Annex 3
Template RT full proposal (step 2)

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CONFIDENTIAL

FULL THEMATIC PROPOSAL (RT PROJECT step 2)

FOOD SAFETY

PFASFORWARD

Research on PFAS contamination in the food chain

Total budget required for the research:	~~€ 400 000~~ € 440.000[i]
Requested research grant and % of the overall budget:	~~€ 400 000~~ € 440.000[i] 100 %
In which case: origin / nature of own contribution:	Not applicable
Proposed start date:	01/06/2023
Proposed duration of the project:	48 months

[i] Cf. PV Beoordelingscomité 16/02/2023

1. IDENTIFICATION OF THE CONSORTIUM

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2. HISTORY OF CHANGES

Have significant changes been made to the full proposal compared to the pre-proposal?
All the remarks received after submitting the pre-proposal have been considered. More specifically:
- The link with PARC is clarified (see 4.3)
- Process factors that will be evaluated are clarified (see 4.1 – WP3)
- FPS agreement before communicating to the popular press is clearly specified (see 3.6)
- Transfer studies are fully described (see 4.1 – WP4)

3. GENERAL INFORMATION

3.1 Title of the project in English, Dutch and French + acronym

Research on PFAS contamination in the food chain

Onderzoek naar PFAS-verontreiniging doorheen de voedselketen

Recherche sur la contamination par les PFAS dans la chaîne alimentaire

PFASFORWARD

3.2 Research questions to be answered in this research project, in English and Dutch or English and French

(1) Which PFAS are most relevant, and in which type of food?
In the past, most studies focussed on the occurrence of some per- and polyfluoroalkyl substances (PFAS), although many more PFAS exist. Therefore, monitoring a wide range of PFAS using sufficiently sensitive analytical methods is essential. Some ongoing projects (including Fluorex) will already provide extensive monitoring data, but other relevant commodities should be investigated further.

(2) What are the behaviour and distribution of PFAS within food?
Little is known about the distribution of PFAS in food (e.g. peal vs fruit flesh/pulp, different parts of the animal). Such knowledge, specifically about edible parts, is essential for policy, establishing risk management measures, and estimating the impact of regulations.

(3) What is the impact of food processing on the occurrence of PFAS?
The European Commission requested information on concentrations of PFAS in raw and processed food from the same batch of raw products to determine processing factors and their impact. In particular, processing factors for cheese, whey powder, fine bakery wares with a high yolk egg content, and liver-meat products are relevant and recommended to be studied.

(4) What are the relevant sources and contamination pathways of PFAS in the food chain?
More knowledge on the origin and transfer of PFAS throughout the food chain should be gained through literature review, experiments or simulations in order to be able to reduce or prevent possible contamination and, as such, guarantee consumers' food safety. Possible PFAS transfer from compost, sewage sludge, irrigation water, well water, soil, feed and feed materials will be investigated.

(1) Quels sont les PFAS les plus pertinents, et dans quel type d'aliments ?
Dans le passé, la plupart des études se sont focalisées sur l'occurrence de quelques substances per et polyfluoralkylées (PFAS), bien qu'il en existede nombreuses autres. Par conséquent, il est donc nécessaire de surveiller une plus large palette de PFAS en utilisant des méthodes analytiques suffisamment sensibles. Certains projets en cours (dont FLUOREX) fourniront déjà des données de surveillance importantes, mais d'autres denrées pertinentes doivent être étudiées plus en détails.

(2) Quels sont le comportement et la distribution des PFAS dans les aliments ?
On sait peu de choses sur la distribution des PFAS dans les aliments (par exemple, dans la peau par rapport à la chair/pulpe de fruit, ou dans les différentes parties d'un animal). De telles connaissances, particulièrement pour les parties comestibles, sont pourtant essentielles pour les politiques, en particulier pour l'établissement de mesures de gestion des risques et l'estimation de l'impact des réglementations.

(3) Quel est l'impact de la transformation des aliments sur la présence de PFAS ?
La Commission européenne a demandé des informations sur les concentrations de PFAS dans les aliments crus et transformés provenant du même lot de produits bruts afin de déterminer les facteurs de transformation et leur impact. En particulier, les facteurs de transformation pour le fromage, la poudre de lactosérum, les produits de boulangerie fine à forte teneur en jaune d'œuf et les produits à base de viande de foie sont particulièrement intéressants et il est recommandé de les étudier.

(4) Quelles sont les sources pertinentes et les voies de contamination des PFAS dans la chaîne alimentaire ?
Il convient d'acquérir d'avantage de connaissances sur l'origine et le transfert des PFAS tout au long de la chaîne alimentaire par le biais d'une analyse documentaire, d'expériences ou de simulations, afin de pouvoir réduire ou prévenir une éventuelle contamination et, ainsi, garantir la sécurité alimentaire des consommateurs. Le transfert possible des PFAS à partir du compost, des boues d'épuration, de l'eau d'irrigation, de l'eau de puits, du sol, des aliments pour animaux et des matières premières pour aliments des animaux sera étudié.

3.3 Executive summary of the project

The main objective of PFASFORWARD is to gain insights concerning the presence, prevalence, behaviour, and distribution of various per- and polyfluoroalkyl substances (PFAS) throughout the entire food chain, including the impact of processing. The pathways by which PFAS can enter the feed and food chain will also be investigated.
PFASFORWARD will focus on the PFAS listed by Commission Recommendation (EU) 2022/1431[1], mainly 4-EFSA-PFAS, carboxylate-PFAS (C4-C14), sulfonate-PFAS (C4-C13), and when possible without compromising method sensibility emerging PFAS (FOSA, DONA, F53B minor and major, HFPO-DA, capstone A and B, fluorotelomers sulfonates and alcohols). Furthermore, untargeted analyses will also be performed for a limited number of samples to extend the analyte scope and envisage a broader range of fluorinated contaminants.
Firstly, the monitoring data obtained within FLUOREX will be further complemented by adding matrices from the market. Afterwards, PFAS behaviour and distribution will be investigated by analyzing different contaminated edible fractions of the same animal (including e.g. fish, pig, cattle and crustaceans) or plant (including e.g. apples, potatoes, carrots, beans,

oilseed rape). Since the concentrations of PFAS can vary between the different parts, the impact of food recipe (product composition/formulation and/or fractionation) will be investigated experimentally (e.g. juice pressing, grain milling, production of dairy products) and theoretically (e.g. fine bakery wares with a high yolk egg content, liver pate or black tripe). It should also be taken into account that this might be influenced by the type of PFAS (e.g. long-chain versus short-chain compounds).
Additionally, more knowledge on the origin and transfer of PFAS throughout the food chain will be obtained through literature review, experiments or simulations in order to be able to reduce or prevent possible contamination and, as such, guarantee consumers' food safety. Information on possible PFAS transfer from compost, sewage sludge, irrigation water, well water, soil, feed and feed materials will be gathered.
PFASFORWARD will not only give an overview of the presence of PFAS on the Belgian market but also respond to issues highlighted in the Commission Recommendation (EU) 2022/1431 on PFAS monitoring in food[1].

3.4 Motivation for submitting the project proposal under this topic

From different angles, there is a request for more occurrence data on PFAS and to increase knowledge on the impact of food processing. The results obtained in PFASFORWARD will be valuable to support policymakers' decisions concerning future European regulation to manage the risks accordingly in order to be able to guarantee consumers' food safety.
Furthermore, the PFASFORWARD project aligns with the Commission Recommendation (EU) 2022/1431 on PFAS monitoring in food[1] and will respond to the highlighted issues: (i) extend the list of PFAS and relevant food, (ii) investigate PFAS distribution within foodstuffs, and (iii) monitor feed and feed material.
Next, this research will generate relevant data to implement Article 2 of Regulation (EU) 1881/2006[2] to define the process factors regarding dried, diluted, and processed compounds in foodstuffs.
Additionally, the results can be of particular importance to the Federal Agence for Safety on the Food Chain (FASFC) since they will give an overview of the presence of PFAS on the Belgian market, and they could also be used to determine the background contamination of the food chain in Belgium, thereby serving as a reference point for future monitoring, as recommended in the SciCom on PFAS (2021/13)[3].
Finally, the results obtained in this project will be directly relevant for PARC[4], specifically in task 4.2. regarding 'Environmental and multisource monitoring'. More details are given in **Chapter 4.3**.

3.5 Context: scientific specificity and setting in relation to existing research

PFAS are manufactured chemicals used for various applications (e.g. textiles, household products, fire-fighting, automotive, food processing, construction, electronics)[5]. Due to their persistent, toxic and bioaccumulative character, exposure to these chemicals may lead to adverse health effects. In 2020, the European Food Safety Authority (EFSA) set a new tolerable weekly intake (TWI) of 4.4 ng per kg of body weight (bw) per week for the sum of four PFAS (4-EFSA-PFAS) (i.e. perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS)) due to the effects observed in humans and animals, toxicokinetics and observed levels in human blood, particularly decrease of immune response to vaccination in human studies (mother/child)[6]. Animal- and plant-based commodities contribute to the consumers' exposure with an exceedance of the EFSA-TWI in Belgium for all age categories (based on a lower-bound average exposure), except for adolescents[6]. FASFC has monitored 4-EFSA-PFAS in the Belgian food chain. Belgian monitoring data and data from hot spot regions can be found in the PFAS reports of the Flemish Government published in 2021-2022, with a continuation of data gathering in upcoming reports (see PFAS-vervuiling | Vlaanderen.be). Recently, the Commission Recommendation (EU) 2022/1431 on PFAS monitoring was published[1]. An extensive PFAS list to be measured in a wide range of foodstuffs to prepare for the future policy was included in this recommendation. Therefore, there is an urgent need to address the simultaneous and sensitive analysis of various PFAS in several relevant matrices.

The agrifood chain's entry, transfer and bioaccumulation of PFAS may originate from different sources. The presence of PFAS in the environment has been described for water, soil, and air. Contaminated water, soil particles (e.g. in the case of poultry with outdoor exposure or via silage in the case of cattle), feed crops and substrate (e.g. bedding material) are among the possible sources of PFAS for animals. The transfer from the placenta and milk could be an additional exposure route for young animals. The uptake and transfer of PFAS from contaminated water and/or soil have been described for plants.
Information on potential PFAS contaminations from compost, sewage sludge, irrigation water, relevant feed materials, and animal feeds is limited, although it might significantly impact consumers' exposure. Moreover, information on transfer to plant- and animal-based commodities is even more limited or lacking. Especially the new fertilizing products Regulation (EU) 2019/1009[7], which allows using recycled and organic materials and has been in force since July 2022, necessitates investigating such matters and poses challenges in this regard. This regulation also applies to using different water (re)sources in agrifeed/-food applications in periods of drought, a phenomenon that has been increasing and will continue to increase in coming years. Concerns on the safe use of water (re)sources in terms of feed/food producing conditions underline this need.

As a result, PFAS have been found in animal- and plant-based food products. The highest PFAS concentrations characterize the former. The PFOS exposure of the Belgian population mainly originates from fish, shellfish, meat and meat products and eggs and egg products[3]. Specifically, high concentrations were found in wild animals and offal meat. Due to the high diversity of PFAS, their behaviour and uptake, the transfer and bio-accumulation of PFAS in plant- and animal-based matrices and their fate during processing can vary widely. In addition, little is known about how PFAS are distributed in foodstuffs and the impact of the food recipe (product composition/formulation and fractionation) on the PFAS content in the final product (process factors), although it is generally assumed that PFAS are stable in food during processing steps like heating. However, very few studies investigated the true impact of processing factors such as fractionation, and this knowledge is essential for policy to establish relevant risk management measures. Furthermore, it is still unclear which fractions of the food contain PFAS and in which concentration, but this information is needed because of Regulation (EU) 1881/2006[2]. Finally, the recent PFOS crisis at the site of 3M in Antwerp has created awareness of the importance of PFAS investigations.

Therefore, it can be concluded that the possible origins and routes through which PFAS may enter the food chain need further investigation. PFASFORWARD aims to provide an answer to these research needs. The methodology presented in **Chapter 4.1** of this proposal can be justified. Indeed, it will not only allow generating data on contamination levels in relevant matrices, but it also looks beyond since possible transfer of PFAS and the impact of product composition/formulation and fractionation on PFAS contamination in feed and food will be investigated. In addition, the project focuses on more than just the 4-EFSA-PFAS for which a

TWI was set in July 2020. More specifically, PFAS, for which there are already indications of their presence in water, food of plant origin and animal origin (and human blood), are included in the analyses foreseen in the current project proposal. Such a broad scope in terms of food/feed matrices, PFAS, their possible origin/sources, the impact of the food recipe (product composition/formulation and fractionation) and their transfer will provide a better insight into aspects for which current data are limited or even lacking.

The consortium has extensive expertise in PFAS-related research. The different partners of the consortium are involved in the following ongoing or future projects:

- FLUOREX' Exposure assessment of perfluoroalkyl substances as follow-up on the concerns raised in the recent draft opinion of EFSA' (FPS Health, Food Chain Safety and Environment, shortened FPS Health, RF 21/6350, 2021-2023). In this project, the dietary exposure of the Belgian population to PFAS is investigated. Different methodologies for an extensive list of PFAS in line with the Commission Recommendation (EU) 2022/1431[1], with low limits of quantification (LOQ), are developed and validated. These sensitive methodologies will also be applied within PFASFORWARD.
- PARC' European Partnership for the Assessment of Risks from Chemicals' (Horizon Europe, 2021-2027)[4]. In PARC, one of the projects in T4.2 on multisource monitoring is focused on PFAS. More information is given in Chapter 4.3.
- Human Biomonitoring Project 'Project milieu en gezondheid- aandachtsgebied 3M' (Flemish Government 2022-2023),
- Development of a biomonitoring tool to estimate risks of perfluoroalkyl acids (PFAS) through consumption of self-cultivated food products (PhD fellowship FWO 2020-2023),
- "Are internal concentrations of micropollutants in aquatic organisms predictive of the ecological quality of water courses?" (FWO 2019-2023),

Other research proposals on PFAS will be submitted. Example is :

- PFASolEau (2023-2027), Development of a framework for assessing PFAS pollution in soil and water in Wallonia, based on the combination of a flexible analytical protocol and in situ toxicity tests (Greenwin project, to be resubmitted in November 2022).

An overview of PFAS research conducted within a Belgian context can be consulted online (Rapport_3_PFAS_opdrachthouder_15.07.2022_myrr5y.pdf (vlaanderen.be)).

3.6 Use of the expected research results

The results obtained in the project will be communicated to the FPS Health and can be used to support policymakers' decisions concerning future European regulation (new matrix and new substances). Furthermore, the results could be used by the FASFC to support the future strategy for the monitoring program. The relevant data will also be reported to EFSA using the SSD format, enabling EFSA to update their PFAS exposure and risk assessment. As for scientific output, the results will be published in peer-reviewed scientific publications and presented in (inter)national scientific symposia. Specific attention will be paid to informing the Belgian population and the food and feed sector by presenting the results to the popular press after the agreement from FPS Health. Finally, all people providing samples will receive individual information on the concentrations in their vegetables, compost, etc. The FPS Health will be involved in communicating the results, and their approval will be requested before publishing.

3.7 Risks

Risks associated with the project proposal are expected to be limited since the expertise of the different partners is complementary. However, one of the critical steps in the project is the

availability of contaminated samples to investigate the distribution of PFAS in food (**WP2**) and the impact of food recipe (product composition/formulation and fractionation) (**WP3**). It has been shown in previous research (e.g. FLUOREX and FASFC monitoring) that most samples on the market have PFAS concentrations below the limit of quantification (LOQ) and, therefore, will not be relevant to support **WP2 & WP3.** Probably, the consortium partners have access to contaminated food (e.g. plant products (fruits, vegetables and cereals), eggs and fish via SC-CONT; plant products, eggs and fish from ECOSPHERE-UA). Nevertheless, contaminated samples in Belgium will only be contaminated with certain relevant PFAS (and not all of them). Alternatively, some types of food processing can also be investigated using fortified batches (e.g. milk), although sampling of naturally contaminated samples will be preferred. The sampling of contaminated samples will be adapted if the number of foreseen samples is insufficient due to significant heterogeneity revealed within a contaminated sample type or matrix. Regarding the transfer experiments in **WP4**, it could occur that the contaminated soil would be either excessively toxic and/or of too low fertility to support the full plant development. The contaminated soil will be mixed with standard organic soil to restore plant growth in such a case.
Finally, the risk associated with the analyses is relatively low since all partners are experienced analytical laboratories, and methods are already available for most of the WP (ECOSPHERE-UA (**WP4**) and SC-CONT (**WP1-4**)). If necessary, advice could always be requested at the EURL. However, it should be noted that adding neutral and positively charged compounds to the method will probably decrease the efficiency of the current purification method and, therefore, the sensitivity.

4. SPECIFIC INFORMATION

4.1 Scientific and operational methodology of the proposed research

The project's main objective is to gain insights concerning the presence, prevalence, behaviour, and distribution of various PFAS throughout the whole food chain, including the impact of processing. The pathways by which PFAS can enter the feed and food chain will also be investigated. An overview of the general work packages (**WP Y-Z**) and technical work packages (**WP 1-5**) of the project is given in figure 1:

The WPs have been restructured to clarify the remarks of the Expert Committee, specifically for WP1 (Identification of relevant samples and sampling). The different tasks of the previous WP1 have been transferred to the different corresponding WPs.

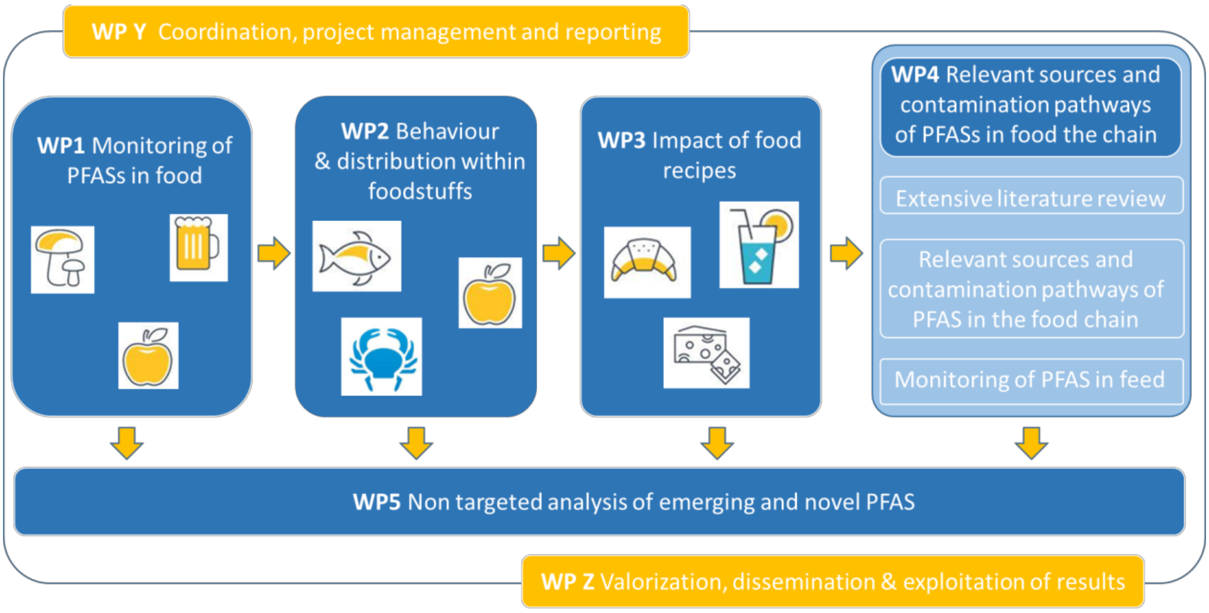

Figure 1: Workflow of the different work packages (WP) of the project

WP1: Monitoring of PFAS in food (RQ1)

Coordination: SC-CONT
Partner: /
Person-months: 7

Monitoring data regarding PFAS in food are already available from different sources (e.g. FASFC yearly control plan, FLUOREX, etc). These data will be further complemented following the Commission Recommendation (EU) 2022/1431[1]. This recommendation advised to monitor compounds: (i) 4-EFSA-PFAS, (ii) compounds which are similar to 4-EFSA-PFAS but have a different alkyl chain (PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PPFTrDS, FOSA), and (iii) emerging PFAS (the acid form of F53B, the acid form of GenX, the acid form of ADONA, capstone A, capstone B, fluorotelomer alcohols and sulfonates). The relevant matrices to monitor according to this recommendation are described in **task 1.1**.

Task 1.1 Sample selection for monitoring of PFAS in food

During the FLUOREX project, 280 individual food items were selected based on an exposure-driven sample collection. However, this sampling was too limited for certain food groups with potentially low contamination (cereals, dairy products) or for potentially contaminated food items with low consumption (mainly wild, gathered or hunted animals and mushrooms, including wild or gathered). Moreover, additional samples will complement the results obtained within the FLUOREX project, hereby also taking into account the organic production. The Commission Recommendation (EU) 2022/1431[1] and results from recent research will guide the sampling that will be discussed and approved by the steering committee. As indicated in the recommendation, the monitoring will include a wide variety of foodstuffs reflecting consumption habits, including fruits, vegetables (including wild and farmed mushrooms), starchy roots and tubers, seaweed, cereals, nuts, oilseeds, food for infants and young children, food of animal origin, non-alcoholic drinks, wine and beer. However, as the FLUOREX results are not yet finalized, a concrete sample plan cannot yet be proposed. In total, 300 samples will be selected on the market in close consultation with FPS Health and analyzed in this **task 1.3**.

Sampling and precaution for sampling will be done according to the Commission Implementing Regulation (EU) 2022/1428[8] and the Commission Recommendation (EU) 2022/1431[1]. After purchase or collection, the samples will be labelled with a unique number, and all available information about the different samples will be collected in a database (e.g. the date and place of purchase, lot number, detailed description of the food item and ingredient list). This database will contain information on product types and characteristics, which is of particular relevance for animal products (wild, gathered or hunted versus farmed non-organic production or farmed organic production; free range or outdoor production versus indoor production methods) and mushrooms (wild or gathered versus farmed) as describes in the Commission Recommendation (EU) 2022/1431[1]. This database can be further extended with other relevant information and will serve as a tool for evaluating and interpreting the results. Furthermore, this will facilitate reporting results in Standard Sample Description (SSD), an EFSA standardized model for data submission (**WP Z**).

Task 1.2 Extension of available methods

Currently, the methods available at SC-CONT include most of the advised substances: 4-EFSA-PFAS, other carboxylate-PFAS (C5-C14), sulfonate-PFAS (C4-C13), and PFAS substitutes (DONA, F53B minor and major forms and HFPO-DA). The method protocol includes a solid/liquid extraction followed by a two-step purification using solid-phase extraction (SPE) on weak anion exchange cartridges in combination with Envicarb cartridges. Analyses are performed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). The actual LOQ of SC-CONT methods are about 0.1 ng/g for eggs, 0.02 ng/g for milk, 0.05 ng/g for other animal origin products and 0.005 ng/g for plant products (fruits, vegetables and cereals). These methods are validated according to the EURL guidance document[9] and ISO 17025 accredited. These methods are sufficiently sensitive to reach at least the required LOQ indicated in the Commission Recommendation (EU) 2022/1431[1] for 24 compounds in milk, fish meat and meat of terrestrial animals, eggs, crustaceans and molluscs, edible offal of terrestrial animals and in fish oil. Moreover, these methods cover the totality of the analyzed matrix in this WP and will be available before the start of PFASFORWARD as they are developed in the FLUOREX framework. Additional developments will be performed to increase the number of compounds in the method, but it will be limited to negatively charged compounds (e.g. fluorotelomer sulfonate). Although it is an analytical challenge, neutral compounds (FOSA, fluorotelomer alcohols) and positively charged compounds (capstone A and B) will be included if the LOQs of negatively charged compounds are not compromised. Additionally, SC-CONT will try to reduce the high and not constant PFBA instrumental contamination to quantify this molecule at concentrations as low as possible.

Task 1.3 Analysis of the samples

Finally, 300 samples from the market, selected in **task 1.1**, will be analyzed. As indicated in the Commission Recommendation (EU) 2022/1431[1], only the edible portion of the foodstuffs will be analyzed. Fruits, vegetables, starchy roots and tubers should be washed before sampling while ensuring not to introduce additional PFAS contamination via the washing water. Food for infants and young children will be analyzed dry or liquid, as marketed.

The validated SC-CONT methods meet the criteria and the recommendations from EURL Guidance document[9] and the Annex C of the Commission Implementing Regulation (EU) 2022/1428[8]. A quality control plan will be implemented for each batch of samples to monitor the daily method's performance and ensure the reliability of the reported results.

A calibration curve ranging from LOQ level to 2 µg/kg will be injected at the beginning of each batch. The deviation of the back-calculated concentrations of the calibration standards from the true concentrations had to be not greater than ± 20 % to ensure proper quantification. Two standards, including the lowest calibration point equal to the LOQ, will be reinjected at the end of each batch to ensure that no loss of sensitivity or instrumental deviation has occurred during the batch (deviation ≤ 30 % for the end batch standards).

Measurements include analysis of procedural blanks to ensure that no contamination occurred during the extraction procedure nor in the LC-HRMS system. The contribution of blank levels will be assessed as ≤ 30 % of the LOQ in the procedural blank accompanying each batch.

Fortified samples will be prepared using a matrix similar to the batch analyzed to ensure no method deviation. Recoveries will be plotted on a control chart for the 4-EFSA-PFAS. Deviation of 20 % for the 4-EFSA-PFAS compounds and 35 % for the others are judged acceptable.

To ensure the experiment's effectiveness, the internal standard recoveries and identification criteria will be assessed for each batch and sample, following the same criteria as the validation (30-140 %). Where overall results fell outside this range, samples were re-analyzed.

Milestones & Deliverables

- *Food list containing detailed information on the samples purchased*
- *Sampling campaign agenda*
- *Determined PFAS concentrations in selected market food samples*

WP2: Behaviour and distribution within foodstuffs (RQ2)

Coordination: SC-CONT
Partners: ECOSPHERE-UA, TBP-InBioS
Person-months: 10

Task 2.1 Sample selection for investigation of behaviour and distribution of PFAS within the food

To explore the behaviour and distribution within the food, PFAS-contaminated samples are needed to allow quantification of PFAS present in different fractions of the food samples when the repartition within foodstuffs could be low in some fractions compared to others. They will be bought on the market or obtained using the network of the consortium developed in previous projects. Overall, six different kinds of food will be profiled and used in **WP2** for a total of about 300 analyses. The investigation plan will be discussed and approved by the steering committee.

Plant products

(i) Contaminated fruits and vegetables (e.g. apples, potatoes, carrots, beans): The selection of commodities will take into account the composition of the food (which hydrophobic and hydrophilic proteins, fats and water) in order to study the hypothesis of the presence of PFAS at the hydrophilic-hydrophobic interface. These samples can be obtained from previous research projects or thanks to the strong network between ECOSPHERE-UA and residents living close to the contaminated 3M site. Previous research has demonstrated that fruits and vegetables cultivated in private gardens in this area are contaminated with PFAS[3]. Relatively high concentrations of different PFAS were already detected in different types of vegetables or fruit from private gardens close to the 3M site. The PFAS profile depended on the type of vegetables or fruit, e.g. different profiles were found in leaves, roots, etc.

(ii) Contaminated plant tissues: a dedicated experimental set-up is available at TBP-InBioS for simulating PFAS transfer and distribution within plant tissues. Three plant-based foods of contrasted nature will be evaluated among : (i) grain from a cereal (wheat, *Triticum aestivum*); (ii) tuber (potato, *Solanum tuberosum*); (iii) pea (*Pisum Sativum*) and (iv) oilseed rape (*Brassica napus*). The experiments will be performed in dedicated indoor growth chambers where the main abiotic factors (temperature, humidity, light quality and intensity, photoperiod and CO_2 concentration) are strictly controlled to prevent any variability due to fluctuating environmental conditions (ULiège Phytotrons). For each species, the conditions and the culture scheme allowing the completion of a culture cycle and the harvesting of the different tissues and consumable parts will be defined beforehand. The transfer of PFAS to the plants will be assessed and compared on standard organic soil and PFAS-contaminated soil (e.g. from the 3M contaminated area), enabling an environmentally-relevant exposure. Two independent experiments (6 to 9 months culture cycle), each including two biological replicates (independent growth chambers), will be carried out.

Animal origin products

(iii) Contaminated fish samples: ECOSPHERE-UA has permission to catch fish from Blokkersdijk near the 3M site. From a former study conducted, it was clear that high concentrations of different PFAS were present in fish caught in Blokkersdijk. Additionally, a collaboration with INBO will allow obtaining fish (e.g. perch and eel) from other contaminated areas in Flanders. These areas will be identified based on the results of the current investigations of the Flemish Environment Agency (VMM).

(iv) Contaminated cattle meat: the autopsy service of the Veterinary Faculty of Liège will provide different pieces of some cattle raised in the open air without any infectious disease. The cattle selection will be guided by ongoing Wallonia research on PFAS in soil, from the PFASolEau project, if approved, or from potential future research in Wallonia.

(v) Contaminated crustacean: The results of the FLUOREX project have demonstrated that the highest PFAS concentrations were found in three subgroups: crab and sea-spiders, fish roe and shrimps and prawns in the fish and other seafood categories. The detection frequencies were higher than 40% for crabs and shrimps categories for at least 7 compounds. Therefore, other crustaceans samples (e.g. shrimps, crabs) will be collected.

(vi) Contaminated pigs: On the one hand, the FASFC will be contacted, and hopefully, the contact details of free-range pig farmers can be provided. On the other hand, hunters will be contacted to obtain pieces of the same wild boars to support the analysis of the Suidae family. In addition, at least 3 different pieces and kidneys of wild boars could be provided from the LECAHUNT project.

A maximum of 300 samples selected in **task 2.1** will be used to investigate the behaviour and distribution of PFAS, which will vary due to their physico-chemical properties. These samples will be carefully selected to guarantee an efficient investigation of the presence of PFAS in the different fractions.

Task 2.2 Samples subdivision

Different strategies will be implemented according to the type of food. This list of fractions for each of the 6 types of food to be considered may be revised/adjusted when starting the WP (based on scientific input and input from the steering committee). If the first results show heterogeneity in a matrix type, the number of samples in that type of matrix could be increased.

<u>Plant products</u>
A batch of about 3 to 10 contaminated samples will be separated into 2 or 3 different fractions (peel, core and leaf) and mixed separately for fruit and vegetable samples (e.g. apples, potatoes, carrots, beans). A minimum of 4 composite samples with 2 or 3 fractions for each of the 4 products will be considered.
Fractions of TBP-InBioS samples from each of the 4 biological replicates will be analyzed among wheat (steam, grain and bran), potato (peel, core and leaf), pea (pea pods, pea seeds and leaf) or oilseed rape (rapeseed, leaf) for the cultures on standard organic soil and PFAS-contaminated soil.

<u>Animal origin products</u>
In contrast, each contaminated sample will be considered individually for animal-origin samples according to their composition.
Three separate fractions will be considered for at least 20 fish samples of different sizes, fishing places and compositions (more or less fatty): filet, skin, and mix of offal.
For 5 beef samples, six fractions will be collected: liver, tongue, kidney, fat, fatty muscle (entrecote/rib steak), and lean muscle (steak). The preliminary liver analysis will enable the selection of the most contaminated beef.
Two fractions will be collected for at least 6 shrimp samples (meat and carcass/shell and three fractions for at least 6 crab samples (meat, offal, carcass/shell).
If the sampling is possible, five edible fractions will be collected for 5 pigs or wild boar samples: liver, kidney, fat, fatty muscle (spare ribs), and lean muscle (ham).

Task 2.3 PFAS analysis in each fraction of selected foodstuffs

A maximum of 300 analyses will be performed in this **WP2**, thereby applying the methods described in **WP1**. In combination with contaminated samples, the highly sensitive methods of SC-CONT will allow quantification of PFAS present in different fractions of the samples, even if repartition within foodstuffs is heterogeneous and is estimated to be low in some fractions depending on the matrice[6].

A summary of the proportion of each of the detected PFAS and total PFAS present in different fractions of each kind of foodstuff will be provided. The percentage of water and lipids in the relevant fractions will also be determined for plant and animal origin products, respectively and linked to the detected PFAS concentrations.

Milestones & Deliverables

- *Food list containing detailed information on the samples purchased and provided by (external) partners*
- *Sampling campaign agenda*
- *Data and knowledge on the behaviour and distribution of PFAS in selected foodstuffs*

WP3: Impact of food recipes: product composition/formulation and fractionation (RQ3)

Coordination: SC-CONT
Partners: ILVO, TBP-InBioS, CART, ECOSPHERE-UA
Person-months: 7.5

Since the concentrations of PFAS can vary throughout the different parts of the plants, fruits, vegetables or animals (**WP2**), the concentrations of end products might be impacted by the food recipe (product composition/formulation and/or fractionation) (e.g. fine bakery wares with a high yolk egg content, liver pate or black tripe, grain milling, juice pressing, production of dairy products). Furthermore, this might be influenced by the type of PFAS (e.g. long-chain versus short-chain compounds). The overall PFAS concentrations are expected not to change significantly during heating processes due to their high stability. Therefore, this will not be studied in this project.

The European Commission recommended collecting information on concentrations of PFAS in raw and processed products from the same batch of raw products and determining processing factors for various processed products, in particular, cheese, whey powder, egg yolk, fine bakery wares with a high egg content and meat products containing liver. Due to the potentially high contamination of starting products, processes including liver products and baked goods rich in eggs will be prioritized. Because of the high Belgian consumption[10], cereal milling fractions will also be given priority. This list of conditions and/or ingredients/matrices to be considered will be revised and adjusted, if necessary, based on scientific input and discussions with the steering committee, before the actual start of the WP.

Task 3.1 Selection of relevant primary ingredients

First, a list of primary ingredients of interest will be compiled based on a market study literature review, results of **Fluorex, WP1** and **WP2** and the Commission Recommendation (EU) 2022/1431[1]. For example, liver or yolk eggs will be selected. The list of primary ingredients will be discussed and adjusted based on input from the steering committee. Subsequently, a market survey will be performed, and a list of products containing the relevant primary ingredients will be established for processes with (**Task 3.3**) and without (**Task 3.2**) fractionation.

Task 3.2 Food recipe - determination by calculation (processes without fractionation)

In case there is no fractionation of an ingredient during the process, calculations will be performed for products composed of high-risk ingredient(s) (as defined in Task 3.1). Based on data on the quantity of PFAS present in such primary ingredients and calculations for different relevant recipes (homemade and industrial), different scenarios, including the worst-case scenario, will be developed. This will help risk managers to estimate which products have priority if a batch of highly contaminated primary ingredients would enter the food chain and estimate possible PFAS exposure through certain food products.

The exposure assessment conducted in the FLUOREX project (see **task 3.1)** allows for identifying the most relevant foods in which a specific primary ingredient is present and might be important for additional exposure. Examples thereof are products in which eggs or egg yolk are used (e.g. (pan)cakes, brioche), as well as products containing liver or animal blood (e.g. black tripe), and fish ingredients (e.g. fish tureen). The list of the relevant composed food products will need to be extended and discussed with the steering committee.

Task 3.3 Food recipe - experimental determination (processes with fractionation)

For processes during which fractionation takes place, it must be determined which fraction(s) is(/are) most relevant because of possible PFAS contamination and how this may impact the contamination in the final food prepared from the ingredients used. Experimental fractionation of the primary contaminated ingredient is necessary, and the differences between manufacturing at home and industrial processes should be considered. The following process factors (and corresponding matrices) will be considered: (i) juice pressing (apples, oranges), (ii) milling (wheat), (iii) brewing for cereals (wheat, barley), (iv) oil pressing (nuts), (v) production of butter and buttermilk, hard and soft cheese, and whey powder (milk). The list of relevant and feasible processes will be discussed with the steering committee.

<u>Sample selection for investigation of the impact of food recipe</u>

In order to investigate the impact of product formulation/composition and fractionation (**WP3**), a maximum of 100 analyses will be performed on contaminated food samples by preference. If contaminated material is unavailable, homogenized fortified starting material (e.g. fortified milk) could be used.

The contaminated samples can be obtained from the following:

(i) Previous research projects

(ii) A collaboration with ERM (<u>ERM - Environmental Resources Management</u>), implicated in the 3M crises and the analysis of fruits, vegetables and eggs from private gardens and maize from farms in the vicinity of 3M.

(iii) Samples needed for the investigation of behaviour and distribution of PFAS in food (**task 2.1**).

<u>Processing of contaminated food samples</u>

Processing experiments will be performed in Living Lab ILVO Food Pilot with kitchen-/labscale food processing equipment. Experiments will be conducted on a kitchen-/labscale basis considering a 1-10 kg starting amount with a minimum of three repetitions each and the inclusion of blank control samples.

<u>Analysis of different process parts at different steps</u>

In this task, 100 analyses, including contamination tests, will be executed by SC-CONT according to the method described in **WP1** for the most relevant processes identified. For each product process, different parts at different steps will be sampled for PFAS analysis.

Before starting the process, precautions, cleaning procedures with different solvents, and material tests will be taken to exclude external contamination due to the migration of PFAS from food contact materials.

Then, for all processes, analyses will be performed (i) on a batch of starting material from the same source where the PFAS concentrations are known, (ii) on the different parts of the end materials, and (iii) on the intermediate preparations, just before cooking, when it makes sense for the process. All other ingredients necessary for the recipe will be analyzed to ensure they are free of PFAS.

Cleaning procedures and material tests will be carried out to ensure that the material is free of PFAS at the end of each treatment batch.

The highly sensitive method of SC-CONT, in combination with contaminated samples, will allow quantification of the partitioning of each PFAS. For example, it is sufficient that PFAS concentration in the raw material is higher than 2 ng/g for eggs, 1 ng/g for other animal origin products and 0.1 ng/g for plant products (fruits, vegetables and cereals) to ensure that 5% is quantifiable with actual LOQ of SC-CONT methods in the end-up fractions. These necessary contamination levels in the starting material correspond to the current contamination level of some samples for some compounds already analysed by SC-CONT and ECOSPHERE-UA in the framework of other projects.

<u>Determination of the process factors obtained by project experiments</u>
Based on the concentrations determined in the starting material and the end products, the influence of process factors will be estimated as recommended in the Commission Recommendation (EU) 2022/1431[1] and summarised in a database. The PFAS determination in intermediate preparations, just before cooking, when it makes sense for the process, will allow us to estimate the influence of process factors during the production of processed food. At the same time, dilution or concentration effects due to the process will be determined from PFAS concentrations in the different end products. For example, worst-case process factors for most contaminated foodstuffs (e.g. liver products and baked goods rich in eggs) will be estimated in priority.

<u>Milestones & Deliverables</u>

- *Data on the presence and concentrations of PFAS in different primary ingredients*
- *Knowledge of possible risks associated with the inclusion of at(-high)-risk ingredients*
- *The impact of food recipe (product formulation/composition and fractionation) determined by calculation or experimentally*

WP 4: Relevant sources and contamination pathways of PFAS in the food chain (RQ4)

Coordination: ECOSPHERE-UA
Partners: ILVO, SC-CONT, CART
Person-months: 12

Task 4.1 Extensive literature review

ILVO will perform an extensive literature review concerning possible PFAS sources, uptake and contamination pathways, bioaccumulation and transfer of PFAS to edible animal-/plant-derived commodities. For the possible environmental sources of PFAS, information on occurrence and concentrations in compost, sewage sludge, irrigation water (from different origins), soil and feed will be searched. A study of the behaviour of PFAS in terms of uptake, transfer, bioaccumulation, bioavailability, etc. will be performed, taking into account the possible variation in terms of PFAS (e.g. physico-chemical characteristics) and feed/food matrix. Sufficient attention will be paid to uncertainties, restraints, sampling set-up, and methodologies' sensitivity for each publication included in this review. Since information will

not always be available, this WP will also allow identifying current knowledge gaps and related research needs to make science-based regulatory decisions.
Due to the recently regained and increasing interest in PFAS, the number of international publications on their presence in feed and food commodities, as well as their behaviour (e.g. transfer) in plant- and animal-based commodities, is rapidly increasing. All partners within the consortium will keep up to date with the most recent publications, and the consortium will construct a database of all relevant publications in the field of PFAS contamination within the food chain.

Task 4.2 Relevant sources and contamination pathways of PFAS in the food chain

In order to better understand the contamination of food, research into relevant sources and contamination pathways of PFAS in the food chain will be performed. In addition to a literature review (**task 4.1**), experiments (**tasks 4.2 and 4.3**) will be conducted.

UA-ECOSPHERE will investigate relevant sources and contamination pathways of PFAS in the food chain by analysis of PFAS in compost and sewage sludge used for fertilization. Samples will be collected primarily at relevant locations for the commercial food chain. However, as was stated earlier, PFAS-contaminated samples (with known or expected contamination) are also needed to allow quantification of PFAS present in the different relevant sources and thus to identify important pathways for PFAS to enter the food chain. Compost, originating from locations close to hotspots such as the 3M site, fire-fighting exercise areas, as well as sewage sludge from wastewater treatment plants, can contain high concentrations of PFAS (unpublished data UA-ECOSPHERE). Consequently, when applied for fertilization, it may contaminate the soil and groundwater, potentially resulting in increased uptake by plants and animals, including vegetables and chickens. Depending on the decisions made at the kick-off meeting with the scientific committee and the literature review (**task 4.1**), the protocol described below will be adapted accordingly to avoid experiments and analyses that have been done before.

More than 250 irrigation water, compost, sewage sludge, well water and soil samples (i.e. 50 samples per matrix) will be collected across Belgium to ensure sufficient variation in PFAS concentrations to enable statistically correlating PFAS concentrations in these matrices to those in the food items. These samples will be analyzed to clarify the possible contribution of these matrices to the transfer of PFAS toward food. These samples will be collected from the commercial sector and analyzed using UPLC-MS/MS. The method to analyze PFAS in environmental matrices is described elsewhere[11]. In addition, samples from private gardens can be included if the targeted number of samples cannot be collected solely from the commercial sector. UA-ECOSPHERE currently has good contacts with owners of private gardens near a PFAS hotspot and has experience recruiting volunteers for less-contaminated sites.

In addition, the bioconcentration factor (BCF) will be determined as the ratio of the concentration of a specific PFAS in a matrix to the exposure concentration. The BCF indicates how probable a chemical is to bioaccumulate from the environment or other sources. Therefore, the BCF can be used to identify relevant sources and contamination pathways of PFAS in fruit, vegetables, fish, poultry and cattle. In contrast to BCFs for most POPs the concentrations will not be expressed on a lipid basis but as the ratio of total concentrations and/or normalized for protein content.

Task 4.3 Feed material and animal feed analysis

Additionally, to better understand food contamination in the experiments conducted from **task 4.2** and the literature review from **task 4.1**, 100 feed samples will be selected to be analyzed by SC-CONT. In correlation with **WP3**, the contamination levels of the premixes and compound feed can be calculated from their constituent contribution. Therefore, this task will focus on the feed constituents that have not been investigated in the other WPs and that could either accumulate the PFAS in their structure or have a fraction of the contamination of the original foodstuffs (according to the Belgian Feed Association 2021 report, 42.7% of the raw feed produced in Belgium were food by-products). Thus, 100 samples will be allocated in consultation with FPS Health. As an initial proposition, the following allocation could be used:

- 40 samples for "vegetable feed", including plant parts and/or plants not intended for human consumption as well as food production waste used as feed ingredients, like alfalfa pellets, barley pellets, soybean meal, beat pulp, nutshell, wheat bran, etc.
- 40 samples for "animal by-products" such as animal and fish flour, dried blood, animal fat, etc.
- 20 samples for minerals used as additives like bleaching earth, sepiolite, copper sulphate, calcium carbonate, etc.

<u>Milestones & Deliverables</u>

- *Food and feed lists containing detailed information on the samples purchased and provided by (external) partners*
- *Sampling campaign agenda*
- *Database of national and international publications on the occurrence and behaviour of PFAS within the food chain*
- *Overview of state-of-the-art information on the occurrence and behaviour of PFAS within the food chain*
- *Identification of relevant sources and contamination pathways of PFAS in food items*
- *PFAS concentrations in selected feed*

WP5: Non-targeted analysis of emerging and novel PFAS

Coordination: CART
Partner: /
Person-months: 0 (PhD student hired from external financial resources)

In order to broaden the scope of the project and not only consider the PFAS selected (legacy and emerging lists[1]) in **WP1**, additional untargeted method developments will be performed. To this end, **WP5** is divided into two tasks.

Task 5.1 Development and optimization of untargeted approaches for emerging PFAS

The CART proposes to use novel methodologies by gas chromatography coupled to trapped ion mobility (TIMS) and high-resolution MS (GC-APCI-TIMS-MS) and liquid chromatography (LC) coupled to ion mobility and high-resolution MS (LC-ESI-TIMS-MS), with the option to run MS/MS experiments. The two approaches we advocate will focus on the untargeted analysis of either PFAS families by GC or LC. The acquisition mode in TOF will also enable the monitoring of potential PFAS metabolites (either volatile or more polar metabolites). The CART will make a new hybrid instrument available that they purchased recently in 2022 (Tims-Tof-Pro2) coupled to GC and UPLC and equipped with several sources (APCI and ESI, both in positive and negative modes). The sample preparation prior to analysis is a rather classical and generic approach for PFAS analysis with a solid/liquid extraction followed by a purification step using solid-phase extraction (SPE) on weak anion exchange cartridges in combination using SPE (Oasis Wax, 6cc). Clean-up can be adapted according to the families of emerging PFAS and is part of the analytical developments ongoing in the laboratory under other projects (FRIA, FNRS projects). PFASFORWARD will benefit from these parallel developments subsidized by other funds to transpose and apply here in the framework of **task 5.1**.

Data processing in an untargeted approach is the bottleneck of the methodology proposed. Thus we have developed an internal software[12] based on Kendrick Mass Defect (KMD) to filter and highlight families of (un)known compounds from very complex mass spectra. The strategy is summarised in Figure 2, where 4 molecular descriptors will be made available from the proposed hyphenated methodologies combined with advanced data treatment to gain more confidence in the identification process of untargeted emerging PFAS.

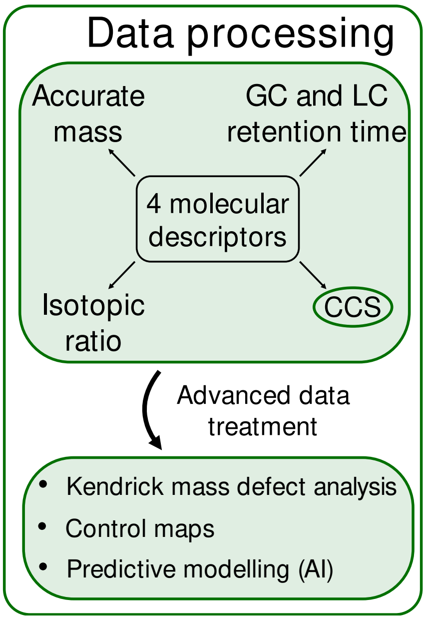

Figure 2: Overview of the data treatment strategy for the hyphenated method proposed here

Task 5.2 Analysis of relevant samples for emerging and novel PFAS identification

In this task, the methodology developed in **task 5.1** will be applied to a subset of 100 relevant samples from **WP1-4** (i.e. the ones with the highest contamination with legacy PFAS or other relevant criteria such as unusual pattern, specific identified hot spot, etc.). This proposed screening will be performed alongside the whole project according to the frequency given in Table 1. The idea here is to develop an extensive screening using non-targeted approaches to identify new families of PFAS that might be present in these relevant samples as much as possible. The analytical effort and data treatment will be focused mainly on the identification process rather than the individual quantification of unknown compounds.

<u>Milestones & Deliverables</u>

- *Method protocols (SOP) for task 5.1*
- *Screening of 100 relevant samples along the project in close connection with **WP1-4***

WP Y: Coordination, project management and reporting

Coordination: SC-CONT
Partners: All
Person-months:3

At the operational level, **WP Y** is designed to ensure that the project will be progressing in conformity with the work plan, particularly regarding the progress, milestones, deliverables, and planned resources.
At the organizational level, **WP Y** aims to achieve maximum efficiency of the infrastructure set-up between the different partners to support the project with particular attention paid to financial, logistics and coordination issues. This efficient organization will be done by organizing project meetings (kick-off, interim and final) to communicate information on the project's progress to the Steering Committee and funder but also to discuss any inputs to ensure good exploitation of the results and resources. Teleconferencing systems will be the partner's favoured communication tool as it allows short-notice scheduling and reduces travel time and costs. This WP will also include the maintenance of the project's information carry-ons after finishing.

<u>Milestones & Deliverables</u>

- *Approved meetings minutes*
- *Interim and final reports*
- *Partners meetings scheduling*
- *Establishment of a contact person for project activities after the end of the project*

WP Z: Valorization, dissemination and exploitation of results

Coordination: SC-CONT
Partner: all
Person-months: 3

This **WP Z** will focus on disseminating the results obtained to a broad audience, mainly policy makers and competent authorities but including also consumers and volunteers who provided samples. The **WP Z** will use a wide range of communication media from a dedicated compartment on the Institutes' website, through press releases, scientific publications and international conferences. After the FPS Health agreement, specific attention will be paid to informing the Belgian population and the food and feed sector by presenting the results to the popular press. After the FPS Health agreement, all people providing samples will receive individual information on the concentrations in their vegetables, compost, etc.

As for scientific output, the results will be published in peer-reviewed scientific publications and presented in (inter)national scientific symposia.

All results will be reported using the SSD, the EFSA standardized model for transmission of chemical occurrence data from different data providers to EFSA. The SSD contains approximately 20 mandatory data elements (e.g. analytical method, limit of detection, result, properties of the sample, etc.) that enable a unique description of each sample[13]. However, any changes in reporting concerning the sample and/or analytical results regarding SSD that entered into force during the project, will be considered.

Milestones & Deliverables

- *Formulation of recommendations for further monitoring*
- *Individual report for people providing samples*
- *Information available on institutions or dedicated websites and press releases*
- *Publications and oral/poster presentations*
- *Reporting of the results of samples in EFSA format*

An overview of the project and the corresponding timeline and budget is given in table 1.
Table 1: timeline and budget

	Year 1				**Year 2**				**Year 3**				**Year 4**				**Total budget per WP***
Code	**t1**	**t2**	**t3**	**t4**	**t1**	**t2**	**t3**	**t4**	**t1**	**t2**	**t3**	**t4**	**t1**	**t2**	**t3**	**t4**	
WP1	**x**	**x**	**x**	**x**	**x**												**60 000 €**
T1.1	x	x															
T1.2	x	x															
T1.3		x	x	x	x												
WP2	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**								**60 000€**
T2.1	x	x	x	x	x	x	x	x	x								
T2.2					x	x	x	x	x								
T2.3					x	x	x	x	x								
WP3							**x**	**x**	**x**	**x**	**x**	**x**	**x**				**80 000€**
T3.1							x	x	x								
T3.2									x	x	x	x	x				
T3.3									x	x	x	x	x				
WP4				**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**120 000€**
T4.1				x				x				x				x	
T4.2					x	x	x	x	x	x	x	x					
T4.3													x	x	x	x	
WP5	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**			**x**	**40 000€**
T5.1	x	x	x	x	x	x	x	x									
T5.2				x	x			x	x			x	x			x	
WP Y	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**20 000€**
WP Z				**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**20 000€**

t: trimester ; * total budget per work package: sum of staffing, operational and general costs

4.2 Structure and organization of the research

Table 2 Structure and organization of the research

Code	Task description	Contracting institution(s)	Required personnel*	PM
WP1	Monitoring of PFAS in food (RQ1)	SC-CONT		**7**
T1.1	Sample selection for monitoring of PFAS in food	SC-CONT	SW10	1
T1.2	Extension of available methods	SC-CONT	SW10	1
T1.3	Analysis of the samples	SC-CONT	SW10	5
WP2	Behaviour and distribution within foodstuffs (RQ2)	SC-CONT, ECOSPHERE-UA, TBP-InBioS		**10**
T2.1	Sample selection for investigation of behaviour and distribution of PFAS within the food	SC-CONT, ECOSPHERE-UA, TBP-InBioS	SW10 PhD	2 2
T2.2	Samples subdivision	SC-CONT	SW10	1
T2.3	PFAS analysis in each fraction of selected foodstuffs	SC-CONT	SW10	5
WP3	Impact of food recipes (RQ3)	SC-CONT, ILVO, CART, TBP-InBioS, ECOSPHERE-UA		**7.5**
T3.1	Selection of relevant primary ingredients	SC-CONT, ILVO	SW10 Scientist	1 0.25
T3.2	Food recipes - determination by calculation (processes without fractionation)	SC-CONT, ILVO	SW10 Scientist	1 0.75
T3.3	Food recipes - determination by project experiments (processes with fractionation)	SC-CONT, TBP-InBioS, CART, ECOSPHERE-UA, ILVO	Technician SW10	0.5 4
WP4	Relevant sources and contamination pathways of PFAS in the food chain (RQ4)	ECOSPHERE-UA, ILVO, SC-CONT, CART		**12**
T4.1	Extensive literature review	ILVO	Scientist	1
T4.2	Relevant sources and contamination pathways of PFAS in the food chain	ECOSPHERE-UA	PhD	7
T4.3	Feed material and animal feed analysis	SC-CONT, CART	SW10	4
WP5	Non-targeted analysis of emerging and novel PFAS	CART		**0**
T5.1	Development and optimization of untargeted approaches for emerging PFAS	CART	**	0

T5.2	Analysis of relevant samples for emerging and novel PFAS identification	CART	**	0
WP X	Coordination, project management and reporting	All partners	SW10	**3**
WP Z	Valorization, dissemination and exploitation of results	All partners	SW10	**3**

** Qualification; WP: work package; T: task; PM: person-months; SW10 - junior scientist;*
*** In addition, two PhD students from the MSLab (FNRS grants) will be involved in these developments in close connection to their PhD research. Thus, CART will ask only for operating costs in the frame of this PFASFORWARD project.*

4.3 Collaboration, complementarity and/or parallel applications

All consortium partners have extensive expertise in PFAS and are involved in numerous research projects and dedicated working groups.

SC-CONT conducts the FLUOREX project (2021-2023). Moreover, SC-CONT participates in a working group dedicated to PFAS of the European Reference Laboratory (EURL), which drafted and published the guidance document on analytical parameters to determine PFAS in Food and Feed in March 2022. Finally, in 2022, SC-CONT obtained an ISO 17025 flexible scope of BELAC to analyze all PFAS in all foodstuffs by LC-MS.

SC-CONT, ILVO and ECOSPHERE-UA are involved in the European Partnership for the Assessment of Risks from Chemicals (PARC, https://www.anses.fr/fr/system/files/PARC_Participating%20institutions%20List.05.2022.pdf [4]), specifically in task 4.2. regarding 'Environmental and multisource monitoring'. SC-CONT is a core team member for laboratory analysis and QA/QC, and one of the selected classes of substances is PFAS. These partners are also involved in the Flemish working group ('Handelingskader'), created due to the PFOS crisis in Antwerp. Furthermore, experts of SC-CONT, CART, ECOSPHERE-UA and ILVO were external members of the working group writing the urgent advice SciCom on PFAS in animal and vegetable foodstuffs (SciCom 2021/13).

The results of the PFASFORWARD project will also be relevant for the **European Partnership for the Assessment of Risks from Chemicals (PARC)**[4]. The Partnership aims to set up an EU-wide research and innovation program to support EU and national chemical risk assessment/management authorities and processes with new data, knowledge and methods to address current or emerging chemical safety challenges. SC-CONT and ECOSPHERE-UA are actively involved in task 4.2. regarding 'Environmental and multisource monitoring'. The first project that will be executed in this task is the establishment of the overall environmental and multisource monitoring process with the help of a pilot study addressing PFAS and EDCs. Currently, the relevant PFAS are selected, not only related to the environment, but also food is taken into account. The data obtained within the framework of PFASFORWARD will be directly relevant for PARC as they will be used to evaluate food's contribution to overall exposure to PFAS. Furthermore, the data will be correlated with the occurrence data of PFAS in the environment and the biomonitoring data obtained in T4.1 Human biomonitoring. Furthermore, the human biomonitoring data obtained in the framework of PARC could also guide the selection of PFAS in the project PFASFORWARD. For example, if certain PFAS are

often found in biomonitoring studies, they will be considered a high priority for PFASFOWARD.
Since PARC[4] activities necessitate co-financing and the activities of PFASFORWARD are beneficial for the case study on PFAS in T4.2, PFASFORWARD could be considered co-financing for PARC. However, PARC is currently inventorying the data available in the different Member States. Next, the data gaps will be identified, and additional investigations will be developed. Currently, it is impossible to evaluate a potential co-financing of the European Commission for PARC. Still, the consortium of PFASFORWARD will try to find synergies between PARC and PFASFORWARD, as this will benefit both projects.

SC-CONT, CART, ILVO and ECOSPHERE-UA have a proven track record as analytical laboratories and research organizations related to contaminants in food. They were involved in many similar projects in this field like TEQ-FOOD (2022-2024), PARCLINTA (2019-2021), MEFURAN (2019-2021), FLAREFOOD (2015-2017) and MINOIL (2015-2018). They also have state-of-the-art analytical instrumentation (liquid and gas chromatography combined with mass spectrometers and ion mobility). Moreover, SC-CONT and CART conjointly manage the Belgian National Reference Laboratory (NRL) for food process contaminants and halogenated persistent organic pollutants (POPs) in food and feed. Further, ECOSPHERE-UA has a long-standing experience analyzing PFAS in several matrices, including water, sediment, biota (fish, eggs, invertebrates, etc.) and vegetables. TBP-InBioS and CART share expertise in analyzing metal and organic pollutant transfer into plant tissues (ECOSOL 2016-2022).

ILVO has multi- and transdisciplinary expertise in chemical food safety and feed/food processing. ILVO is involved in the projects CYANTIR (2021-2023), ALLERSENS1 (2016-2020), PATPOM (2017-2018), MYCOTOPLUIM (2010-2011) and AMRESMAN (2017-2021). Its Living Lab ILVO Food Pilot is an application and analysis center for the entire food sector, established together with Flanders' Food. It offers scientific advice, pilot trials with semi-industrial and kitchen-/labscale food processing equipment, and laboratory analyses for different food matrices.

4.4 Own publications in peer-reviewed international journals in this field of research in the past five years

A maximum of 5 most relevant publications are listed for each partner.

SC-CONT

- McGrath TJ, Limonier F, Poma G, Bombeke J, Winand R, Vanneste K, Andjelkovic M, Van Hoeck E, **Joly L**, Covaci A. 2021 Concentrations and distribution of chlorinated paraffins in Belgian foods. *Environmental Pollution*, 291, 118236
- Fraiture MA, **Joly L**, Vandermassen E, Delvoye M, Van Geel D, Michelet JY, Van Hoeck E, De Jaeger N, Papazova N, Roosens NHC. 2021. Retrospective survey of unauthorized genetically modified bacteria harbouring antimicrobial resistance genes in feed additive vitamin B2 commercialized in Belgium: Challenges and solutions. *Food Control*, 119, 107476
- McGrath TJ, Poma G, Bombeke J, Limonier F, Van Hoeck E, **Joly L**, Covaci A. 2021. Optimization and validation of an analytical method for the quantification of short- and medium-chained chlorinated paraffins in food by gas chromatography-mass spectrometry. *Food Control*, 119, 107463

- Toumi K, **Joly L**, Vleminckx C, Schiffers B. 2020. Biological monitoring of exposure to pesticide residues among Belgian florists. *Human and Ecological Risk Assessment*, 26(3), 636–653
- Szternfeld P, Marchi J, Malysheva SV, **Joly L**. 2019. Modular Method for the Determination of Polycyclic Aromatic Hydrocarbons in Spices and Dried Herbs by Gas Chromatography–Tandem Mass Spectrometry *Food Analytical Methods*, 12(10), 2383–2391

ECOSPHERE-UA

- Byns C, Teunen L, Groffen T, Laster R, **Bervoets L**. 2022. Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: distribution and human health risk implications. *Environmental Pollution*, 311, 119907
- Lasters R, Groffen T, Eens M, Coertjens D, Gebbink WA, Hofman J, **Bervoets L**. 2022. Home-produced eggs: An important human exposure pathway of perfluoroalkylated substances (PFAS). *Chemosphere*, 308, 136283
- Teunen L, **Bervoets L**, Belpaire C, De Jonge M, Groffen T. 2021. PFAS accumulation in indigenous and translocated aquatic organisms from Belgium, with translation to human and ecological health risk. *Environmental Sciences Europe*, 33, 39
- Groffen T, Eens M, **Bervoets L**. 2019. Do concentrations of perfluoroalkylated acids (PFAAs) in isopods reflect concentrations in soil and songbirds? A study using a distance gradient from a fluorochemical plant. *Science of the Total Environment*, 657, 111 – 123
- Groffen T, Lasters R, Lemiere F, Willems T, Eens M, **Bervoets L,** Prinsen E. 2019. Development and validation of an extraction method for the analysis of perfluoroalkyl substances (PFASs) in environmental and biotic matrices. *Journal of Chromatography B*, 1116, 30-37

CART

- Goscinny S, McCullagh M, Far J, De Pauw E, **Eppe G**. 2019. Towards the use of ion mobility mass spectrometry-derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food. *Rapid Communication in Mass Spectrometry*, 33 (S2): 34-48
- Kune, C, McCann, A, LaRocca, R., **Eppe G**, Quinton, L, Far, J, De Pauw, E. 2019. Rapid visualization of chemically related compounds using kendrick mass defect as a filter in mass spectrometry imaging, *Analytical Chemistry*, 91(20),13112–13118
- Zanuttini C, Gally F, Scholl G, Thomé J-P, **Eppe G**, Das K. 2019. High pollutant exposure of the largest community of bottlenose dolphins in the English channel, *Scientifc Reports*, 9(1), 12521
- ten Dam, G., Pussente, I.C., Scholl, G., **Eppe, G**, Schaechtele, A., van Leeuwen, S. 2016. The performance of atmospheric pressure gas chromatography–tandem mass spectrometry compared to gas chromatography–high resolution mass spectrometry for the analysis of polychlorinated dioxins and polychlorinated biphenyls in food and feed samples, *Journal of Chromatography A*, 1477, 76-90
- Goscinny S, Joly L, De Pauw E, Hanot V, **Eppe G**' 2015. Travelling-wave ion mobility time-of-flight mass spectrometry as an alternative strategy for screening of multi-class pesticides in fruits and vegetables' *Journal of Chromatography* A, 1405, 85-93

ILVO

- Tangni EK, **Van Pamel E**, Huybrechts B, Delezie E, Van Hoeck E, Daeseleire E. 2020. Carry-over of some *Fusarium* mycotoxins in tissues and eggs of chickens fed experimentally mycotoxin-contaminated diets. *Food and Chemical Toxicology*, 145, 111715
- El Hajj Assaf C, De Clercq N, Van Poucke C, Vlaemynck G, Van Coillie E, **Van Pamel E.** 2019. Effects of ascorbic acid on patulin in aqueous solution and in cloudy apple juice. *Mycotoxin Research*, 35, 341-351
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- Bernaert N, Van Droogenbroeck B, **Van Pamel E**, De Ruyck H. 2018. Innovative refractance window drying technology to keep nutrient value during processing. *Trends in Food Science & Technology*, 84, 22-24
- Daeseleire E, **Van Pamel E**, Van Poucke C, Croubels S. 2017. Chemical Contaminants and Residues in Food: Veterinary Drug Residues in Foods. In: *Chemical Contaminants and Residues in Food.* Schrenk, D. & Cartus, A. (eds.) second edition ed. Woodhead Publishing Limited, 117-153, 36

TBP-InBioS

- Amini S, Arsova B, **Hanikenne M.** 2022. The molecular basis of zinc homeostasis in cereals. *Plant Cell Environ*, 45, 1339-1361
- Thiriet-Rupert S, Gain G, Jadoul A, Vigneron A, Bosman B, Carnol M, Motte P, Cardol P, Nouet C, **Hanikenne M**. 2021. Long-term acclimation to cadmium exposure reveals extensive phenotypic plasticity in Chlamydomonas. *Plant Physiol,* 187, 1653-1678
- Amini S, Arsova B, Gobert S, Carnol M, Bosman B, Motte P, Watt M, **Hanikenne M**. 2021 Transcriptional regulation of ZIP genes is independent of local zinc status in Brachypodium shoots upon zinc deficiency and resupply. *Plant Cell Environ* 44, 3376-3397.
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- Schvartzman Echenique MS, Corso M, Fataftha N, Scheepers M, Nouet C, Bosman, B, Carnol M, Motte P, Verbruggen N, **Hanikenne M**. 2018. Adaptation to high zinc depends on distinct mechanisms in metallicolous populations of *Arabidopsis halleri*. *New Phytol*, 218, 269-82

5. BUDGETARY INFORMATION

5.1 Budgetary overview table

Type of cost	*Sciensano SC-CONT*	*UA ECOSPHERE*	*ULiege CART*	*ILVO*	*ULiege TBP-InBioS*	**Total per item**
Staffing	€ 182,644	€ 30,000	€ 0	€ 25,030	€ 0	€ 237,674
Operational	€ 34,092	€ 42,000	€ 40,000	€ 2,467	€ 20,000	€ 138,559
General	€ 18,264.40	€ 3,000	€ 0	€ 2,503	€ 0	€ 23,767
Total per partner	€ 235,000	€ 75,000	€ 40,000	€ 30,000	€ 20,000	€ 400,000
Own contribution	€ 0	€ 0	€ 0	€ 0	€ 0	€ 0
% own contribution	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
FPS Grant	€ 235,000	€ 75,000	€ 40,000	€ 30,000	€ 20,000	€ 400,000
% FPS Grant	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%

5.2 Detailed budget proposal

4.1.	**Staffing costs**			seniority in years	number of person-months	budget in euros		**€ 246,074**
			year					
4.1.1.	**Sciensano - SC-CONT**						**182,644**	
4.1.1.1.	xxx							
	Pay scale	SW10	2023	*4*	*7*	26,280		
		SW10	2024	*5*	*12*	46,807		
		SW10	2025	6	6	24,298		
			2026	7	6	25,209		
4.1.1.2.	Double holiday pay, employer contributions (social security, insurance), year-end bonus, other					60,050		
4.1.2.	**University of Antwerp - ECOSPHERE**						**38,400**	
4.1.2.1.	N.							
	Pay scale	PhD	2023	*0*	*2*	8,100		
		PhD	2024	*1*	*3*	12,700		
		PhD	2025	*2*	*4*	17,600		
4.1.2.2.	Double holiday pay, employer contributions (social security, insurance), year-end bonus, other					0		
4.1.3.	**ILVO**						**25,030**	
4.1.3.1.	Ilse Becue							
	Pay scale	Scientist	2024	*18*	*0.25*	1,474		
		Scientist	2025	*19*	*0.75*	4,577		
		Scientist	2026	*20*	*0.75*	4,814		
		Scientist	2027	*21*	*0.25*	1,646		
4.1.3.2.	N.							
	Pay scale	Technician	2026		*0.5*	6,425		
4.1.3.3.	Double holiday pay, employer contributions (social security, insurance), year-end bonus, other					6,094		

4.2.	**Operational costs**				**€ 129,319**
4.2.1.	**Sciensano - SC-CONT**			**34,092**	
4.2.1.1.	Standard operational costs (flat-rate)		27000		
4.2.1.2.	Specific operational costs		7092		
4.2.1.2.1.	*Sampling*	*1000*			
4.2.1.2.2.	*Analysis PFAS (reagents, standards, lab consumables)*	*6092*			
4.2.2.	**University of Antwerp - ECOSPHERE**			**32,760**	
4.2.2.1.	Standard operational costs (flat-rate)		3840		
4.2.2.2.	Specific operational costs		28920		
4.2.2.2.1.	*Analysis PFAS (reagents, standards, lab consumables)*	*27000*			
4.2.2.2.2.	*Transport field work*	*1920*			
4.2.3.	**ULiège-CART**			**40,000**	
4.2.3.1.	Standard operational costs (flat-rate)		0		
4.2.3.2.	Specific operational costs		40000		
4.2.3.2.1	*Standard, reference materials*	*8000*			
4.2.3.2.2	*Small laboratory equipments (glassware, columns, ...)*	*7000*			
4.2.3.2.3	*Chromatography and mass spectrometry consumables*	*20000*			
4.2.3.2.4	*Outreach, open access publication*	*5000*			
4.2.4.	**ILVO**			**2,467**	
4.2.4.1.	Standard operational costs (flat-rate)		2467		
4.2.5	**ULiège TBP-InBioS**			**20,000**	
4.2.5.1	Standard operational costs (flat-rate)		0		
4.2.5.2	Specific operational costs		20,000		
4.2.5.2.1	*Plant growth in controlled chambers (occupation fees, consumables, soil tr*	*20000*			
4.3.	**General costs**				**€ 24,607**
4.3.1.	**Sciensano - SC-CONT**			**18,264**	
4.3.1.1.	Overheads (flat-rate)		18264		
4.3.2.	**University of Antwerp - ECOSPHERE**			**3,840**	
4.3.2.1.	Overheads (flat-rate)		3840		
4.3.3.	**ILVO**			**2,503**	
4.3.3.1.	Overheads (flat-rate)		2503		
Indicate own contributions with *					
TOTAL					**€ 400,000**

6. ADMINISTRATIVE INFORMATION

6.1 Proposal for a guidance committee (minimum 8 persons, mentioning the institution and e-mail address)

Title – First name – Name	Institution	E-mail
Dr.	FOD Volksgezondheid	[redacted]@health.fgov.be
Dr.	FOD Volksgezondheid	[redacted]@gezondheid.belgie.be
Ir.	FAVV-AFSCA	[redacted]@favv-afsca.be
Ir.	FAVV-AFSCA	[redacted]@afsca.be
Mrs	INBO	[redacted]@inbo.be
Dr.	VPO, departement Omgeving	[redacted]@vlaanderen.be
Dr.	VPO, departement Omgeving	[redacted]@vlaanderen.be
Dr.	OVAM	[redacted]@ovam.be
Ir.	VMM	[redacted]@vmm.be
Pr.		
	University of Antwerp	[redacted]@uantwerpen.be
Dr.	VITO	[redacted]@vito.be
Dr.	VITO	[redacted]@vito.be
Pr.	University of Amsterdam	[redacted]@uva.nl
Ir.	RIVM	[redacted]@rivm.nl
Mrs.	Spaque	[redacted]@spaque.be
Dr.	ERM Environnemental ressource management	[redacted]@erm.com

6.2 Name and identification of the persons who must sign the contract if the project is eligible for a research grant

Institution coordinator	Located at	Name representative institution coordinator	Position
Sciensano	J. Wytsmanstraat 14 1050 Elsene	Christian Léonard	General Director
		Name coordinator	Position
		Laure Joly	Senior Scientist
Institution promoter 2		Name representative institution promoter 2	Position
University of Antwerp	Prinsstraat 13, 2000 Antwerp	Ronny Blust.	Vice Rector…..

Institution promoter 3		Name representative institution promoter 3	Position
University of Liège	7 Place du XX Aout, 4000 Liège	Anne-Sophie Nyssen	Rector
Institution promoter 4		Name representative institution promoter 4	Position
ILVO Instituut voor Landbouw-, Visserij- en Voedingsonderzoek	Burgemeester Van Gansberghelaan 92 bus 1 9820 Merelbeke	Joris Relaes	Administrateur-generaal
Institution promoter 5		Name representative institution promoter 5	Position
University of Liège	7 Place du XX Aout, 4000 Liège	Anne-Sophie Nyssen	Rector

6.3 Identification and bank details of the coordinating institution as to be included in the contract, subject to selection for funding

Company registration number	:	
Establisment unit registration number	:	
IBAN	:	BE18 6790 0011 3265
BIC	:	PCHQ BE BB
Name and address of the account holder	:	Sciensano Rue Juliette Wytsman, 1050 Bruxelles

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Date, name and signature of the coordinator,
as representative of the consortium

On 23 September 2022, Laure Joly
Amended on 09th January 2023, Laure Joly