF
OCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD
OCDD
B
fraction of total TEQ (%)
citrus pulp 1998
Feed 1998
milk
50
45
40
35
30
25
20
15
10
5
0
2,3,7,8-TCDF
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF
OCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD
OCDD

Figure 1

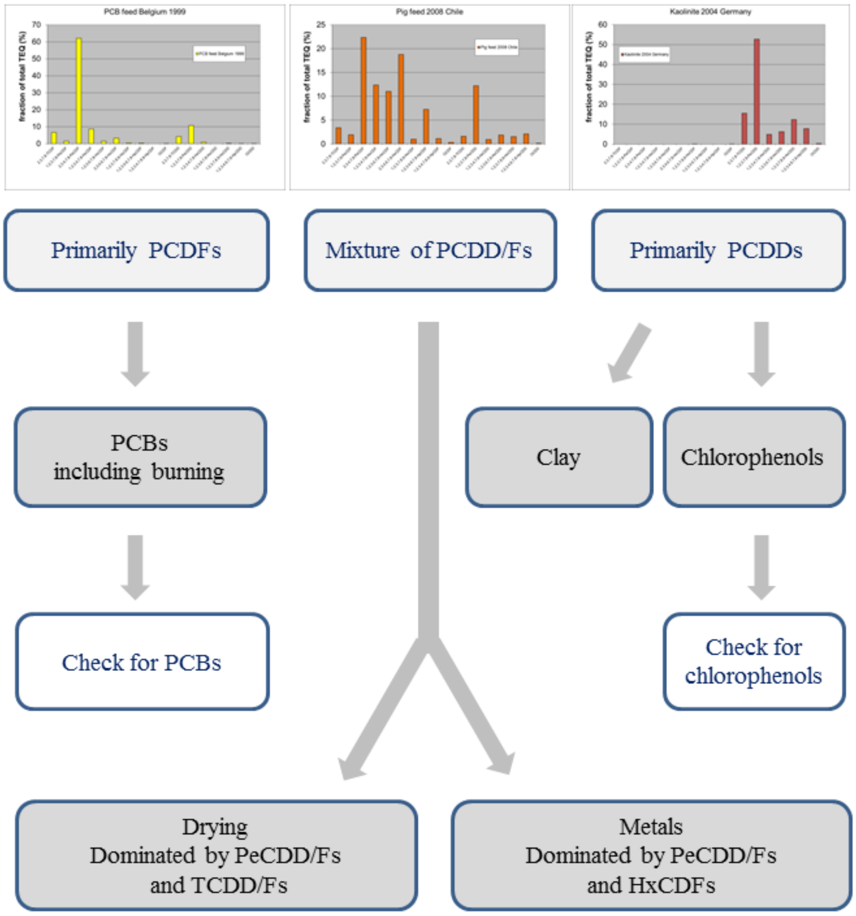
PCB feed Belgium 1999
Pig feed 2008 Chile
Kaolinite 2004 Germany
Primarily PCDFs
Mixture of PCDD/Fs
Primarily PCDDs
PCBs
including burning
Clay
Chlorophenols
Check for PCBs
Check for
chlorophenols
Drying
Dominated by PeCDD/Fs
and TCDD/Fs
Metals
Dominated by PeCDD/Fs
and HxCDFs

Figure 2