Annex 1
Research topics

Research topics RT-projects

	TOPICS	Maximum duration (months)	Maximum grant
	Animal Health		
1	Gene analysis on social behaviour of honeybees in the fight against varroasis (GENOVARR)	36	€ 300,000
2	Brucellin skin test in pigs as confirmatory test in the event of positive serology for *Brucella suis* (BRU-PIG)	12	€ 100,000
3	Effect of altered antibiotic use in food-producing animals on antimicrobial resistance in animal and human pathogens (AB-changeR)	24	€ 200,000
	Plant health		
4	Risk analysis of harmful bark and ambrosia beetles in the Belgian context (SCOLIBE)	36	€ 250,000
5	Design of a statistically sound and risk-based survey plan for the detection of *Xylella fastidiosa* in Belgium (RIBSURX)	12	€ 75,000
	Food Safety		
6	Occurrence and exposure to dioxins, furans and halogenated biphenyls in foodstuffs (TEQFOOD)	24	€ 250,000
7	Mycotoxins in vegetarian protein-rich and fibre-rich food (MYCOPROF)	18	€ 150,000
8	Intake monitoring of food flavourings (INFLAVOUR)	36	€ 300,000

Research topics RI-projects: plant health - Euphresco

	TOPICS	Maximum duration (months)	Maximum grant[i]
	Plantengezondheid		
2021-C-368	Heat- (incl. hot water) treatments	24-36	€ 100,000
2021-A-373	Fast detection methods for quarantine Tephritidae (TEPHRIFADE)	24-36	€ 100,000
2021-A-378	Inventory and validation of quality control procedures for the extraction of nucleic acids used for diagnosis	12-24	€ 100,000

[i] The FPS Health foresees **€ 200,000** to the transnational call. A maximum of € 100,000 can be requested per topic. Since three topics are included, this means that there may be insufficient budget for one of the three topics.

1. Gene analysis on social behaviour of honeybees in the fight against varroasis (GENOVARR)

Context

Honeybees are essential pollinators and are vital to both the food chain and the health of ecosystems. Significant losses to honeybee populations[i] have been recorded in recent years due to a combination of different stressors, including the use of pesticides (neonicotinoids), single-crop farming, climate change, bacterial and viral pathogens and (non-native) parasites. The Varroa destructor mite appeared in Europe in the 1980s and has since become endemic. Beekeepers try to control this mite in various ways, either through biotechnical methods or by using veterinary medicinal products. Unfortunately, there is currently no adequate treatment and resistance to various veterinary medicinal products has been confirmed. In recent years, research has been conducted on the genetic potential of bees in order to build up natural resistance to Varroa. Recent scientific publications reveal that honeybees have the genetic potential for resistance to Varroa, to pathogens and possibly to other stressors.

The finalised VARRESIST project[ii], funded by the FPS, focused on reducing mite reproduction, in particular the reproduction of the mite in the drone brood (drone brood resistance). Although the Varroa mite can reproduce in both drone brood and worker brood, it has been observed that drone brood becomes more infested with Varroa. However, no further research has been conducted to identify which characteristics are responsible for this, in order to reduce the infection pressure of the V. destructor mite in the worker brood and on the bees present in the hive.

The hypothesis of reducing mites through the social behaviour of worker bees was not studied in the VARRESIST project financed by the FPS. However, Broeckx *et al.* (2019)[iii] state that social and individual characteristics of the honeybee influence the defence mechanism against the V. destructor mite. These social characteristics are expressed by the detection, opening and removal and cleaning of the affected combs. This is described as "*Varroa sensitive hygiene"* (VSH) behaviour. These social characteristics are independently inheritable and are expressed by the worker.

The ongoing MAS-BEE-VAR project[iv] continues the VARRESIST project and investigates whether genetic markers of the phenotype 'reduced mite reproduction' are present in the Belgian bee population. This study of a single phenotype can be complemented by a study of multiple social characteristics (e.g. detection of infected cells, opening of the infected cells, active removal of the infected pupae, etc.), which must be present simultaneously in order resistance to this Varroa mite can be expressed.

Important conditions for the usability of this genetic potential are

(1) the identification of relevant genes,
(2) the identification of underlying genomic variants and
(3) the development of minimally invasive testing methods.

[i] HealthyBee monitoring programme of the FASFC: http://www.afsca.be/bijenteelt/dierengezondheid/#HealthyBee

[ii] RT 13/4 VARRESIST - Study of Varroa tolerance in honeybees in Belgium.

[iii] Broeckx, B.J.G., De Smet, L., Blacquière, T. et al. Honeybee predisposition of resistance to ubiquitous mite infestations. Sci Rep 9, 7794 (2019). https://doi.org/10.1038/s41598-019-44254-8

[iv] RF 19/6336 MAS-BEE-VAR - Marker-assisted selection in honeybees, *Apis mellifera*, for higher Varroa resistance

The ultimate goal is to develop rapid and easily deployable tests based on genetic and genomic research for both monitoring and targeted selection. The availability of such test(s) will also have a positive contribution to a durable solution to the problem of Varroa and the related bee mortality.

Objectives

- Identifying genomic variants associated with genes focusing on the social behaviour of the honeybee worker which is relevant for resistance to the *Varroa destructor* mite.
- Developing a minimally invasive test method for the social behaviour of honeybees based on genomic variants. This test method will be used by beekeepers in the field in selection programmes in order to improve the resistance of honeybees to varroasis.

Maximum budget: € 300,000

Maximum duration: 36 months

2. Brucellin skin test in pigs as confirmatory test in the event of positive serology for *Brucella suis* (BRU-PIG)

Context

Belgium has been free from *Brucella suis* in domestic pigs for many years. However, *Brucella* is endemic to the wild boar population. Sporadic cases of brucellosis in pigs have been reported in some EU Member States.

The existing serological tests for brucellosis in pigs detect antibodies directed against the lipopolysaccharides of the external membrane. Some other bacteria, such as *Salmonella enterica* serotype Urbana, *E. coli* O:157, *E. coli* O:116, *Pseudomonas maltophilia* and especially *Yersinia enterocolitica* O:9, share the same epitopes on the O-polysaccharides, resulting in cross-reactions.

Breeding boars used in artificial insemination centres must be tested for brucellosis, among other things, before introduction. Seropositive reactions are regularly declared. Only additional and sometimes time-consuming tests can prove that it relates to a false positive. In many cases, boars are slaughtered for bacteriological research. Pending the results of the additional studies, the movement of breeding pigs and semen is prohibited. This leads to significant economic losses. Specific feeding strategies influence the gut microbiota but are probably unable to prevent infection with *Y. enterocolitica*, among others. Isolating bacteria such as *Y. enterocolitica* does not rule out an infection with *Brucella suis* either.

In Belgium, the brucellin skin test is used as a confirmatory test for brucellosis in bovines.

Objectives

- The aim of this project is to develop and validate a brucellin skin test in pigs. The results of the available serological tests need to be compared with the results of the newly developed skin test.
- A workshop for experts should be organised for performing the skin test and interpreting the results.

Maximum budget: € 100,000

Maximum duration: 12 months

References

3. Effect of altered antibiotic use in food-producing animals on antimicrobial resistance in animal and human pathogens (AB-changeR)

Context

In recent years, efforts have been made, both by the government and by sectoral organisations, to reduce the use of antibiotics in food-producing animals to the necessary level, with a view to tackling antimicrobial resistance (AMR). Tackling AMR means protecting public and animal health. In 2016, a covenant[i] was concluded between the government (Minister of Agriculture and Minister of Health) and the relevant partner organisations to work within a framework of co-regulation towards achieving three targets by the end of 2020, namely a reduction as compared to 2011 of (1) 50% in the use of antibiotics by 2020, (2) 50% in the use of antibiotics in medicated feed by 2017 and (3) 75% in the use of critically important antibiotics by 2020. The last two targets were successfully achieved; only the 50% reduction in the use of antibiotics still needs to be achieved.

The relevant partner organisations are willing to continue tackling AMR in the coming years, primarily by less use and more responsible use of antibiotics. This has been laid down in a second covenant that will run from 2021 to 2024. The covenant itself is an integral part of the strategic objective "One-Health Governance" of the One-Health National Action Plan against AMR (OH NAP AMR) which still needs to be validated at the political level.

The proposed topic is part of the strategic objective "Innovative and targeted research: targeted and innovative research projects for more effective control measures and a better understanding of the transmission sources of resistant micro-organisms between humans, environment, food chain and animal populations" and the operational objective '70. Fund or encourage research projects to fill knowledge gaps on AMR and ensure effective implementation of policies to tackle AMR, in line with the One Health approach'.

At present, there is no clear understanding on the impact of reducing antibiotic use in food-producing animals on public and animal health, translated into the impact on resistance in animal pathogens and human pathogens whose resistance has a link to animals. The aim of this study is to evaluate the outcome of the policy decision, namely focusing on a reduced and more responsible use of antibiotics, and to draw lessons for the period after 2024. The results of the ongoing RU-BLA-ESBL-CPE[ii] research project can be used to evaluate the trend in the occurrence of resistance genes and profiles in humans and animals.

Research questions

- What are the trends in the resistance profiles and genes in major animal pathogens in food-producing animals over the last 5 years? What is the impact of altered antibiotic use in food-producing animals on these trends?

  The food-producing animals covered by this research question are pigs, veal calves, broilers and laying hens.

[i] Covenant between the Federal Government and all the relevant sectoral partners on reducing the use of antibiotics in the animal sector.
https://www.amcra.be/swfiles/files/NL_FR%20convenant%20AB%2020160630_9.pdf

[ii] RF 17/6317 RU-BLA-ESBL-CPE – Emergence or decline of classical beta-lactamases (BLAC), of cephalosporinases (BLAAmpC), of extended spectrum beta-lactamases (BLAESBL), and of carbapenemases (BLACPE) amongst coliform enterobacteria from bovines: encoding gene identification and antibody neutralization.

- What are the trends in the resistance profiles and genes with a link to animals in human pathogens in the last 5 years? What is the impact of altered antibiotic use in animals on these trends?
  The research should not be limited to zoonotic human pathogens but should focus on (genotypic) resistance profiles of human pathogens with a (molecular) link to the animal sector.
- What recommendations can be formulated for future policy in the context of the responsible use of antibiotics in food-producing animals, the use of critically important antibiotics, the development of cross-resistance, etc.?

Maximum budget: € 200,000

Maximum duration: 24 months

4. Risk analysis of harmful bark and ambrosia beetles in the Belgian context (SCOLIBE)

Context

In recent decades, bark beetles and ambrosia beetles have attracted worldwide attention, due to introduction in many new areas, with significant damage being observed in certain cases.

Various names are used to designate this large group of insects, due to a recent change in the taxonomic classification. Based on morphological characteristics, these insects were previously considered to be a separate family, the Scolytidae. However, recent phylogenetic research classifies these beetles as a subfamily, the Scolytinae, under the Curculionidae (EFSA, 2020[i]). In a recent study from the European and Mediterranean Plant Protection Organization (EPPO, 2020[ii]), the Platypodinae subfamily of the Curculionidae was also designated as ambrosia beetles.

At the time of the implementation of the plant health legislation, the bark and ambrosia beetles which are considered quarantine for the EU were still listed in Annex II of Implementing Regulation (EU) 2019/2072[iii] as "*Scolytidae* spp. (non-European) [1SCOLF]". In addition, a number of them have also been included in the list at species level (*Pseudopityophthorus minutissimus*, *Pseudopityophthorus pruinosus*, *Pityophthorus juglandis*, …), and some have already been proposed for inclusion in the first revision of this list (*Euwallacea fornicatus* sensu lato, …). From horizon scanning (EPPO alert list, EFSA Plant health Newsletters, etc.) other species have been deemed important for further analysis (*Dendroctonus valens*, *Xylosandrus crassiusculus*, *Xylosandrus compactus*, etc.).

In view of a revision of the standardisation and listing of non-European Scolytidae on Coniferae in Directive 2000/29/EU Annex IIAI, the EFSA has already worked on a categorisation at species level for non-EU Scolytinae on conifers (EFSA pest categorisation[i]).

For the complete revision of the current list, the EFSA is considering conducting an analogous study on host plants other than Coniferae, in the near future.

EPPO also recently listed a number of representative bark and ambrosia beetles that are considered as examples for introduction or spread via imports of non-coniferous wood[ii].

There is a need for more data and a risk analysis specific for Belgium of this large group of insects, in order to support future regulation and a more targeted monitoring and control of the identified quarantine species.

[i] EFSA Panel on Plant Health. 2020. Pest categorisation of non-EU Scolytinae of coniferous hosts. EFSA Journal 18(1):5934. https://doi.org/10.2903/j.efsa.2020.5934

[ii] EPPO Technical Document No. 1081, EPPO Study on the risk of bark and ambrosia beetles associated with imported non-coniferous wood. EPPO Paris https://www.eppo.int/media/uploaded_images/RESOURCES/eppo_publications/TD-1081_EPPO_Study_bark_ambrosia.pdf

[iii] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019.

Research questions

- Which species of bark and ambrosia beetle pose the biggest risk for Belgium? This must include a study of the potential for establishment, the possible impact, the available host plants and existing introduction pathways based on an analysis of the lists compiled by the EFSA (non-EU Scolytinae on conifers) and the EPPO (case studies of bark beetles and ambrosia beetles on non-coniferous wood), including other non-EU Scolytinae and Platypodinae on deciduous trees that may be proposed by the EFSA in the future.
- What is the pest status of selected Scolytinae and Platypodinae in Belgium?
  Preference is given to Scolytinae and Platypodinae that have already been reported to a limited extent (in the EU) or where, on the basis of the risk analysis, there is the greatest likelihood of wider spreading, with the aim of underpinning an EU versus non-EU list. The status should be determined according to the IPPC standards, based on a representative and targeted survey, on sites identified as risk areas. Organisms that have recently been included in a phytosanitary status determination in Belgium should not be included again in the survey.
- Which elements can be identified to organise the continuous monitoring of Scolytinae and Platypodinae species (traps, sites, etc.), with a view to preventing introduction or early detection of possible outbreaks?
  The aim is to achieve, where possible, more generic monitoring plans at risk sites in the future.
- Are (rapid) detection and identification methods available at the species level, depending on intercepted stages?

  If relevant, additional work in this regard can be included in the proposal.
- Which control measures for the relevant Scolytinae and Platypodinae species for Belgium can be proposed? An inventory of measures that are or have been applied elsewhere can serve as a basis in this regard.

Maximum budget: € 250,000

Maximum duration: 36 months

5. Design of a statistically sound and risk-based survey plan for the detection of *Xylella fastidiosa* in Belgium (RIBSURX)

Context

Article 2 of Implementing Regulation (EU) 2020/1201 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.) obliges Member States to conduct annual surveys in their territory to detect the possible presence of this organism. This survey must be carried out on the basis of the risk level. These surveys must take place outdoors, as well in plots, orchards and vineyards, as in nurseries, garden centres and/or shopping centres, in nature reserves and in other relevant locations. Samples must be taken from plants, and from plants intended for planting, and must be tested for the presence of *Xylella fastidiosa*. In this regard, the EFSA guidelines[i] for statistically sound and risk-based surveys of *Xylella fastidiosa* must be taken into account.

From 2023 onwards, it will be mandatory that the sampling scheme used could detect a level of infection in 1% of the plants, with at least 80% reliability. The RiBESS+ tool developed by the EFSA must be used in this regard.

The aim of the study is to develop such a survey plan for Belgium. The results of research projects already completed (RT 15/7 XYLERIS[ii]) and still ongoing (RF 18/6323 XYFABEL[iii], RF 19/6331 Xfast[iv]) can provide valuable input in this respect.

This study will also provide useful experience for the design of future statistically sound risk-based surveys for other quarantine organisms. This meets the objective of the European Commission.

Research questions

- What are the relevant host plant species of *Xylella fastidiosa* for Belgium and what is their part in the host plant population?
- What is the sensitivity of the sampling and analysis methods used?
- What are the relevant epidemiological units and inspection units to design the survey plan?
- What are the relevant risk factors for Belgium, what are their risk levels and what is the relative risk of each level?
- How should the samples calculated with the RiBESS+ tool need to be allocated in the survey area, taking into account the available information about the target population and risk factors in order to develop an efficient survey plan in terms of costs and manpower whilst ensuring the required reliability?

Maximum budget: € 75,000

Maximum duration: 12 months

[i] https://doi.org/10.2903/sp.efsa.2020.EN-1873

[ii] RT 15/07 XYLERIS - Study on Xylella fastidiosa plant hosts and vectors in Belgium and the influence of specific plant growth conditions on disease development

[iii] RF 18/6323 XYFABEL - The fate of Xylella fastidiosa in common woody plant species in Belgium and the analysis of communities of endophytic xylem-inhabiting bacteria as possible markers for its presence and lifestyle

[iv] RF 19/6331 Xfast - Biological characteristics of potential vectors of Xylella fastidiosa to support sampling and containment procedures

6. Occurrence and exposure to dioxins, furans and halogenated biphenyls in foodstuffs (TEQFOOD)

Context

Persistent organic pollutants (POPs) are widely present the environment. Various studies have been devoted to chlorinated dioxins and furans (PCDD/F). However, there are very few studies on the occurrence of brominated dioxins and furans (PBDD/F) and other halogenated compounds.

Dioxins and furans are halogenated organic compounds which are essentially formed during incomplete combustion processes.

The presence of brominated dioxins could be explained mainly by the presence of brominated organic compounds in waste, and more specifically brominated flame retardants. Over the last 30 years, brominated flame retardants have been widely used in consumer products (plastics, textiles, electrical and electronic equipment, mattresses, etc.), and at their end of life, they are either recycled or incinerated.

PBDD/Fs are primarily found in emissions from waste incinerators, from accidental fires and when plastic is recycled. Three main formation pathways are described: formation from precursors, de novo synthesis and the presence of PBDD/F contained in brominated flame retardants in the form of impurities. In addition to anthropogenic sources of brominated dioxins, the literature also highlights a biological formation pathway. Some lower brominated PBDDs (Tri-PBDD and Tetra-PBDD) therefore appear to be formed via precursor pollutants present in the aquatic environment and bioaccumulated in certain aquatic species including fish and shellfish.

The co-combustion of materials containing bromine and chlorine (e.g. accidental fire involving products containing brominated flame retardants and PVC) leads to the formation of PXDD/F, i.e. dioxins and furans substituted by both chlorine and bromine.

Investigations carried out in various countries have confirmed the presence of PBDD/F in all environmental matrices (outdoor air, soil, water, sediment, the food chain, dust and indoor air). As PBDD/Fs can be present in terrestrial and aquatic environments (marine and freshwater sediments), they are likely to contaminate both the terrestrial and aquatic food chain.

A non-exhaustive compilation of the concentrations observed in different foodstuffs was produced by INERIS in 2020. These data show high concentrations in green vegetables and fresh fruits (maximum of 4.46 pg toxic equivalent (TEQ)/g of fresh matter) despite a low fat content (<1%). Offal and meat (with a maximum of 2.04 and 3.5 pg TEQ/g fat), fish (with a maximum of 1.87 pg TEQ/g fat) and seafood (with a maximum of 0.23 pg TEQ/g fresh matter) are among the products with the highest concentrations of PBDD/F.

PBDD/Fs have also been measured in human biological matrices. A study carried out by the Belgian National Environment-Health unit as part of a campaign by the World Health Organization (WHO) between 2006 and 2009 on POP concentrations in breast milk in Belgium allowed to quantify PBDD/F at a concentration of 0.67 pg/g of lipids. Concentrations of PXDD/F were below the analytical limit of quantification at 0.03 pg/g for each congener.

PXBs, i.e. biphenyls substituted with both chlorine and bromine, are also found in foodstuffs such as eggs, milk, meat, offal, fish and shellfish.

Biomonitoring data show that the Belgian population is also exposed to brominated dioxins. A study of the concentrations in foodstuffs on the Belgian market therefore appears necessary.

In humans, cases of chloracne have been reported following exposure to TBDD. There is currently no toxicological reference value for brominated dioxins and furans. The mechanism of action and type of toxicity of brominated compounds are deemed to be similar to those of chlorinated compounds. As with chlorinated compounds, it has been shown that most of the biological and toxic effects are mediated by the Ah receptor (AhR).

The main route of exposure to dioxins is though food. In 2018, the EFSA (European Food Safety Authority) revised the tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs (DL PCBs) downwards. Exposure of the population exceeds the new TWI of 2 pg TEQ per kg body weight per week. With a view to eliminating the uncertainties regarding exposure, the EFSA recommends revising the toxic equivalency factors (TEFs). It is therefore necessary to perform congener-specific analyses, so that in the future the TEQ values found can be converted using possibly revised TEF values.

In the absence of specific data for each brominated congener, Van den Berg *et al.* (2013) reported that the WHO and the United Nations Environment Programme (UNEP) recommend provisionally applying the TEF values calculated for chlorinated compounds to brominated derivatives, pending specific values for brominated derivatives.

Brominated dioxins give a response in biological tests for dioxin-like substances because they are an agonist of the AH receptor (EFSA, 2018). For example, brominated dioxins and PXDD/F and PXB DL are not currently included in the TEQ principle for dioxin-like substances. This could lead to an underestimation of exposure to persistent agonists of the AH receptor (EFSA, 2018). The recommendations of the EFSA in the 2018 opinion on dioxins and dioxin-like PCBs include:

- There should be an evaluation of the relative exposure contribution of other persistent chemicals, acting as agonists on the AH receptor, taking into account their toxic potencies.
- To improve human exposure estimation to dioxins and DL PCB, more occurrence data are needed on foodstuffs of plant origin, especially where individual results of certain foodstuffs indicate potential higher contamination.

In a previous Belgian research project on the intake of dioxins and dioxin-like PCBs by the Belgian population, there was a contribution from food of plant origin, which has not been entirely clarified, e.g. the contribution of chocolate spreads (Windal *et al*., 2010). As concentrations change significantly over time, the contribution of foodstuffs of animal and plant origin could be revised. A recent study (Test Aankoop / Test Achats, 2021) also highlighted the role of certain foodstuffs of plant origin in the total exposure in Belgium.

Considering the use of brominated flame retardants and the potential formation of PBDD/F and mixtures of bromochloro-dioxins and furans (PXDD/F), the fact that these compounds act on the AH receptor, and the fact that there are some gaps in the occurrence data for dioxins and DL PCBs, there is a need for conducting a study on the occurrence in the food chain of dioxins and DL PCBs, brominated dioxins and a mixture of bromochloro-dioxins in foodstuffs in order to determine the exposure levels of the Belgian population. This study should include mixtures of bromochlorobiphenyls (PXB).

The data can be used to revise the exposure assessment when the new TEF are available. They are necessary in order to take risk management measures to reduce exposure.

The EFSA (2010) reports that the risk of exposure of the European population to polybrominated biphenyls (PBBs) via food is not of concern. Given that PBBs are no longer produced in Europe and taking into account the declining concentrations in the environment, the EFSA concludes that PBBs are a low priority for research and monitoring programmes.

Objectives

1) Development and validation of a congener-specific method of analysis for PBDD/F, PXDD/F and PXB in foodstuffs. This method must make it possible to analyse chlorinated and brominated congeners and mixtures, to ascertain the impact of the TEF and to calculate a TEQ concentration.
2) Study of the concentrations of PCDD/F, PBDD/F, PXDD/F as well as PCB and PXB in foodstuffs (animal and vegetable) on the Belgian market.
3) Estimation of the intake by the Belgian population (children, adolescents, adults) of PCDD/F, PBDD/F, PXDD/F, PCB and PXB; estimation of the contribution of PBDD/Fs to the total intake.

The occurrence data collected in the context of the research project must be transmitted to the EFSA.

Maximum budget: € 250,000

Maximum duration: 24 months

References

, Institut national de l'environnement industriel et des risques (https://www.ineris.fr/sites/ineris.fr/files/contribution/Documents/Rapport-Ineris-19-177734-00120B_Dioxines%20et%20furanes%20brom%C3%A9s-v1.0.pdfLin Y, Le S, Feng C, Qiu X, Xu Q, Jin S, Zhang H, Jin Y, Wen Y, Xu H, Liu P, Rao Q, She J, Lu D. 2021. Exposure and health risk assessment of secondary contaminants closely related to brominated flame retardants (BFRs): Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) in human milk in shanghai. Environ Pollut. 268:115121.

, Wall R.J., Fernandes A., Rose M., Bell D. R., Mellor I. R. 2015. Characterisation of chlorinated, brominated and mixed halogenated dioxins, furans and biphenyls as potent and as partial agonist of the aryl hydrocarbon receptor. Environment International 76, 49-56.

7. Mycotoxins in vegetarian protein-rich and fibre-rich food (MYCOPROF)

Context

In light of the current protein transition, plant proteins are being steadily used in foodstuffs. Increased consumption of these products requires research on the contaminants that might be present. Vegetarian protein-rich ingredients are used not only in meat substitutes, but also in sports nutrition and in meal replacements for weight control. These products have not been deeply studied for mycotoxins.

The protein transition is also going hand in hand with the valorisation of by-products and the reduction of waste. The protein fractions that were used previously more frequently in animal feed are now more often found in food for human consumption. Well known examples of this are whey, oilseed pressed cakes. When the by-products are valorised, there is sometimes insufficient attention paid to the possible presence of contaminants.

The most recent dietary guidelines also advise consumption of fibre-rich rather than refined cereal products. There is also a trend towards a larger variety of fibre-rich foods, such as a wider range of cereals types consumed (spelt, etc.). At the same time, there is a growing trend in valorisation of fibre-rich by-product fractions (hemp cake, fruit pulp from pressing fruit juice, etc.) in the context of a circular economy.
When food is fractionated, contaminants may be unevenly distributed among the different fractions. For example, starch is known to be a pure fraction, while contaminants concentrate in bran and gluten. In this context, it is necessary to gain insight in the current risks of mycotoxin contamination associated with these changing consumption patterns.

This research is in line with the transition targeted by the European 'Green Deal' and 'Farm to Fork Strategy'. In this respect, it is important that the transition is not at the expense of food safety.

Some concrete examples of indications of contamination:

- In 2020, Starch Europe revealed that significant levels of ergot alkaloids can be present in wheat gluten. In this fraction, the issue of ochratoxin A concentrations has been known for some time and was included in the contaminants regulation.
- The EFSA opinion from 2020 on ochratoxin A[i] highlighted the need for further research on ochratoxin A contamination in cheese. There are indications that the danger is primarily in the edible cheese rind, and its presence in processed cheese, grated and ground cheeses. This needs to be further studied, in order to take an appropriate policy decision.
- The project RF 16/6308 CITRIRISK[ii], funded by the FPS Public Health, revealed that vegetarian alternatives to meat are a source of citrinin intake. Further research is recommended.
- If cured meats are replaced by peanut butter as spread, the intake of aflatoxins increases. The EFSA opinion from 2020 on aflatoxins identified peanut butter as a relevant food group.

[i] EFSA Journal 2020;18(5):6113, https://doi.org/10.2903/j.efsa.2020.6113
[ii] RF 16/6308 CITRIRISK - The incidence of citrinin in the Belgian food and feed chain and the risk for human and animal health

In the case of deoxynivalenol, the study should include all congeners included in the EFSA's tolerable daily intake (TDI) group: DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside. More data is also needed on this series of mycotoxins in whole-grain wheat products, to ascertain whether it is necessary to better protect consumers who consume whole-grain products, by extending the standard for DON to total DON. Up-to-date data are required in this regard, as fungal populations evolve under the influence of climate change. A comparison with existing data can shed light on the evolution over time.

In the study, it is advisable to consider good practices for the prevention and reduction of mycotoxin contamination for the studied foodstuffs, such as the sorting of mouldy products.

The research should be policy oriented and is not intended to verify compliance with current legislation and standards (such as ochratoxin A in wheat gluten). Nor should its aim be the preparation of a novel food dossier. The results may be valorised by the Government in the context of developing new standards for combinations of mycotoxins and food groups (e.g. ergot alkaloids in wheat gluten). In this context, the research should develop new knowledge.

Objectives

- On the basis of targeted sampling and analysis: study into mycotoxin contamination of vegetarian protein-rich foods and vegetarian protein-rich ingredients, including ochratoxin A in processed cheese, grated and ground cheese.
- On the basis of targeted sampling and analysis: study into mycotoxin contamination of fibre-rich by-products and cereal products valorised as a foodstuff, from cereals which are not widely consumed.
- On the basis of targeted sampling and analysis: study into total deoxynivalenol levels in cereal products (pasta, wholemeal wheat bread, etc.): DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside.
- Based on the results of the study: identification of high-risk combinations of mycotoxin/foodstuffs to be monitored and possible critical factors of good practice.

Maximum budget: € 150,000

Maximum duration: 18 months

8. Intake monitoring of food flavourings (INFLAVOUR)

Context

The European Commission is currently developing a "Guidance for Monitoring of the Consumption and Use of Food Additives and Food Flavourings". This document is a first step in establishing monitoring guidelines for food flavourings, which is mandatory under Article 20 of the Flavouring Regulation (EC) No 1334/2008. The aim is that Member States establish risk-based systems to monitor the consumption and use of the flavourings set out in the Community list (Annex I of the Flavouring Regulation) and the consumption of the substances listed in Annex III. Member States must collect information on the consumption and use of flavourings in order to assess, via intake estimates, whether the intake is safe.

The RT 18/08 MULTIMADD[i] project has shown that analytical methods can be developed to measure a whole range of additives at the same time. In the ongoing project RT 19/07 FLAVOURAN 1[ii], a multimethod approach is also being developed, to analyse (potentially) genotoxic flavouring substances in food. Multimethods also proved successful for analysing plant toxins, mycotoxins and pesticides.

For this first monitoring ('pilot monitoring'), the aim is to use a multimethod approach to analyse relevant flavourings, calculate a preliminary intake and carry out a risk assessment. In selecting flavourings, the priorities listed in the Commission's guidance document, analytical capabilities and other relevant information should be taken into account. The selected flavourings for this pilot monitoring should be from Annex III of the Flavouring Regulation 1334/2008 and from the EU list of approved flavourings (Annex I). Smoke flavourings are excluded from the study.

Objectives

1. Selection of priority flavourings for intake monitoring using (the working document of) the European guidance document and other relevant information (flavouring groups, concerns for intake above the threshold of concern, analytical capabilities, intake model in the EFSA opinion, etc.).
2. Developing a sampling plan so that a preliminary intake estimate can be calculated (analysis of different foodstuffs in different food categories). In this regard, it can also be investigated whether the analysis of intermediate flavouring preparations could be used instead of the analysis of end products.
3. Development and validation of (several) multimethod(s) for the analysis of selected flavourings in different foodstuffs.
4. Perform preliminary intake calculations based on the analysis data.
5. Carry out a risk assessment (compare the calculated intake with the intake taken into account in the EFSA evaluation for that flavouring).
6. Enter the findings of the study into standardised templates to facilitate the transfer of the data to the European Commission.

[i] RT 18/08 MULTIMADD - Development of a multi-method for the analysis of additives in foodstuffs.
[ii] RT 19/07 FLAVOURAN – Analysis of genotoxic flavouring substances in foodstuffs.

7. Evaluation of the applied methodology for intake monitoring with a view to future monitoring programmes.

Maximum budget: € 300,000

Maximum duration: 36 months

2021-C-368 Heat- (incl. hot water) treatments

Short description

Hot water treatments can be used on Vitis against *Viteus vitifoliae* (EPPO Standard PM 10/16), against Grapevine flavescence dorée phytoplasma (EPPO Standard PM 10/18) and considered efficient against *X. fastidiosa* (EFSA, 2015). The question was raised whether other time-temperature combinations should be used to reduce plant mortality.

It would be useful to compare how these treatments are done in practice in different countries. Heat- treatments can also be used on strawberry plants to control *Aphelenchoides besseyi* and *Aphelenchoides fragariae* (EPPO Standard PM 10/19). Hot air treatments have been shown to eliminate *Verticillium dahliae* from Olive plants (Morello et al., 2016). The use of these treatments should be investigated for other pest/host combinations (e.g. on Olive plants against *X. fastidiosa*). These treatments could be used for the exportation or circulation of plant reproductive material from infected areas, or in the context of certification schemes[i].

Description of the end product

Validation of heat-treatments as phytosanitary measures.

Provisional other funders

- European and Mediterranean Plant Protection Organization, France (Contact: Ms Françoise Petter, fp@eppo.int)
- Council for Agricultural Research and Economics, Italy (contact: Mr Luca Riccioni, luca.riccioni@crea.gov.it)
- Ministry for Primary Industries, New Zealand (contact: Ms Aurélie Castinel, Aurelie.Castinel@mpi.govt.nz)
- Canadian Food Inspection Agency – Plant Research & Strategies, Canada (contact: Ms Brittany Day, brittany.day@canada.ca)
- Department for Environment Food and Rural Affairs, United Kingdom (contact: Mr Iain dummett, Iain.Dummett@defra.gov.uk)
- Direction-General for Food and animal health, Portugal (contact: Ms Paula Cruz Decarvalho, pcarvalho@dgav.pt)
- Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel (contact: Ms Yael Meller Harel, YaelM@moag.gov.il)
- All Russian Plant Quarantine Center, Russian Federation (contact: Mr Yuri Schneider, yury.shneyder@mail.ru)
- Ministry of Food Agriculture and Forestry, General Directorate of Food and Control, Turkey (contact: Mr Yunus Bayram, yunusbayram@tarimorman.gov.tr)
- Department of Agriculture, Water and the Environment, Australia (contact: Mr Con Goletsos, ACPPO@agriculture.gov.au)

Provisional project duration

24-36 months

[i] Pour les recherches pour lesquelles un subside du SPF Santé publique est sollicité, les demandeurs doivent se limiter aux organismes de quarantaine et aux mesures relevant de la compétence du SPF Santé publique.

2021-A-373 Fast detection methods for quarantine Tephritidae (TEPHRIFADE)

Short description

Non-European Tephritidae are categorised as quarantine pests (EU 2019/2072, annex II A). Furthermore, *Anastrepha ludens, Bactrocera dorsalis, Bactrocera zonata* and *Rhagoletis pomonella* have been included in the list of priority pests (EU 2019/1702). Identification of intercepted and detected Tephritidae to genus or species level is important for adequate follow-up, risk assessment and evaluation of measures.

The list of non-European Tephritidae was analysed in more detail by EFSA (2020). The EFSA pest categorization is taken on board in the ongoing discussions on the revision and possible amendment of the EU quarantine pest list, preferring a classification at species (or genus) level. If a modification to genus/species listing enters into force, it is even more important to have diagnostics adapted to that level for all life stages and in particular for the most intercepted ones (larvae). Morphological identification methods exist for adult and later larval stages, whereas identification of the most intercepted earlier stages currently requires upfront rearing or sequencing. Alternative methods that are faster and potentially applicable on-site are under development on a national level and in European projects (e.g. FF-IPM). Moreover, fast detection methods are preferred as the majority of interceptions relate to perishable goods.

Potential objectives

- Compilation of an international inventory of fast diagnostics (for example but not exclusively LAMP tests) for Tephritidae genera and species that are currently available or being developed.
- Transnational exchange of protocols and best practices, and organisation of interlaboratory tests among the partners for specific fast detection methods.
- Compilation of an overview of available sequences necessary for (more classical) diagnostics for Tephritidae genera and species and identification of gaps.
- Collecting type species and performing sequencing experiments in order to fill the identified gaps.

Description of the end product

Inventory, enhanced knowledge and knowhow of fast methods for the detection of Tephritidae at genus and species level.

Expanded panel of available sequences for Tephritidae species.

Provisional other funders

- Canadian Food Inspection Agency – Plant Research & Strategies, Canada (contact: Ms Brittany Day, brittany.day@canada.ca)
- Federal Ministry for Sustainability and Tourism, Austria (contact: Ms Sylvia Bluemel, sylvia.bluemel@ages.at)
- Ministry for Primary Industries, New Zealand (contact: Ms Aurélie Castinel, Aurelie.Castinel@mpi.govt.nz)
- Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel (contact: Mr Abed Gera, AbedG@moag.gov.il ; Ms Yael Meller Harel, YaelM@moag.gov.il)
- Department for Environment Food and Rural Affairs, United Kingdom (contact: Mr Iain dummett, Iain.Dummett@defra.gov.uk)

- Ministry of Foreign Trade and Economic Relations Administration of Bosnia & Herzegovina for Plant Health Protection, Bosnia and Herzegovina (contact: Ms Ajla Dautbasic, ajla.dautbasic@uzzb.gov.ba)
- Federal Ministry of Food and Agriculture, Germany (contact: Ms Silke Steinmöller, silke.steinmoeller@julius-kuehn.de)
- Ministry of Agriculture, Tunisia (contact: Mr Mohamed Lahbib Ben Jamaa, benjamaaml@gmail.com)
- US department of Agriculture, Animal and Plant Health Inspection Service, USA (contact: Ms Jennifer Nicholson, jennifer.s.nicholson@usda.gov)
- Ministry of Agriculture Forestry and Food, Slovenia (contact: Ms Erika Oresek, erika.oresek@gov.si)
- Department of Agriculture, Water and the Environment, Australia (contact: Mr Con Goletsos, ACPPO@agriculture.gov.au)
- National Plant Protection Organization, Netherlands Food and Consumer Products Safety Authority, Netherlands (contact: Mr Martijn Schenk, M.Schenk1@nvwa.nl)

Provisional project duration

24-36 months

2021-A-378 Inventory and validation of quality control procedures for the extraction of nucleic acids used for diagnosis.

Short description

Diagnostic activities for phytopathogenic organisms concern organisms with DNA genomes such as fungi, bacteria or certain families of plant viruses, but also other organisms whose genome is composed of RNA, such as the majority of plant viruses or viroids. The titer of these organisms in infected tissues can sometimes be high, but in many cases involving bacteria, phytoplasmas or viruses infecting seed lots, dormant tubers and lignified tissues, the titer can also be very low, close to the detection limit of diagnostic tests. Given this diversity of situations, quality control of the extraction is an important element required to deliver a negative diagnosis on a sound and standardised basis.

To date, the different control procedures for the extraction step are not always applicable or relevant and when they are, they are rarely validated and formalised in the form of recommended procedures and threshold values. The aim of this project is to take stock of the extraction procedures used in the participating laboratories and in the literature. These procedures will be tested and compared on a wide range of plant matrixes infected with pathogens of interest in order to formulate recommendations for diagnostic laboratories.

Description of the end product

Results of the comparative tests carried out in the different laboratories. The participants will formulate recommendations in the form of a written communication to the diagnostic laboratories.

Provisional other funders (*to be completed in a later stage*)

- Federal Office for Agriculture, Switzerland (Contact: Mr Andreas von Felten, andreas.vonfelten@blw.admin.ch)
- Council for Agricultural Research and Economics, Italy (contact: Mr Luca Riccioni, luca.riccioni@crea.gov.it)
- Ministry for Primary Industries, New Zealand (contact: Ms Aurélie Castinel, Aurelie.Castinel@mpi.govt.nz)
- Central Institute for Supervising and Testing in Agriculture, Czech Republic (Mr Michal Hnizdil, michal.hnizdil@ukzuz.cz)
- Federal Ministry of Food and Agriculture, Germany (contact: Ms Silke Steinmöller, silke.steinmoeller@julius-kuehn.de)
- Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel (contact: Ms Yael Meller Harel, YaelM@moag.gov.il)
- Ministry of Food Agriculture and Forestry, General Directorate of Food and Control, Turkey (contact: Mr Yunus Bayram, yunusbayram@tarimorman.gov.tr)
- US department of Agriculture, Animal and Plant Health Inspection Service, USA (contact: Ms Jennifer Nicholson, jennifer.s.nicholson@usda.gov)
- All Russian Plant Quarantine Center, Russian Federation (contact: Mr Yuri Schneider, yury.shneyder@mail.ru)
- Ministry of Agriculture Forestry and Food, Slovenia (contact: Ms Erika Oresek, erika.oresek@gov.si)
- Forestry Commission, United Kingdom (contact: Ms Joan Webber, joan.webber@forestresearch.gov.uk)

- Department of Agriculture Food and the Marine, Ireland (contact: Ms Maria Laura Destefanis, Maria.Destefanis@agriculture.gov.ie)
- Department of Agriculture, Water and the Environment, Australia (contact: Mr Con Goletsos, ACPPO@agriculture.gov.au)
- National Institute for Agricultural and Veterinarian Research, Portugal (contact: Ms Leonor Cruz, leonor.cruz@iniav.pt)

Provisional project duration

12-24 months