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Labinfo

Newsletter for the approved food safety laboratories

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NRL NATIONAL REFERENCE LABORATORIES

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Newsletter for the approved food safety laboratories

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Labinfo Editorial

Dear reader,

As announced in the previous issue, Labinfo has now become an annual publication which will be published only in English. Labinfo n°18 for the year 2019 is thus the first issue in English only.

The years go by, but nothing much seems to change. In 2018 and early 2019, the Agency was once again confronted with different crises or incidents such as African swine fever, avian influenza and the detection of Xylella in olive trees imported from Spain. As in 2018, the Agency could count on the support and collaboration of the NRLs and some other approved laboratories. I would like to thank all the parties involved for their support.

As you are also aware, the political situation in Belgium has become quite complex following the May elections. We are consequently still waiting for a new government and a new minister. We are looking forward to their food safety policy and to knowing what resources we will have available to accomplish our mission.

A new European regulation (EU 2017/625) on official controls on food safety, the OCR (Official Control Regulation), will be partially implemented from 14 December 2019. Some points concern the designation of official laboratories and NRLs (National Reference Laboratories). Our Royal decree on "approvals" already contains many of these requirements, including the ISO 17025 Accreditation (only applicable from 2022), but this is an opportunity to start a revision of our decree. A new version is being prepared and will be published in 2020.

The articles published in this issue cover a variety of subjects: the research projects Specenzym and Metrofood, antibiotic residues in the agricultural environment, alkaloids and mineral oil in foodstuffs.

I hope you will enjoy reading this eighteenth issue of Labinfo.

Bert Matthijs
Director-general of DG Laboratories

Antibiotic residues and resistance genes in the agricultural environment: One Health in practice

Rasschaert G., Daeseleire E., Heyndrickx M.

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO)

Belgium has one of the highest livestock concentrations, which results in a correspondingly large amounts of animal manure. The raw manure, mainly from cattle and pigs, is often used as fertilizer on grasslands and fields of maize, cereals, potatoes and vegetables (VLM, 2018). In addition to environmental problems associated with the use of manure, concern is growing about the dissemination of antibiotic residues and antibiotic resistance genes in the environment. The occurrence of antibiotic resistance is a natural phenomenon, but enhanced by the use of antibiotics as antibiotic resistance is selected under antibiotic pressure.

This is especially problematic in Belgium, as the antibiotic use in Belgian livestock farming is high compared to the European average (Chantziaras et al., 2014; ESVAC, 2016). Various studies have shown a correlation between antibiotic use in livestock animals and the degree of antibiotic resistant bacteria in the intestines of these animals (Aarestrup et al., 2008; Dunlop et al., 1998; Dewulf et al., 2007). Such resistant bacteria carry DNA coding for resistance to one or more antibiotics, the so-called antibiotic resistance genes. In this way, these bacteria can act as a reservoir for antibiotic resistance genes.

These antibiotic resistant bacteria may be pathogens such as Salmonella that may cause disease in animals or humans. But most often, harmless bacteria such as most strains of E. coli can carry such antibiotic resistance genes. When these bacteria reach the human gut flora, they can transfer their resistance genes to other gut related bacteria - including pathogens - which can lead to problems with the antibiotic treatment of infectious diseases.

Besides the direct transfer of antibiotic resistant bacteria from animals to humans via direct contact with the animal or via the consumption of undercooked meat, there is also an important indirect route via the environment, for example via the manure used to fertilize agricultural lands. Immediately after fertilization, most of the bacteria present in the soil originate from manure, which shows that the bacterial density in manure is extremely high. The manure-associated bacteria die off quickly as they cannot survive long outside the intestinal environment, but before dying they can possibly transfer their resistance genes to other bacteria (Leclercq et al., 2016).

Manure used as fertilizer on the arable fields may also lead to fecal contamination of vegetables and crops and possibly also of the groundwater and watercourses, each possessing their own microbial community (Radhouani et al., 2014). These niches are all in close contact with each other, and can be considered as a melting pot where antibiotic resistance genes can be exchanged (One Health principle, Figure 1).

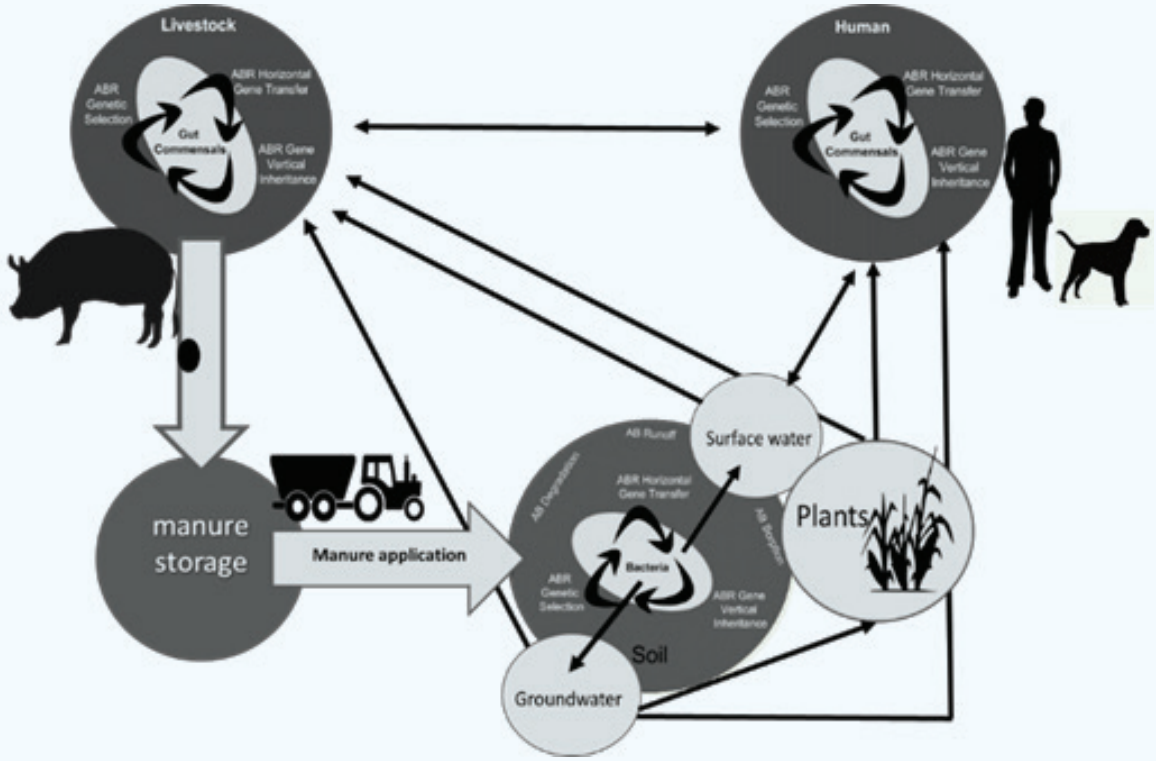

Figure 1: Possible transmission routes of antibiotic residues and resistance genes between humans and animals (adapted from Chee-Sanford, 2008).

In addition to selection for antibiotic resistant bacteria via the use of antibiotics in the animal itself, selection may also occur outside the animal, such as in the manure pit, in (manure-)fertilized soil, in watercourses or on the plant. When animals are treated via antibiotics, antibiotic residues are excreted via the feces or urine, and these residues may also end up in the environment.

Furthermore, some studies have demonstrated that low antibiotic concentrations may already select for resistant bacteria, even if those concentrations are lower than the threshold values used for specific bacterial species to distinguish resistant strains from sensitive strains, called the "minimum inhibitory concentration" (MIC) for that bacterial species. These resistances may remain present in the bacteria even after the selective pressure is released (van der Horst et al., 2011).

A recent Belgian study, carried out by ILVO on behalf of Flanders Environmental Agency (VMM), showed that in 96 out of 100 samples from pig and calf manure, between 1 and 15 antibiotic residues could be detected. The antibiotic residues doxycycline, lincomycin and sulfadiazine were most frequently detected.

The antibiotic residues neomycin, oxytetracycline, doxycycline and sulfadiazine were found in the highest concentrations, meaning above 1000 µg/kg of manure (Rasschaert, 2018). In addition, the degree of antibiotic resistance in the indicator organism E. coli was quite high; resistance to 12 antibiotics within one E. coli strain could be detected. A second study (Van den Meersche, 2019), examined the length of time that certain antibiotic residues and resistance genes could be detected in the soil after fertilization. This was dependent on both the stability of the antibiotic itself and the soil type.

For example, doxycycline could already be detected in clay soil before fertilization of the soil (30-50 µg/kg soil). After fertilizing this soil with manure containing 1600 µg of doxycyline per kg of manure, the doxycycline concentration in the soil did not increase significantly and remained more or less constant over the study period of four months.

It was also noteworthy that doxycycline was only detected in the upper 60 cm of the soil, indicating a strong binding between doxycycline and the clay particles. In sandy-loam soil, on the other hand, no doxycycline was detected before fertilization. After fertilization with manure containing approximately 10,000 µg of doxycyline per kg of manure, the doxycycline concentration increased significantly in the soil and there was a 100-fold reduction in the concentration four months after fertilization.

Remarkably, doxycycline could be detected deeper in sandy-loam soil than in clay soil. Regarding the antibiotic resistance genes studied, these resistance genes could already be found in the soil before fertilization.

After fertilization, this concentration of resistance genes increased sharply for most antibiotic resistance genes and evolved back to the original concentration in the four months thereafter.

Remarkably, for the control soil fertilized with manure without antibiotic residues, a similar trend was noticed. It is therefore not yet clear to what extent antibiotic residues in the manure or soil also contribute to the selection for antibiotic resistance. In the near future, leek will be cultivated in soil fertilized with manure containing certain antibiotic concentrations (FOD AMRESMAN project).

That study will investigate if antibiotic residues accumulate in the plants and if antibiotic resistance in plant-associated bacteria increases.

Mineral oil in food: A Belgian market survey

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Introduction

In the last few years, migration of mineral oil from packaging materials into various foods has been reported (Vollmer et al. 2011; EFSA CONTAM Panel 2012; Foodwatch 2015). However, mineral oil can enter the food via different routes: (i) certain mineral oils are allowed as additives (e.g. E905a), (ii) as a pollutant from atmospheric precipitation or aquatic pollution, (iii) due to processing of food (e.g. use of machine oils and anti-dusting products) and (iv) as a residue coming from ingredients from pesticides, or components from printing inks on paper and board packaging. Occurrence data of mineral oil in food sold on the Belgian market are lacking.

Mineral oils are extremely complex mixtures of linear, branched and cyclic hydrocarbons with varying carbon numbers and structures. Among the many different substances present in mineral oil, two main types can be distinguished: the saturated hydrocarbons (MOSH) and the aromatic hydrocarbons (MOAH). Since these two fractions have a different toxicological relevance, it is important to quantify them separately. However, the analysis of MOSH and MOAH is very challenging since they form 'humps' of unresolved peaks in the chromatograms with the same range of volatility. The most popular technique for the analysis is online coupling of liquid chromatography (LC) and gas chromatography (GC) with flame ionisation detection (HPLC–GC–FID). An example of a result of the analysis of chocolate is given in Figure 1:

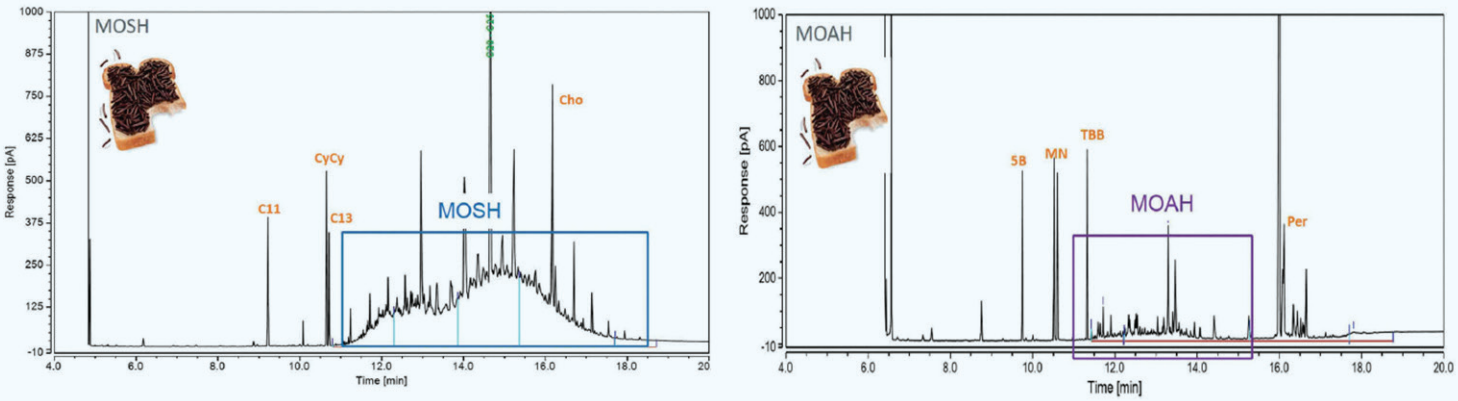

Figure 1: Analysis of MOSH and MOAH in chocolate flakes.

Market Survey

A survey of the Belgian market was conducted. Samples were selected using a well-defined strategy. Food items which were suspected to contain mineral oil and items which are highly consumed both in quantity and in frequency were targeted. An overview of the selected samples is given in Figure 2.

Figure 2: Samples selected for the market survey

The developed methodology was applicable for 198 samples and the analyses were conducted on the food as purchased. MOSH was detected in 142 samples with concentrations up to 84.82 mg kg−1 (i.e. sweets) while a concentration below LOQ was determined for 56 samples. It should be noted that these MOSH concentrations were generally lower compared to previous studies.

For MOAH, 175 samples had a concentration below the LOQ. However, 23 samples contained MOAH with a concentration ranging from 0.6 to 2.24 mg kg−1. The highest concentration was found in coffee. A summary of the results is given in Figure 3.

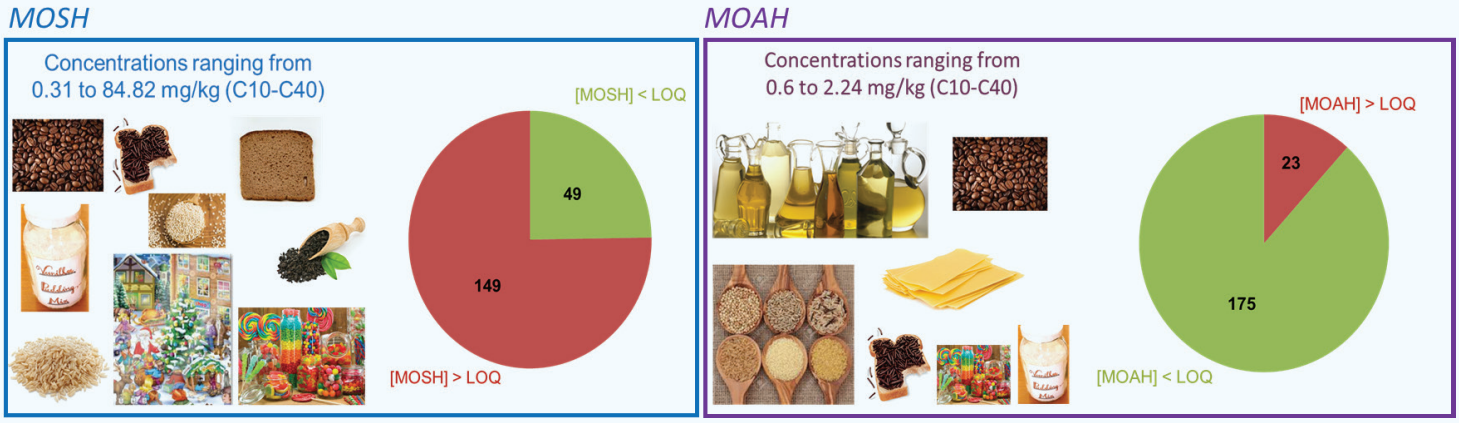

Figure 3: Results of the market survey

Comparison with the action thresholds of the Scientific Committee of FAVV-AFSCA

To date, there are no official regulatory limits at European or national level. In 2017, the Scientific Committee (SciCom) of the Belgian Food Safety Agency (FASFC) published an advice with action thresholds for MOSH and MOAH.

(SciCom, 2017) The proposed thresholds are based on the available information and possible risks. Different thresholds varying from 5 to 150 mg kg−1 were established for MOSH depending on the food type, while the available toxicological information for MOAH was too limited to propose a threshold. Therefore, the analytical detection limit of 0.5 mg kg−1 was set as action threshold.

The results from the Belgian market survey were compared with the action thresholds. The results are provided in Table 1. Only one sample exceeded the threshold for MOSH, while the threshold for MOAH was exceeded in 23 samples. For the samples exceeding the action threshold, further investigation is needed to identify the contamination source.

Table 1: Comparison of the results of the market survey with the action thresholds (AR) set by SciCom of FASFC.

Category	MOSH			MOAH		
	AR*	< AR*	> AR*	AR*	< AR*	> AR*
	mg/kg	# Samples	# Samples	mg/kg	# Samples	# Samples
Animal and vegetable fats and oils	100	13	0	0.5	5	8
Grain and grain-based products	15	102	0	0.5	95	7
Vegetables and vegetable products (incl. fungi)	20	12	0	0.5	11	1
Legumes, nuts and oilseeds	150	31	0	0.5	31	0
Snacks, desserts and other foods	20	5	0	0.5	3	2
Sugar and similar, confectionery and desserts	30	21	1	0.5	17	5
Fish and other seafood	60	7	0	0.5	7	0
Meat and meat products (incl. edible offal)	30	6	0	0.5	6	0
Total		**197**	**1**		**175**	**23**

AR = Action Threshold defined by SciCom of FASFC*

Conclusion

Consistent with previous studies, this study demonstrated that mineral oil is present in food. However, the measured concentrations are lower compared with previous market surveys. When comparing the results with the proposed action thresholds of the SciCom, it can be concluded that most of the samples are in compliance. However, a few samples show high concentrations and thus further investigation is needed.

Acknowledgments

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More information can be found in the following publication:
Van Heyst A, Vanlancker M, Vercammen J, Van den Houwe K, Mertens B, Elskens M, Van Hoeck E. 2018. *Analysis of mineral oil in food: results of a Belgian market survey.* Food Add Contam. https://doi.org/10.1080/19440049.2018.1512758.

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(3) Scientific Committee of the Federal Agency for the Safety of the Food Chain (SciCom of FAVV-AFSCA). 2017. Advice 19-2017 action thresholds for mineral oil hydrocarbons in food.
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Pyrrolizidine alkaloids in food… the good, the bad and the ugly !

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Introduction

Pyrrolizidine alkaloids (PA) are natural toxins, exclusively biosynthesized by a wide variety of plant species (> 6000), the great majority of them belong to the Asteraceae, Fabaceae and Boraginaceae families. PAs are typical plant secondary metabolites against herbivores and are believed to be one of the most widely spread natural toxins. They can affect wildlife, livestock and humans via food consumption. Outbreaks in farm animals can cause economic losses to farmers and diverse cases of human poisoning in Afghanistan, India, South Africa and the former USSR are documented and are related to animal grazing on toxic plants, fed with contaminated feed/forage or to bread contaminated with PA-producing plant seeds.

Viper's Bugloss field (Echium vulgare) in Cevennes National Park, South of France, PA-producing plants close to beehives and very attractive to honeybees.

Human poisoning is mainly caused by the consumption of contaminated traditional herbal remedy, contaminated milk (cow grazing PA plants) or honey (transfer from bees). Poisoning caused by PA toxins is characterized by acute and chronic liver damage, sometimes by pulmonary hypertension, cardiac hypertrophy or kidneys degenerative injuries, and can lead to death.

From a chemical point of view, most of the naturally occurring PAs are esterified necines (retronecine, heliotridine, otonecine or platynecine) or alkaloids N-oxides (PANOs), with necic acids. The common structural features of toxic PAs are the presence of an insaturation in 1,2 position and at least one ester bond; those compounds are in fact protoxins which can be activated in the liver to reactive pyrrole metabolites by the hepatic cytochrome P450.

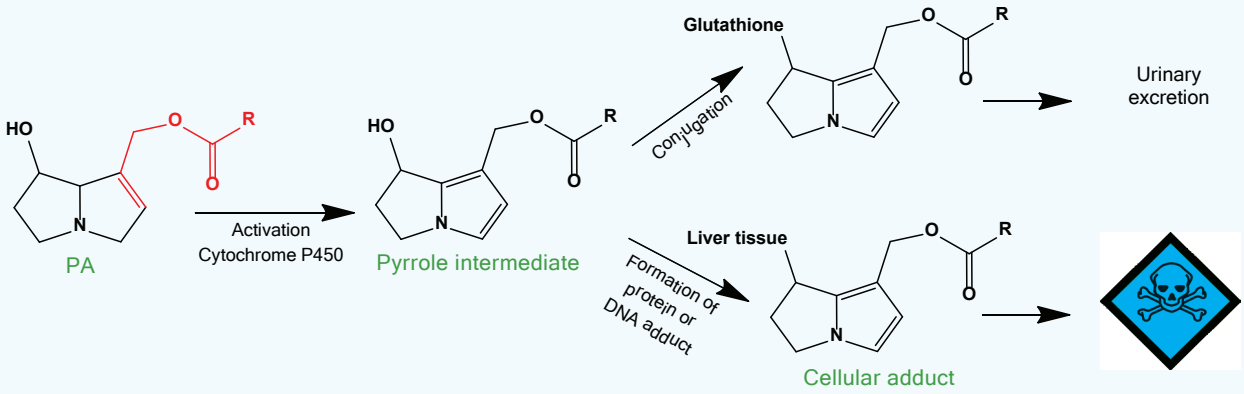

Figure 1. (De)toxification routes of pyrrolizidine alkaloids (PAs).

One cannot exclude that PAs and PANOs can become a significant public human health problem from the intake of contaminated food of botanical or animal origin. However, previous exposure assessments reported by EFSA (European Food Safety Authority) have been hampered by data gaps, and no occurrence levels have been reported for the Belgian market.

Therefore, the development of efficient analytical methods was required to accurately quantify and provide profiles of the PA content in a wide range of food matrices. This task was particularly challenging for PAs because of their variety, their widespread nature and their different forms. Indeed, more than 600 different PA structures are known so far and 50% of them are recognized as being toxics.

Moreover, they can exist in 2 different forms: the tertiary base amines and the corresponding N-oxides, which behave differently in many analytical systems. PAs are classified in 4 main families according to their structural similarities which are also associated to the botanical origin of the compounds: lycopsamine-type, monocrotaline-type, heliotrine-type and senecionine-type. In addition, a critical point in quantitative assessment of PA-contamination is the limited availability of analytical standards of PAs and PANOs. Indeed, only about 30 PA compounds are commercially available in Europe so far, in small quantities and are very expensive.

Therefore, the Federal Public Service Health, Food Safety and Environment has funded two projects, PAS-FOOD and PASHERBS (2015-2018), that were carried out at Sciensano. The main objectives of these projects were the reporting of occurrence levels of pyrrolizidine alkaloids (PAs) and N-oxides (PANOs) in targeted food items on the Belgian market in order to perform a dietary intake assessment to these natural hepatotoxic

protoxins for the Belgian population. The generated data are the basis to establish Belgium's position at the European Commission in the ongoing discussions between the European Member States in order to determine the relevant PA/PANO that should be monitored with their corresponding maximum levels in pertinent food items.

Analytical method

In Sciensano, different analytical methods were developed for the simultaneous determination of 16 PAs and 14 PANOs in highly diversified food matrices:

- honey and honey based snacks/candies
- meat and processed meat products, prepared dishes, sauces
- milk and dairy commodities
- plant based products (dry teas, herbs, salads…)
- infusions, ice-teas
- food supplements

The methods are based on an acidic extraction of the analytes, followed by SPE clean-up and subsequent analysis by liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS).

As the extent of the contamination was unknown, the developed analytical methods should be able to detect PA/PANOs at very low concentrations. The targeted LOQs were 1 ng/g for honey, 0.01 ng/g for milk and 0.1 ng/g for other food matrices

It is noteworthy that the developed gradient for this UHPLC method and the choice of a suitable injection solvent led to chromatographic resolution of most of the isomers, and particularly the 3 PANOs isomers intermedine N-oxide, lycopsamine N-oxide and indicine N-oxide, which was a huge challenge.

The elution of analytes took place using a gradient of mobile phases composed of 0.1% aqueous NH3 and acetonitrile, the effective run time was 8.8 min for a total run time of 12 min was remarkably short to separate the 30 targeted compounds.

Nine analytical methods were validated in-house and the validation parameters met the critical validation criteria allowed by Directive 2002/657/CE and SANTE/11945/2015 in the vast majority of cases.

The methods were fit for purpose, and the LOQs achieved were often very low: from 5 pg/g in milk (**ppt level**) to 1 ng/g (**ppb level**) in dry plant products. The quantification procedures were based on matrix-matched calibration curves if blank matrices could be found and if they were representative of the real samples compositions in term of matrix effect. For more specific samples, a standard addition strategy was chosen.

Afterwards, the validated methods were used for the analysis of more than **1200 samples** sold on the Belgian market covering 7 food groups.

The targeted food commodities were broadly diversified: honey (N=490), honey based snacks (N=29) & sweets (N=13), milk (N=129), dairy products (N=34), (processed) meat and liver products (N=68), pre-packaged salads (N=17), (herbal) teas (N=173), ice-teas (N=28), beehive products based food supplements (N=28), plant based food supplements (N=70), fresh herbs (N=11), frozen herbs (N=10), individual dry herbs (N=41), mixes of herbs & spices (N=67), salted snacks with herbs (N=4), ready-to-eat dishes (N=16) and sauces (N=15). It is noteworthy that we have had the opportunity to access all honeys produced in Belgium (N=374 among the 490 honey samples).

Results of the Belgian market survey

Whereas 91% of the retail honey samples were contaminated, PAs/PANOs were found in 67% of the honey samples produced in Belgium. Retail honeys were thus more contaminated than Belgian samples, and high levels of europine were detected for the first time in this matrix. The PAs observed in positive samples among the Belgian honeys were in agreement with the recurrent flora in the country: the dominance of senecionine-type compounds is associated to the ubiquitous presence of Senecio species in Belgium, one of the largest PA-producing plant genius. Honey based sweets & snacks were barely contaminated.

In contrast with the data reported by EFSA, the contamination in food items of animal origin (milk, dairy products & meat products) was dominated by N-oxides, which calls in question the results reported in previous studies that stated that PANOs would be degraded or converted to PAs in the course of digestion of the plant material by rumen. However, the levels detected in these matrices were low (ppt range).

Consistently with the main target organ of PAs/PANOs, liver products were the most contaminated amongst the meat samples, and mainly products based on duck meat.

Teas & herbal teas frequently contained high levels of a wide range of PA contaminants as a possible consequence of mechanical co-harvesting of PA-producing plant species, sometimes up to ppm levels.

An original spiking experiment enabled to highlight the fact that the transfer of PAs/PANOs from dry (herbal) tea to the infusion in the course of the brewing process was not total: only 16 to 28% of the contamination was transferred to the infusion. This new approach to quantify PAs/PANOs in (herbal) infusions contrasts significantly with the conventional strategy used by EFSA, that only apply a dilution factor from the concentration in the dry plant product to evaluate the contamination of the infusion.

As a result, major discrepancies with previously reported concentration values appeared: the contamination levels are lower but much more realistic.

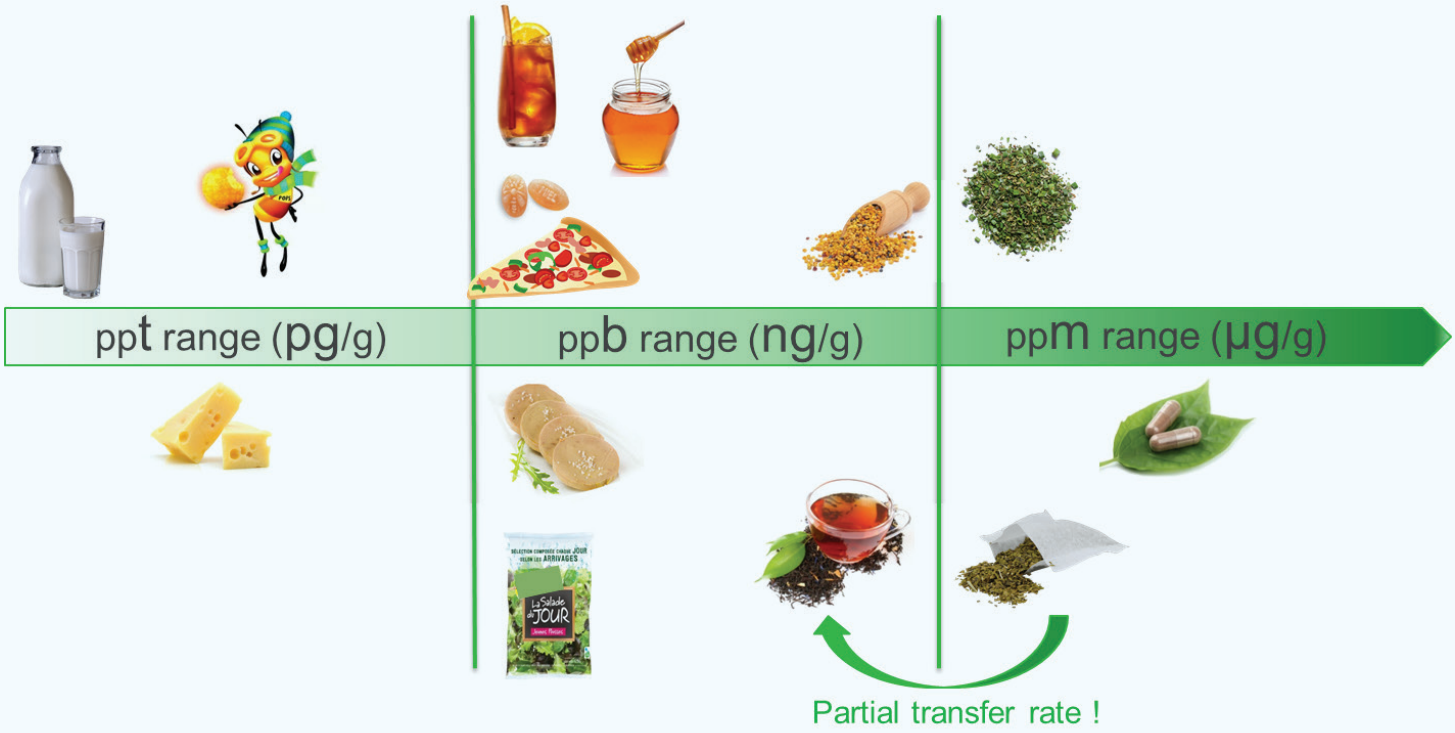

Figure 3. Illustration of the maximum occurrence PAs/PANOs levels in each food matrix (sum of 30 compounds).

Plant based food supplements contained massive concentrations of PA contaminants, but their concentration is highly dependent of the presence of PA producing plants. Indeed, some illegal botanicals according to AR 29/08/97 were found in food supplements bought online and FASFC has been notified for exceeding PA tolerated levels in a herbal tea containing borage, which is a prohibited herb on the Belgian market.

Belgium is indeed one of only few European countries having set maximum levels for PAs in botanical preparations (4 µg/kg). The contamination in beehive products based food supplements was dominated by pollen samples; other formulations were largely less contaminated. Although at low levels, some salad mixes were also contaminated with unexpected PA producing botanicals. For all studied matrices, the major contributors were by far senecionine-type and lycopsamine-type PAs/PANOs.

Surprisingly, very high concentrations of heliotrine-type compounds (ppm range) were detected in herbs mixes for pizza & spaghetti. To the best of our knowledge, only very few studies have focused on the detection of PAs and PANOs in herbs/spices and the number of herbs samples was limited.

No PAs/PANOs were detected in individual fresh herbs in pot or cut. In other words, contamination in frozen herbs, dry herbs and meals with herbs could be attributed to PA plants accidental co-harvesting or intended adulteration. Individual dry herbs and mixes of herbs were the most contaminated herb types (up to 5240 ng/g in an Italian mix).

Oregano was by far the most problematic aromatic herb regarding PAs contamination: up to 2200 ng/g in an individual oregano sample and the 10 most contaminated herbs mixes samples were all comprised of oregano. Heliotrine-type compounds (europine, heliotrine, lasiocarpine and related N-oxides) were the most recurrent contaminants in this follow-up study too (64% of the overall contamination).

Five herbs samples were in common in both research projects (same brands, same ingredients) with sampling respectively performed in August 2016 and October 2017. The PAs concentrations for both identical samples sometimes differed with time, but the contamination profiles were in very good agreement. This clearly showed that PA contamination in culinary herbs is recurrent, independently of time and lot number.

Conclusion

The Good….

Nine analytical methods were developed at Sciensano, allowing an accurate quantification of pyrrolizidine alkaloids in a wide range of food matrices. More than 1200 samples available on the Belgian market were analyzed.

The Bad…

PAs/PANOs are frequently found in all food categories, ranging from ppt to ppm levels. Very high concentrations were detected in plant based food supplements and (herbal) teas, but also in unexpected matrices such as aromatic herbs. Given those results, tolerated levels should be set up.

The Ugly…

Pyrrolizidine alkaloids are currently not regulated at European level, mainly because of occurrence data gaps. The study has generated a large number of meaningful analytical data that are now available as a basis to establish Belgium's position in the ongoing discussions at the European Commission for the setting up of maximum levels of PAs/PANOs in food commodities.

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SPECENZYM : A project to study the purity of food enzymes

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Commercial production of food enzymes (FE) started in the European Union (EU) before the middle of the 20th century. Since then, the use of enzymes in different commercial food applications has been growing continuously. FE can be used in various ways. In most cases, FE preparations of high quality are placed on the market.

FE preparations (and not single pure active enzymes) are used to provide the intended effect during food production. In some instances, FE preparations may contain a blend of several active enzymes that may be derived from a variety of biological sources, such as plants, animal tissues, or microorganisms, including genetically modified ones. FE obtained from these sources are formulated with intentionally added ingredients (diluents, preservatives, stabilizers or other substances suitable for use in food) [1].

Currently, dossiers for 300 FE have been submitted to the European Commission, who submitted the applications to the European Food Safety Authority (EFSA) for safety evaluation [2]. In this context, several data are confidentially communicated by the applicants to EFSA, related for instance to the source material, the characteristics of the genetic modification if applicable (including the sequences), the growth and fermentation conditions and the extraction procedures.

The producing industry, considering the safety and quality of their products as of paramount importance, is responsible for the quality of the enzymes introduced on the food market [2, 3].

Currently, FE preparations are tested by the producers to comply with the applicable JECFA and FCC requirements before being released to the market. In enforcement laboratories in Belgium, or even in Europe, no strategy exists currently for an efficient and accurate analytical procedure for the detection of possible contaminants in FE preparations. The SPECENZYM project proposes to collect information related to FE and the available methods existing in Belgian enforcement laboratories to detect FE impurities or other unwanted material, including the presence of Genetically Modified Micro-organisms (GMM).

The available methods validated on FE preparations will be integrated in a single workflow that will be tested via a pilot monitoring study including FE preparations from the Belgian market. The evidence-based results obtained from the pilot monitoring study and other outputs of the project will be used by the project consortium to propose recommendations to the competent authorities (FPS responsible for Food Safety and the Federal Agency for the Safety of the Food Chain (FASFC)), to help them to take the appropriate actions (e.g. control programme) in order to guarantee the safe use of FE in the food chain.

In addition, the knowledge and networking will be shared with the FPS and the FASFC through taskforces and a workshop. During the project, the competent authorities will have the possibility to consult scientists with a broad range of expertise on FE, chemical and biological contaminants and GMM detection. This will be relevant for (inter)national discussions on the development of the legal framework and the measures which could be taken to implement the new legislations.

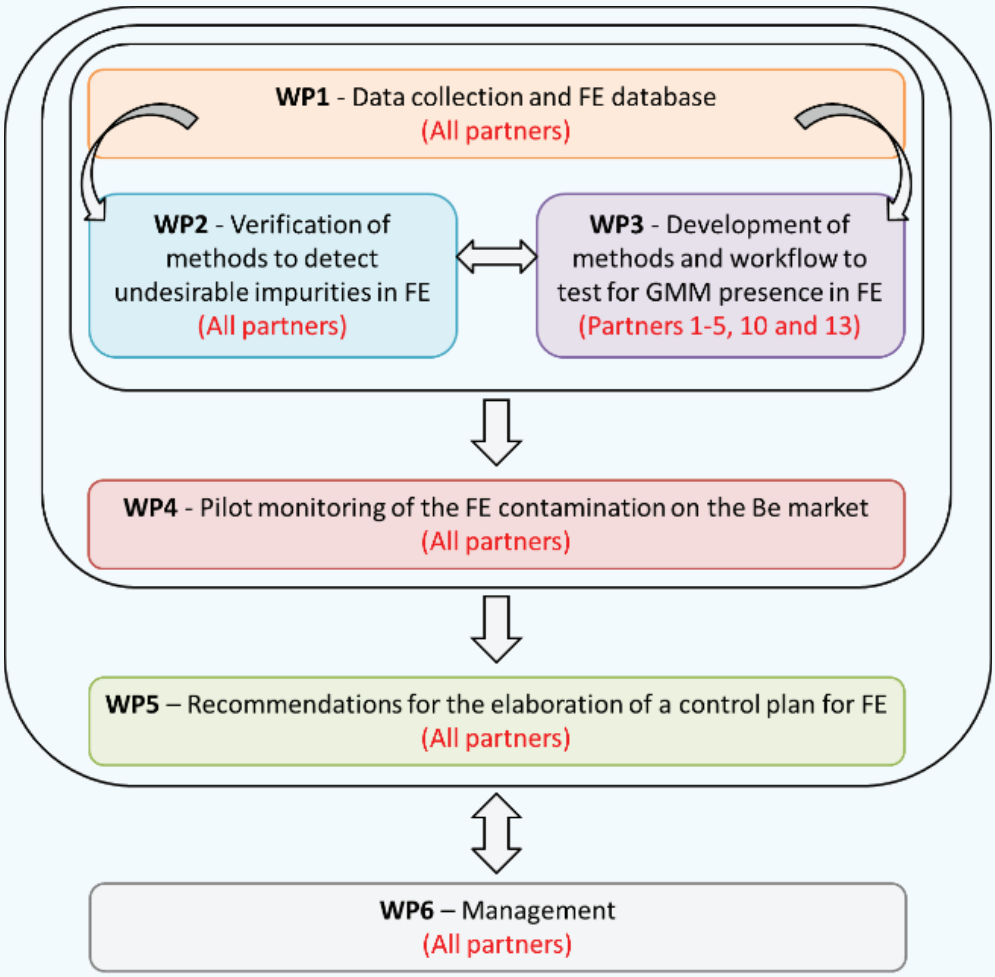

Figure 1: General overview of the work packages (WP) and their interactions of the SPECENZYM project.

WP1 Data collection and FE database construction aims at facilitating the inventory of the relevant information and their analysis regarding possible FE contaminants and the detection methods for these contaminants.

WP2 Verification of methods to detect undesirable impurities in FE proposes to verify the applicability on FE and FE preparations of methods already available in the laboratories of the partners involved in this project as well as the inventory and the prioritization of methods that should be developed to improve the detection of undesirable impurities.

WP3 Development of methods and workflow to test for GMM presence in FE focuses more on the specific issue of GMM detection. The information on FE collected in WP1 and the methods verified and developed in WP2-3 will allow the development of a customized workflow that will be tested on representative samples of FE and FE preparations from the Belgian market.

WP4 Pilot monitoring of the FE contamination on the Belgian market. This WP will have the objectives (1) to elaborate and to test an efficient workflow to identify the FE impurities in the FE & FE preparations (that can be the basis for an effective control in the future); (2) to apply this workflow on representative FE and FE preparations samples form the Belgian market (pilot study).

The pilot monitoring of WP4 will be used as evidence-based results to provide recommendations to the competent authorities for a control plan and specifications regarding FE in WP5 Recommendations for the elaboration of a control plan for FE.

A separate WP is dedicated to the Management and communication (WP6) of this multi-partner project and provides a project management structure matching with the complexity, the multidisciplinarity and the scientific requirements of this project in order to ensure an optimal achievement of the project's objectives.

Structure and organisation of the research describe also how the structure of the project maximizes the input of the different consortium expertises.

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[2] Food enzyme applications submitted to the Commission within the legal deadline (from 11 September 2011 to 11 March 2015). URL < https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_enzymes-applications.pdf >

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Metrofood: A new global research infrastructure for promoting metrology in food and nutrition

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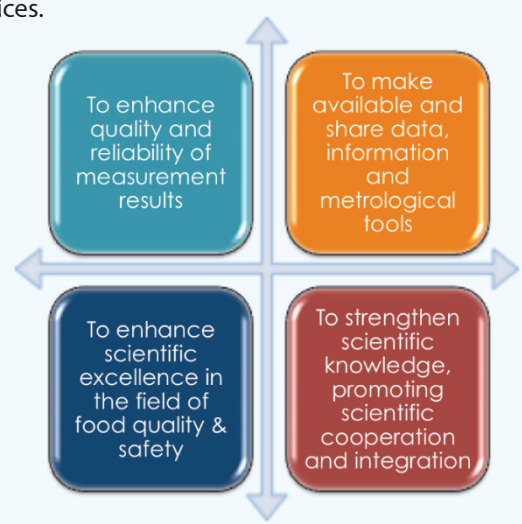
The agri-food sector is a strategic asset of all the European Countries and one of the largest and most important economic activities, with particular social relevance: it is vital to ensure employment, preserve rural public goods, supply healthy and quality food, and facilitate the integration of SMEs into the international food chain. Food quality, authenticity, nutrient profile and health benefits have now become a focus of consumer's requirements all over the world. Food traceability and safety are key factors to ensure food quality and to protect consumers' interests. (WHO, 2015).

However, despite all efforts within the EU there is still a significant fragmentation with regard to food analysis and research in Europe and worldwide. A huge variety of scientific disciplines and types of organizations are involved; data from controls and surveys are worldwide distributed over a large number of different databases. This divergence may lead to inefficiency by duplication of data and research; on the other hand certain knowledge gaps may not be identified and addressed (Rychlik et al., 2018).

In this perspective, a new "Research Infrastructure (RI) for promoting Metrology in Food and Nutrition" – METROFOOD-RI - was introduced within framework of the European Strategy Forum on Research Infrastructures (ESFRI Roadmap 2018 - domain Health and Food). Its mission is to enhance quality and reliability of measurement results and share data, information and metrological tools, to enhance scientific excellence in the field of food quality and safety and to promote scientific cooperation and integration throughout the food chain (Fig 1).

An important goal is to harmonize and coordinate high quality metrology services in food and nutrition by developing standardized procedures and providing suitable reference materials (RM) to insure full traceability to SI Units. In food analysis RMs play an essential role and in view of the emerging challenges for food safety (such as application of nanotechnology, food adulterations…) there is an urgent need for new multi-parameter RMs in various food matrices.



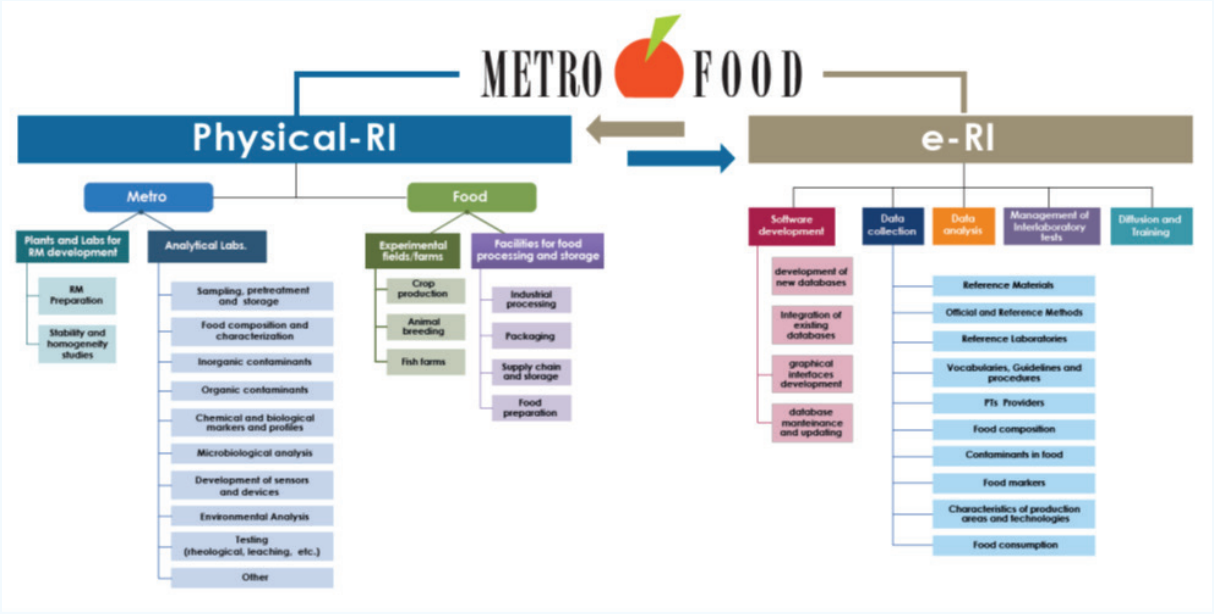


Figure 2: Structure of METROFOOD-RI

The METROFOOD-RI combines a physical infrastructure (p-RI) and a virtual one (e-RI) (Fig 2). This structure is intended to provide distributed scientific services and to create a shared base of data, through a network of interlinked providers and users. The services may range from the development and validation of methods for food or environmental analyses (Fig 3), provision of Proficiency Testing schemes and RM, pilot plants for food production, packaging, storage and distribution, experimental field/farm sites (Fig 4).

Figure 3: METROFOOD-RI will provide high quality metrological services for food and nutrition

Figure 4: METROFOOD covers the food chain from primary production to consumer

The network is destined to serve a wide range of users starting from researchers and technicians from universities and research institutes, public and private analytical laboratories, up to industrial users, policy makers, food inspection and control agencies, consumer organizations and citizens. An important mission of the RI is also to supply high-quality training for academics and professionals at different educational levels and to organize dissemination actions addressed to a wide public.

METROFOOD-RI was cited as emerging project in the "Health and Food" domain of the ESFRI roadmap 2016 and completed its early phase in 2017 upon the EU-funded project PRO-METROFOOD (H2020 INFRDEV-02-2016). It has now been included as an active project within the ESFRI Roadmap 2018 and is approaching the next phases (Preparatory Phase, Implementation and Operation).

The Implementation Phase is planned to start in 2021 with full operation foreseen for 2024 (ESFRI, 2018). The project is coordinated by the Italian Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) and currently involves 48 partners from 18 European countries.

In each country METROFOOD-RI is being organized in national nodes and some dedicated Joint Research Units have been established (e.g. Italy, FYROM, Greece, Portugal, Slovenia). The Belgian node (METROFOOD-BE) is under development and is being represented by the scientific health research institute Sciensano. At present our national partners are the National Metrological Institute (FPS Economy, Metrology, National Standards) and the Food Pilot of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO). The Belgian node is still expanding and is open for interested partners to build a solid Belgian research infrastructure on metrology in nutrition, food safety and quality.

It is clear that the METROFOOD-RI initiative addresses an urgent and important need within the community to provide an interconnected worldwide distributed network of high quality scientific services, knowledge and information, metrological tools and standardized procedures in the domain of health and food, covering all aspects of the whole food chain from primary producers to consumers where all relevant data are findable, accessible, interpretable and re-usable according to the FAIR data principles. In particular the combination of data related to food safety will allow new approaches for risk assessments.

For more information see also:

- https://www.metrofood.eu
- http://www.prometrofood.it/project
- http://roadmap2018.esfri.eu

Reference List

[1] ESFRI (2018) Strategy report on research infrastructures, European Strategy Forum on Research Infrastructures, Brussels

[2] Rychlik, M., Zappa, G., Anorga, L., Belc, N., Castanheira, I., Donard, O. F. X. et al. (2018). Ensuring Food Integrity by Metrology and FAIR Data Principles. Front Chem., 6, 49.

[3] WHO (2015). European Food and Nutrition Action Plan 2015-2030. World Health Organisation Regional Office for Europe, Geneva

Workshops & Symposia

The trainings for the approved laboratories organized by the FASFC in co-operation with the National Reference Laboratories are available on the website of the FASFC (www.favv.be > Home > Business Sectors > Laboratories > Seminars & workshops).

The schedule is updated regularly, it is therefore recommended to check the website from time to time.

Other interesting workshops and symposia are mentioned below.

Date	Subject	Place	More information (website)
6 November 2019	Studiedag : Welke kwaliteitssystemen zijn van toepassing in de voedingssector?	Leuven, Belgium	https://www.kvcv.be/index.php/nl/activiteiten-en-nieuws/kalender/538-studiedag-welke-kwaliteitssystemen-zijn-van-toepassing-in-de-voedingssector
18 - 20 November 2019	Vibrio 2019	Montreal, Canada	https://icbv2019vibrio.wixsite.com/mysite
29 - 31 January 2020	16th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-16)	Ghent, Belgium	www.htc16.com
7 - 10 September 2020	FOODMICRO 2020	Athens, Greece	http://foodmicro2020.com/
18 - 20 May 2020	EuroResidue IX	Egmond aan Zee, The Netherlands	www.euroresidue.nl

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