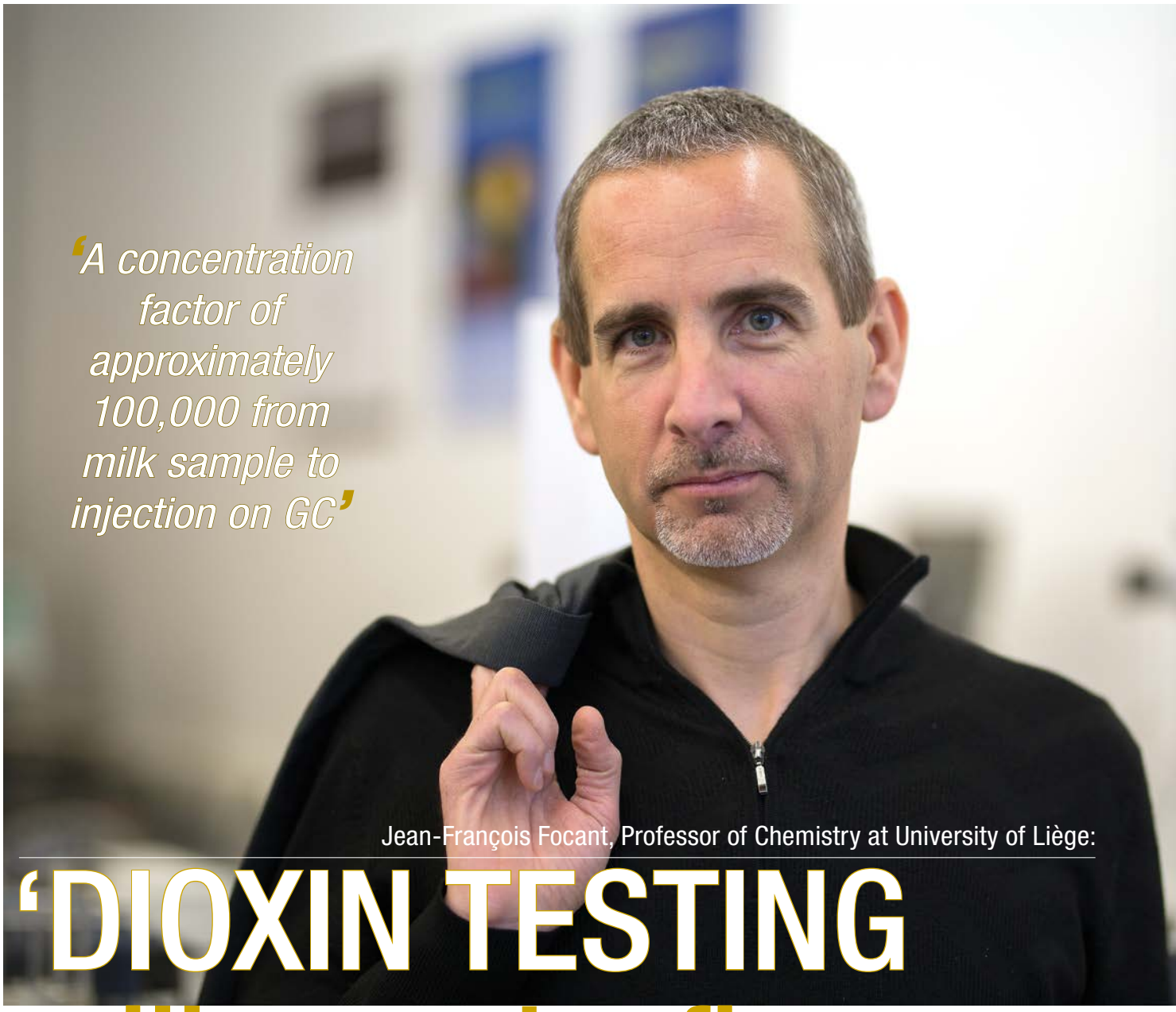

Jean-François Focant, Professor of Chemistry at University of Liège:

'DIOXIN TESTING will soon be five times more sensitive'

Some of the world's leading research into dioxin analysis is happening at the University of Liège Faculty of Sciences, where Chemistry department head Jean-François Focant and his team are spearheading studies on dioxin testing using triple quad GC-MS. This method will enable labs to meet the stricter analytical standards the EU is set to impose.

University of Liège Professor Jean-François Focant: "We're confident we'll soon be able to detect these ultra-low level traces of dioxin, at least in some matrices."

Dioxin affair

Focant is one of the top seven authorities in dioxin analysis worldwide. He is modest about this accomplishment: "I sort of stumbled on the dioxin problem during my PhD in 1999 and 2000. Dioxin had been present in food and feed for 35 years, but it suddenly erupted into a big health scare when dioxin-contaminated chicken meat and eggs were found in Belgium. At an oil-and-fat-recycling plant, transformer oil was mixed in with other fats, and in this way contaminated the fat used in animal feed with PCBs. The ensuing health scare seriously affected consumer trust. An extensive recall of chicken meat and eggs followed. In the middle of my PhD track I had to start measuring dioxins and PCBs for the Belgian ASCFA-FASFC, the Federal Agency for the Safety of the Food Chain. We had no choice but to automate the analysis straightaway, because there were thousands of samples waiting to be analyzed. That was a steep learning curve!"

Femtogram level

The presence or absence of dioxins can be determined using bioassays, but quantitative analysis can only be done by GC-MS. Once a rapid test has established the presence of dioxins, this must be confirmed by further chemical analytical tests. GC-MS analyses can determine about 30 different dioxin compounds, says Focant. "You have to do a separate analysis for every compound. In terms of molecular weight, dioxins are in the 300 to 500 range, which classifies them as slightly to moderately volatile and means they can be separated using gas chromatography. The problem is that they are lipophilic, so you have to extract them from the fat fraction. We can now detect dioxins at the pico and femtogram level, but regulators are tightening the maximum permissible concentrations. As stricter regulations are adopted, we need to detect smaller and smaller trace levels. And the guidelines might be tightened even further in the future, so we need to make sure our detection methods are not just adequate for today, but future-proof too."

'Stringent dioxin regulations are causing quite a buzz in the European reference lab network'

Triple quad GC-MS is used more and more to replace magnetic sector-mass spectrometry, which was the only authorized method for dioxin determinations until about five years ago. Even Focant's lab still uses these enormous machines. Their performance is excellent, but analysts need specialist knowledge to use them and they have a huge footprint. By contrast, a triple quad-mass spectrometer fits on a lab bench, costs half, is more user-friendly, and works just as well as magnetic sector MS. Focant's research group has been working on triple quad analytical methods for years. Initially, they developed pesticide analytical methods, later they focused on dioxins. Dioxin determination poses additional challenges in terms of sensitivity. Focant's group has developed methods for carrying out high-throughput dioxin analyses in the femtogram range. Several of these have been adopted by the Center for Analytical Research and Technology (CART) in Liège, which carries out contract research.

Cleanup

There is little room for improvement in the pre-analytical processing of samples. Protocols have been standardized. The Focant group uses an automatic fat extraction system with a FMS cleanup system for solid phase extraction. During sample prep, the original sample volume is concentrated using prepa-

TRIPLE QUAD VS. MAGNETIC SECTOR MS

Postdoc Flavio Franchina with Jeol's triple quadrupole mass spectrometer..

Triple quadrupole mass spectrometry is not exactly a new technology. According to the Journal of the American Chemical Society, the first commercial triple quad MS was developed at Michigan State University in the late 1970s. However, affordable benchtop models have been around only for the last couple of years. All major manufacturers are eager to jump on the bandwagon in this emerging market. Jeol and Thermo are the only manufacturers still making sector-mass spectrometers for a fast-shrinking market. One of the problems formerly associated with triple quad technology was that too few ions reached the detector, limiting its sensitivity. Improved ionization techniques have significantly increased its sensitivity. Triple-quads can now compete quite well with magnetic sector mass spectrometers, which are not only twice as expensive, but also bulky and difficult to operate.

Magnetic sector-MS is still more sensitive than triple-quad-MS, but the gap is closing quickly, according to Professor Jean-François Focant, head of the Chemistry department at University of Liège's Faculty of Sciences. Magnetic sector-MS still outperforms triple quad MS in simultaneous, high-sensitivity determination of several compounds, but this is relevant mainly in human biomonitoring, where no regulatory limits apply and detection levels have to be ultra-low. Triple quads are sensitive enough for 'target analysis', where maximum permissible concentrations have been defined for food and feed. By programming the mass spectrometer for the monitoring of specific ions, which are specifically fragmented, triple quad MS can be used for highly specific product ion analysis. This compensates somewhat for the lack of spectral resolution and creates adequate sensitivity for detecting dioxin compounds at pico and femtogram levels. Focant's lab is also experimenting with changes to the analytical method to make it suitable for next generation triple-quad MS. One of the lab's tricks is working with large-volume injection—5µl instead of 1-2 µl—which can help to detect the lower limits for dioxins in food and feed that are in the offing when the EU next revises its dioxin guidelines.

rative liquid chromatography. "You start with 50 to 100 ml of sample, for example whole milk. After extraction you end up with 2 to 3 grams of fat, which contains the contaminants. Eventually you end up with a dioxin concentration of 5 micrograms per liter, which amounts to a concentration factor of approximately 100,000 from your milk sample to injection of your sample on GC."

Affordable

Until about five years ago, magnetic sector MS was the only authorized analytical method. Once triple quad-mass spectrometry came onto the scene, it became clear that a second method would be authorized. In the run-up to his professorship, Focant researched various hybrid platforms to develop new dioxin analytical methods. "As you can see, we still have two magnetic sector mass spectrometers in use, but they're gigantic, very expensive and difficult to operate. Until recently, a dioxin determination cost about 1000 Euro. Now we can do it for 250. Streamlining sample prep and using less expensive next-generation automated systems and triple quad has made things much more affordable."

Method development

Focant had been working with Jeol's flight time-mass spectrometer for research into the analysis of volatile degradation products resulting from diseases and decomposition. When Jeol developed a new hybrid platform for GC-triple quad-MS, the JMS-TQ4000GC, they asked Focant to test and validate the system. "Initially they asked us to test and validate the system for a list of pesticides in Japan. We showed that the instrument did what it had to do and scored well on sensitivity. It took six

Top: Brazilian PhD student Eliane Lazzari and Italian postdoc Flavio Franchina working on developing an analytical method using Jeol's JMS-TQ4000GC..

Middle: FMS cleanup system used to extract target analytes from fat.

Bottom: Heidolph evaporators concentrate the dioxin samples after cleanup..

months for Flavio Franchina, one of my post-docs, to complete the project. Then we hit upon the idea of using the platform to develop an analytical method for dioxin testing for validation in the EU market. We had already gained some experience with validations using an Agilent triple quad, though I must admit this is easier for pesticides than for dioxins. The detection limits for dioxins are much lower. The instrument had to be able to detect quantities of 10-12 to 10-15, so in the pico to femtogram range. We already knew that the platform was very sensitive and now Eliane lazzari, a Brazilian PhD student here, is working on validating this triple quad for food and feed according to EU regulations. We will really put the instrument to the test, to make sure the equipment meets all requirements. We are now at 95%, but it's going to take quite a bit of QA-QC to get to the one hundred percent mark."

Magic number

Demonstrating that the device is fit for purpose is essential, Focant stresses. "If a ship docks in Antwerp and is waiting to see whether it is allowed to unload its cargo, the test results have to be absolutely unambiguous. Missing a dioxin contamination can have enormous consequences. If it gets into animal feed, it's going to contaminate the livestock and end up in consumer products. A consumer product recall is a major operation, not to mention how it affects the population and public confidence in food safety."

Focant expects the dioxin validation to be finished by May 2019. He hopes to present the results at the annual dioxin conference, the Symposium on Halogenated Persistent Organic Residues, whose 39th edition will take place in Kyoto this year. "Obviously, it is very important to Jeol to be able to show that the method has been validated." Based on the analytical results so far, Focant is confident that the method will be sensitive enough to detect the lower dioxin limits set by the EU. "The maximum permissible concentration is currently 2.5 picograms per gram of milk fat. This magic number has been retropolated to the number of people who can ingest this concentration without adverse health effects, or in other words, human weekly permissible intake. The EFSA is proposing to reduce this WPI by a factor of 5 or 10. Every dioxin lab in Europe is sweating about whether they can meet this reduction, but we think we're almost ready to detect these ultra-low level traces, at least in some matrices. In any case, this is causing quite a buzz in the European reference lab network."