POPs : Stay Off! Stay Away! Stay Alive!

Human Exposure and Food Control

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Humans all over the world are exposed to chemicals during their lifetime. Among the thousands of existing anthropogenic compounds are the persistent organic pollutants (POPs), of which polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are some of the most toxic representatives. Although peak exposure to those anthropogenic chemicals happened in the 1970s, PCDDs, PCDFs, and PCBs are ubiquitous and can, still today, be found in virtually any matrix from any origin on our planet. For human beings, consumption of food of animal origin represents the major route of exposure. If a population is exposed to levels below the estimated tolerable monthly intake (TMI), part of that population may still exceed the TMI because of the lifetime bioaccumulation of background levels that are present in what we eat. Controlling human exposure, therefore, translates to a matter of food control. This results in the implementation of strict regulations based on the setting of maximum residue levels (MRLs) for PCDDs, PCDFs, and PCBs in foodstuffs, and also in feed for animals, such as cattle.

Implementing food/feed control is easier to say than to timely and properly perform. All together, depending on the position and number of chlorine atoms present in the molecule, PCDDs, PCDFs, and PCBs represent more than 400 individual molecules (congeners). Only a subset of 29 molecules is of prime interest, and those molecules have to be separated from each other, and other congeners, to ensure distinctive quantification and proper estimation of the global toxicity of the mixture (TEQ, toxic equivalent approach). Target levels are as low as pico- or femtogram per gram of matrix, with matrix-related interferences present in concentrations that are orders of magnitude higher than the analytes of interest. For those reasons, a complex multi-step approach is required to 1) extract the analytes from the matrix core, 2) separate undesirable interferences, and 3) isolate, separate, and quantify analytes of interest. This results in long processing times that are not acceptable in the context of food/feed control, as foodstuffs cannot stay under inspection for long periods of time without generating unaffordable economic losses. More importantly, food safety agencies need fast response strategies to deal efficiently with potential dioxin contamination incidents like the one that recently happened because of feed/fat contamination at some feed manufacturers in Germany in late 2010.

Because the proper management of dioxin incidents must be pursued with urgency and effectiveness, accredited reference laboratories must perform with a certain level of automation. Once alert systems trigger a potential issue, the precautionary principle is enforced and usually results in blocking a few thousand farms, again with tremendous economic issues. Proper traceability mechanisms and liberation of non-incriminated farms can only start if full analytical data sets become available. It practically means hundreds of complex sample matrices to be analyzed in matter of days, if not hours. As much as networking of national reference laboratories ensures broad geographic screening and large capacity, coupling and hyphenation of the various analytical steps ensures rapidity. For example, pressurized liquid extraction (PLE) coupled to automated solvent reduction-exchange devices can produce sample extracts that are further cleaned up in-line via a multi-step liquid chromatography (LC) setup. Such an LC setup includes multi-layer silica columns (acid, neutral, basic), basic alumina columns, and other columns containing, for example, carbon dispersed on Celite. Those sorbents allow the separation of the cleaned extract in subgroups of compounds (PCDDs, PCDFs, PCBs) depending on their polarity and geometry. The fractionated extracts can be further evaporated using the hyphenated solvent reduction-exchange device to satisfy the required concentration factor and, then, transferred to GC injection vials for separate GC/MS determinations. Following such approaches, classical throughputs for medium size laboratories are easily up to the production of 20 reports per day, including the congener-specific data (29 analytes) necessary for patterning and source identification.

In the past few years, efforts also focused on the development of alternative measurement tools, namely gas chromatography coupled to mass spectrometry (GC/MS), to speed up and simplify the process while maintaining a high level of quality assurance/quality control (QA/QC). As always, it is, however, a matter of compromise. If you want to go away from high resolution sector instruments (HRsectorMS), you have a good chance of success by shifting to tandem-in-space MS on triple quadrupole systems (qqqMS/MS). The loss in mass resolution is, in that case, at least partially compensated by the gain of specificity due to the monitoring of product ions. The low fg level instrumental limits of detection (iLODs) of HRsectorMS can be maintained when using qqqMS/MS. You rather want to improve your separation and thus simplify the fractionation step? Then go for comprehensive two-dimensional gas chromatography (GCxGC), where separation and peak capacity are enhanced. However, because of cryogenic zone compression (CZC) during modulation, peaks become very narrow and cannot be properly reconstructed using slow-scanning reference HRsectorMS instruments that are already operating in selected ion monitoring mode (SIM) to ensure high sensitivity for target analyses. Then, alternative analyzers like time-of-flight TOF-MS or fast scanning quadrupole qMS can be used, but at the price of losing some sensitivity (low pg iLODs) and mass resolution (typically unit mass in TOF and q). The full mass range data acquisition mode of TOF-MS however opens the possibility to screen for other compounds in addition to those under current regulation (organochlorine pesticides, brominated flame retardants, perfluorinated chemicals, e.g.), without additional work or sensitivity sacrifice. An interesting recent coupling between GCxGC and HRTOF-MS nicely puts this approach one step further, as it allows acquisition of elemental composition data. Furthermore, such a system offers a tremendous improvement in sensitivity (back to low fg iLODs) by operating with negative chemical ionization (NCI), rather than electron ionization (EI). If you ultimately search for the most sensitive instrumentation, go for CZC on sector instruments. In fact, due to mass conservation, CZC not only reduces peak widths but also increases peak heights. Coupling CZC to HRsectorMS instruments thus allows low ag iLODs. Because of dwell time issues related to limited electric jump frequency, the sector instrument is pushed to its limits in terms of scan rate. This potentially impacts the performance of the analyzer that is already working at the ag level, where the number of molecules actually reaching the detector starts to be a limitation for proper ion statistics to take place. CZC-GC-HRMS could nevertheless be the perfect tool for human biomonitoring using very small volumes of sample. How appealing would dioxin analyses in a dried-blood spot (DBS) be?!

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Although so-called 'dioxin analyses' have been performed since the mid-1970s, the need for controlling our exposure is still a current imperative. Analytical procedures have evolved to make such measurements more rapid and straightforward. Improving sensitivity and expanding the task to other emerging compounds are probably the two most important challenges now. Several alternative approaches do exist and offer some interesting features, but none of them can, so far, respond to both challenges. The analytical chemistry of POPs has still some work ahead.

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