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The dioxin problem

(Abstract of the lecture given by Mr Fraisse at CECALAIT's Annual General Meeting 2000)

Structure and chemical properties

Dioxins, or polychlorinated dibenzo-*p*-dioxins or PCDD are a family of molecules based on the structure given in fig. 1, p. 1 in La Lettre de CECALAIT. The two benzene molecules may be substituted by Cl atoms at 8 different places, thus building a family of 75 congeners. The word « dioxin » also refers to another family : the furans or polychlorinated dibenzofurans or PCDF, with a relatively similar structure (cf fig. 2, p. 1 in La Lettre de CECALAIT). These molecules may also be substituted, building a family of 135 congeners.

Among these 210 (75 + 135) different molecules, some are 2-3-7-8 substituted. Their very planar and stiff structure gives them an extreme resistance towards chemical and biochemical processes. Consequently they persist in the environment and bioaccumulate in the food chain. 10 furans and 7 dioxins, called the dirty dioxins, have that conformation, in particular the most toxic and studied congener, 2,3,7,8 TCDD – *Seveso dioxin*-.

These molecules are not very soluble in water (the more Cl atoms, the lower solubility), not very volatile and have a high chemical and thermic stability. They are lipophilic, soluble in organic solvents and degraded by UV.

Origin

Dioxins are formed during combustion processes of organic products in the presence of chlorine : volcanic eruptions, forest fires and mostly domestic waste incineration. Moreover, they are unwanted byproducts of industrial processes : preparation of herbicides and bactericides, bleaching of paper pulp. Finally, they may also be produced accidentally in chlorophenol preparation (Seveso accident) or when strongly heating polychlorinated biphenyls (PCB) widely used industrially because of their dielectric properties.

They are found in the air, water and earth. In the air, their half-life is a few days, but after deposition, they may contaminate waters and earth, where their half-life is about 1 to 3 years on the surface and more than 10 years when below. Accumulation in plants was never observed, but it is the case in seaweed. Animals and man are contaminated mainly through food and dioxins tend to be adsorbed in fatty tissues. The evaluation of human exposure considers either all possible environmental sources or, as exposure from food is the main pathway –90%-, dietary intakes.

Toxicity and human exposure

The toxicity of dioxins may come from several, still unknown ways. At the biomolecular level, there is, however, one well-known way : dioxins enter the cell and bind to an arylhydrocarbon receptor. This complex can then bind to a dioxin-responsive element on the DNA, which results in altered gene expression.

A broad spectrum of toxic and biochemical effects was observed in laboratory animals, especially for chronic exposure. TCDD is a carcinogenesis promoter, but sensitivity depends on species (for example, guinea pigs develop liver tumours whereas rats remain unaffected) and sex (for example, female rats develop liver tumours faster than males).

For human health, subtle biological and biochemical alterations may occur after low exposure, but their clinical significance is not yet known. Exposure to high levels, observed after industrial accidents (Seveso explosion) or accidental food contamination (Yusho rice oil) result in cloracne, neurological, developmental reproductive effects and increased cancer risks.

The general population is currently exposed to levels of dioxins several magnitudes lower than accidental levels, but exposure is always linked to complex mixtures of different dioxin congeners. So the concepts of TEQ (toxic equivalency quantity) and TEF (toxic equivalency factors) have been developed to facilitate risk assesment. The value of the TCDD TEF, the most potent and well studied dioxin congener, is 1. Other TEF are evaluated on the basis of toxicological database. TEQ of a given sample is calculated by the following expression :

$$\mathbf{ITEQ = \Sigma\ TEF_i \times C_i}$$

where C_i is the concentration of congener i.

TEF of the 17 dirty dioxins range from : 1 for 2,3,7,8 TCDD and also for 1,2,3,7,8-PeCDD** (pentachlorodioxin), to 0.0001 for 1,2,3,4,6,7,8,9 OCDD (octachlorodioxin) or from 0.5 to 0.0001 for furans.

** *in the WHO 1997 list.*

TEF schemes are regularly revised. For example, EPA 1987 or NATO 1988 lists included only PCDDs and PCDFs and the TEF value of pentachlorodioxin was 0.5. However, since 1997, WHO and EPA recommend the inclusion of some dioxin-like PCBs (cf fig.3, p.3 in La Lettre de Cecalait). This is a group of 14 molecules (among the 209 PCB congeners) which have a coplanar conformation similar to PCDD or PCDF and also bind to arylhydrocarbon receptors. The TEF of PCBs are far lower than those of the PCDDs, but as their concentrations in the

environment are higher, they are likely to contribute significantly to the TEQ.

For the next revision (2003), WHO also recommends the inclusion of polybrominated dibenzodioxins.

Now, WHO recommends a tolerable daily intake of 1 to 4 pg/kg of body weight/day. This is calculated for a chronic exposure, ie a daily intake for a life for a 70 kg adult.

Determination of dioxins

It is well-known that it requires special expertise and sophisticated instrumentation. The most specific and sensitive method is gas chromatography coupled to high resolution mass spectrometry. For quantification, isotopic dilution with ^{13}C analogs is necessary.

Isotopic dilution is adding a known mass of a labeled compound to an unknown mass, to be determined, of the same native compound. This technique is recommended when high losses of the analyte may occur during extraction and purification steps. Indeed, both the native and the labeled compound will come through the same steps and have the same losses. Usually, samples are spiked with a mixture of the 17 ^{13}C labeled « dirty dioxins » immediately after grounding, sieving or homogeneization. But , for high-fat food, including dairy products, fat is spiked after extraction.

Fat contaminants are then separated and concentrated, either through different chromatography columns or through an activated charcoal column, which is longer, but enables to purify high fat quantities (about ten grams or more) and to detect very low dioxin concentrations.

Further cleaning up, especially for the removal of non « dioxin-like » PCBs requires other chromatography column steps. The final fraction should only contain PCDDs, PCDFs and some « dioxin-like » PCBs and is analysed by high resolution gas chromatography coupled to high resolution mass spectrometry. Any accidental contamination of samples or reagents must be avoided. The fraction is spiked with another labeled standard, as a recovery standard prior to the gas chromatography, in order to check that the losses are under 50%.

Chromatographic profiles are obtained for native and labeled congeners, allowing the calculation of the initial concentration of each congener. Each is then multiplied by its corresponding TEF, and the sum leads to the TEQ of the tested compound.

There are other analytical methods, but which are not as sensitive. Screening and determining some PCBs, which is generally easier and faster than dioxin determination, as tracers of dioxin contamination is valid only if the source of the dioxin contamination is PCBs. Otherwise, no link between PCB and dioxin concentration could be shown.

The only current European standard is for dioxin determination in emissions. An ISO standard for determination in food is under preparation based upon the EPA 1613 method. Meanwhile, the method used for any dioxin determination must be carefully described.

Over the last few months, in several countries, there was a high concern with the estimation of dietary intakes of dioxins, resulting in several reports : one issued by the European Commission, 3 reports in France …. (see bibliography).

abbreviations

NATO : North Atlantic Treaty Organization
PCB : polychlorinated biphenyl
PCDD : polychlorinated dibenzo-*p*-dioxin
PCDF : polychlorinated dibenzofuran
WHO : World Health Organization

For other abbreviations and bibliography, please see page 4 in La Lettre de CECALAIT n° 35