Annex 3
Template RT full proposal (step 2)

CONFIDENTIAL

Targeted Research, step 2
RT FULL PROPOSAL

FOOD SAFETY

RT 24/5 IMPOFAD

Impurities in oil- or fat-derived food additives and compound foods
Onzuiverheden in van olie of vet afgeleide levensmiddeleandditieven en samengestelde levensmiddelen
Impuretés dans les additifs alimentaires dérivés de l'huile ou de la graisse et les aliments composés

Total duration	42 months
Total budget	€ 400,000
Requested grant	€ 400,000

If appropriate:

percentage of own contribution	**0.00** %
origin / nature of own contribution	
Proposed start date[i]	**1st of April 2024**

1. IDENTIFICATION OF THE CONSORTIUM

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2 HISTORY OF CHANGES

Have significant changes– based on the recommendations formulated in the invitation for step 2 – 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:

- We have increased the number of samples for WP2 from 150 to 180 samples
- We have clarified the sampling plan related to food for infants and young children
- We have clarified the specific case of fat-soluble mycotoxins analysis
- We have specified and detailed the Person-Month (PM) across the different Work Packages (WPs) and Tasks

In addition, a fourth WP regarding the dissemination and scientific valorization has been introduced.

3 GENERAL INFORMATION

3.1 Description of the context of the project proposal

During the deodorisation of refined edible oils, glycidyl fatty acid esters can be generated as process contaminants. They are subsequently hydrolysed within the human digestive tract to glycidol. The European Food Safety Authority (EFSA) has concluded that glycidol has a genotoxic and carcinogenic potential. Margins of exposure (MoE) ranged from 11300 to 102000 for the mean exposure of different population groups, while only MoEs of 25000 or higher are considered a low health concern. However, existing maximum levels for glycidol fatty acid esters in food are rather limited and relate exclusively to oils and fats. Regarding fat or oil-derived food additives (FA), currently, glycidyl fatty acids (expressed as glycidol) are only recently regulated in three FA (E471, E475, E476), while others are under discussion at the European level. Indeed, a possible and significant contribution through certain additives for which no maximum levels of glycidol fatty acid esters exist may lead to a composite food containing unacceptable levels of these food process contaminants (FPC). Furthermore, these additives may contain traces of other impurities, such as glycidyl fatty esters (GEs), esters of 3-MCPD, PAHs, PFAS, PCBs, PCDD/Fs, MOSH/MOAH, heavy metals, fatty acids, fatty acid oxidation products, fat-soluble mycotoxins or pesticides, for which little or no occurrence data are available so far. The resulting exposure to impurities from the consumption of FA could, however, be substantial. An intake assessment for the combined exposure to multiple FA in the MULTI-EXP-ADD project, based on EFSA exposure estimates to FA in Belgium, revealed that if metals would be present in FA at concentrations near the European Union (EU) specification limits, the estimated dietary intake through the consumption of FA in food would be 2-12 times higher than the general dietary exposure estimates for these metals. This implies that the actual concentration levels in FA are much lower than the specification limits and/or that the general dietary exposure estimates insufficiently take into account food products that contain FA. Direct measurements of these impurities in additives are needed to support the research questions asked in section 3.3.

In addition, there is no consensus on the possibility of the formation of glycidyl fatty acid esters and 3-MCPD in composite food preparations that undergo thermal processes. The heat treatment of compound foods containing lipids, in the presence of carbohydrates, can contribute to the formation of those compounds or alter their level. The inclusion of certain additives in the compound food formulation may also favour the formation of those FPC.

3.2 Executive summary of the project

The IMPOFAD project brings together a unique consortium in Belgium to address the issues raised in Europe by the lack of data on impurities in food additives derived from oils or fats. We propose to quantify about 280 impurities from legacy and emerging contaminants by using 10 analytical workflows using state-of-the-art technologies to achieve the required analytical performances for 16 additives. All the analyses will be carried out within the consortium to measure the impurities in 136 (+14) food additive samples during WP1. We complete WP1 by estimating the potential contribution of the intake of FA impurities to the total exposure for adults, infants and children.

However, certain additives can make a disproportionate contribution to glycidyl fatty acid esters in foodstuffs, which makes it difficult to establish a standard on the basis of existing standards in proportion to these individual ingredients. In WP2, we propose to analyse 180 samples distributed over 19 food categories that are under discussions at European level.

An innovative WP3 proposes to study the formation of MCPDs and GEs in thermal processing of food containing lipids in the presence of carbohydrates through key parameters such as formulation and selected processing conditions. We propose to work on two models, i.e. biscuits and extruded snacks, produced at laboratory scale for a total of 67 trials performed in duplicates. Finally, we conclude WP3 by proposing a validation step where we will compare the levels of impurities in emulsions that contain several relevant additives and oils with its formulation processed at the laboratory scale.

3.3 Research objectives

The research objectives of this project are (i) to obtain information on the occurrence of impurities in a specific selection of FA and the corresponding exposure; (ii) to generate occurence data of 3-MCPD esters (3-MCPDEs) and GEs in not yet regulated compound foods; (iii) and to study the impact of thermal processing on GEs and 3-MCPDEs formation during the manufacturing of biscuits/cookies and extruded snacks. The research objectives have been converted into four specific research questions:

1. What are the levels of impurities, including GEs and 3-MCPDEs, in selected oil- or fat-derived FA?
2. What is the potential contribution of the intake of these impurities through the considered additives to the total intake of these impurities through food?
3. What are the levels of GEs and 3-MCPDEs in compound foods under discussion at European level?
4. How does thermal processing impact GEs and 3-MCPDEs formation during the manufacturing of the two selected compound food models, i.e. biscuits/cookies and extruded snacks?

Les objectifs de ce projet sont (i) d'obtenir des informations sur la présence d'impuretés dans une sélection spécifique d'additifs alimentaires et l'exposition correspondante ; (ii) de générer des données sur l'occurence des esters de 3-MCPD et des GEs dans les aliments composés non encore réglementés ; (iii) et d'étudier l'impact du traitement thermique sur la formation de GEs et 3-MCPDEs au cours de la fabrication de biscuits/cookies et d'en-cas extrudés. Ces objectifs ont été convertis en quatre questions de recherche spécifiques :

1. Quels sont les niveaux d'impuretés, y compris les GEs et les 3-MCPDEs, dans certains additifs alimentaires dérivés d'huiles ou de graisses ?
2. Quelle est la contribution potentielle de l'ingestion de ces impuretés par les additifs considérés à l'ingestion totale de ces impuretés par l'alimentation ?
3. Quels sont les niveaux de GEs et de 3-MCPDEs dans les aliments composés faisant l'objet de discussions au niveau européen ?

4. Quel est l'impact du traitement thermique sur la formation d'esters de GEs et de 3-MCPDEs au cours de la fabrication des deux modèles d'aliments composés sélectionnés, à savoir les biscuits et les snacks extrudés ?

3.4 Justification of the submission under this topic

The unique consortium and complementary expertise of the IMPOFAD project partners not only cover the minimum requirements of the proposal but **will go well beyond** what is requested in the call for project descriptions. In fact, with regard to the list of contaminants, 2-MCPD (unbounded), 2-MCPD esters, 3-MCPD (unbounded) will be analyzed with the requested 3-MCPD esters and GEs. For inorganics, Cd and As will be analyzed with Pb (to make the link with MULTI-EXP-ADD and Metalfood@, see section 2.3). The list of PFAS monitored will not be restricted to the 4 compounds mentioned in the call (PFOS, PFOA, PFHxS, PFNA) but extended to the complete list of PFCA (C_5-C_{14}), PFSA (C_4 to C_{13}) and emerging PFAS (acidic form of F53B, GenX, ADONA). Among 159 regulated fat-soluble pesticide residues (regulation 396/2005/EC), about 120 will be analysed by LC or GC-MS/MS according to their physico-chemical properties. The list of PCBs will cover dioxin-like (DL-PCBs) and non dioxin-like PCBs (NDL-PCBs).

In terms of fat oxidation, primary and secondary fatty acids oxidation products will be monitored. The indicator for primary oxidation will be the peroxide value, measured using a colorimetric method, while about 10 individual secondary oxidation products (aldehydes and ketones, including malondialdehyde) will be measured by Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS). Erucic acid and trans fatty acids will be determined as well using Gas Chromatography coupled to Mass Spectrometry (GC-MS).

Regarding PAHs, the monitoring will not be limited to the 4 PAHs but extended to all the 15+1 PAHs that were required in Recommendation 2005/108/EC. As well as both fractions of mineral oil, namely MOSH and MOAH, will be monitored, providing the toxicologically relevant distribution according to the number of aromatic rings, and in particular 1-2 rings and 3-7 rings, thanks to the use of LC coupled to comprehensive multidimensional GC (GCxGC) and FID/MS detector (LC-GC×GC-FID/MS).

3.5 Framework of the project proposal

One of the specific features of the IMPOFAD project is the multi-disciplinary nature of the research proposed in the call to answer the scientific questions raised. To set up the research project, I (as coordinator) wanted to surround myself with Belgian experts in the field with several research groups belonging to two public institutions. Integration and coordination will be facilitated by the fact that the ULiège and Sciensano research teams have a long experience in the analysis of contaminants entering the food chain. The laboratories have been working together for many years. They share the National Reference Laboratory (NRL) for contaminants. They are also deeply involved at EU level through their active participation in the EURL networks, EFSA Panel on FA and Flavourings (Dr. [redacted]), but also in Belgium through the scientific committee of Federal Agency for the Safety of the Food Chain (FASFC - Prof. M-L Scippo) and in France through the expert committee on assessment of physical and chemical risks in food (CES-ERCA) of Agence Nationale de SEcurité Sanitaire (ANSES - Prof. [redacted] and Prof. [redacted], see brief descriptions of all partners expertise here below).

The demand in terms of analytical needs to cover the families of impurities and contaminants is extremely vast and challenging. It requires expertise that I have tried to bring together in this consortium to provide relevant answers to the scientific questions (see paragraph 3.3). The expertise is not only limited to the long-standing experience of the promotors but also the state-of-the-art instruments required to achieve the required analytical performances. As an example,

UL-MSLAB is the only public laboratory in Belgium that can perform PCDD/Fs analysis by the gold standard gas chromatography coupled to high resolution mass spectrometry (**GC-HRMS**). MOSH and MOAH will be determined using **LC-GC×GC-FID/MS** (UL-ACL), to also distinguish between the number of MOAH rings, and in particular rings 1-2 and rings 3 and above, thus providing the most relevant data for toxicological questions. Under no circumstances will the data obtained by an approach as mere and restrictive as GC-FID analysis, as often described in technical reports and in the scientific literature, provide more than incomplete answers to the questions raised by MOSH and MOAH contamination in additives and compound food. All the bonus analytes as presented in the call are covered here in our full proposal and we would like to point out that **no analysis will be subcontracted to an external commercial laboratory as part of this project**. The exhaustiveness of the outcomes of WP1 (i.e. assessment of the contamination of impurities in FA derived from oils or fats and of the associated exposure) will enable us to provide an original response that is not covered, or only to a limited extent, by the current scientific literature with regard to the contaminants coverage proposed here. Our direct and collaborating contacts (UL-SAF) with the agri-food industry and key manufacturers in Belgium will allow us to have access to **all the 14** additives listed in this call for the general population, as well as the **15th additive** specific to baby food, **E304(i)** ascorbyl palmitate, to **a 16th additive** that is the most used fat-soluble colour extracted from plant, the **E160a** β-carotene and including lecithin and hydrolysed lecithin (**E322**) from animal and vegetal origin to answer the question raised during the pre-proposal assessment. As NRL for Food Process Contaminants, UL-MSLAB and SC-OCA will be able to interact directly with ongoing discussions at European level (e.g. European Reference Laboratory - EURL meetings) on the selection of food matrices and may suggest potential modifications/adaptations to be discussed at the annual meetings of the guidance committee (see section 6.1) to carry out the sampling plan proposed in WP2.

A **novel work package** is proposed in WP3 in order to respond to the 3rd point of this RT proposal. UL-SAF is equipped with a laboratory scale production line to study, on the one hand, the **impact of formulation** and, on the other hand, **the impact of processing parameters** on the level of MCPDs and GEs in compound food products, focusing on **biscuits and snacks**. Finally, a **validation** is proposed in task 3.3 based on key compound foods selected from the list of WP2 and produced at lab scale. Among them, emulsions (like margarines, mayonnaise, dressings, spreads…) will be of particular interest as, beside the oil phase, several additives (for instance emulsifiers) are needed to formulate such compound foods. The produced samples will then be analyzed by all the partners of WP1 to measure the levels of the impurities. This will allow to compare the actual levels (from WP1) with the predicted (calculated) levels on a limited number of key compound foods in WP3.

Briefly, the expertises of the consortium is summarized here below:

UL-MSLAB - [redacted] (Coordinator): is Full Professor in Analytical Chemistry at ULiège and has about 25 years experience in the field of analysis of food contaminants. He is currently the director of the MSLab (www.mslab.uliege.be) and also the CART, an ISO 17025 accredited platform for MS based analytical methods recognized at national and European levels. Since 2007, UL-MSLAB and SC-OCA have been appointed as National Reference Laboratory (NRL) by the FASFC in Belgium to assist the competent authorities for chemical substances entering the food chain. Among the list of contaminants, FPC such as glycidyl esters, 2-MCPD, 3-MCPD, furan and acrylamide are part of their duties. [redacted] was coordinator of the MEFURAN project (RT19/05) and is currently the coordinator of the TEQFOOD project (RT22/06). (See publications: https://orbi.uliege.be/simple-search?query=[redacted]).

UL-ACL - [redacted] is a Professor of Analytical Chemistry and Head of the Analytical Chemistry Laboratory at Gembloux Agro-Bio Tech, University of Liège. She has 20 years of experience in the field of food contaminants and in particular, in hydrocarbon analysis, including polycyclic aromatic hydrocarbons and mineral oil hydrocarbons (MOSH&MOAH). She is an expert in hyphenated techniques, in particular, LC-GC and GC×GC coupled with different detectors. She is part of the Core Working Group MOH within the EURL/NRL-PC network, vice-chair of the analytical chemistry division of the American Oil Chemical Society (AOCS) and WG 1 Leader of the DAC-EuChems Sample Prep Study Group. (See publications: https://orbi.uliege.be/simple-search?query=[redacted])

UL-SAF - [redacted] is a senior scientist with 25 years of experience in Food Technology, she belongs to the Food Science and Formulation (SAF) laboratory of GxABT (Head: Prof. C. [redacted] (full professor)), whose experience relates to food science, physico-chemistry and formulation of food systems with the aim of improving food quality. She is currently working on the mitigation of process-induced contaminants. (see publications: https://orbi.uliege.be/simple-search?query=[redacted] and https://orbi.uliege.be/simple-search?query=danthine)

UL-LADA – [redacted] is a Full professor of chemical food safety, head of the laboratory of food analysis, of the food Science department of the Faculty of Veterinary medicine of ULiège. She has a 30 years of expertise in the field of various chemical compound analysis including contaminants and nutrients such as PAHs, dioxins and PCBs using in vitro assays, biogenic amines, fatty acid profiles, short chain fatty acids, etc. She is a member of the scientific committee of FASFC and of expert working groups of ANSES. Publications: https://orbi.uliege.be/simple-search?query=Scippo)

SC-OCA - [redacted] is a senior scientist with more than 15 years of experience in the analysis of pesticides and contaminants, and she is involved in numerous committees and working groups at the Belgian and EU levels. The unit is the Belgian NRL for process and environmental contaminants in collaboration with UL-MSLAB and Belgian NRL for pesticides. The unit, accredited ISO 17025, has state-of-the-art analytical instrumentation to analyze residues and contaminants in food (pesticides, PFAS, mycotoxines for example). [redacted] is a senior scientist with more than 10 years of experience with LC-MS method development and analysis, and he currently conducts research on the myco-, phyco-, phyto- and bacterial toxins quantification in food, human and environmental matrices.

SC-RHIA - [redacted] is a senior scientist, with more than 15 years of experience in metal analysis and related dietary exposure, and risk assessments. Recently, she worked on the MULTI-EXP-ADD project (2021-2022, FPS HFCSE), in which she estimated the potential dietary exposure to metal impurities in FA. She will be coordinator of the Metalfood@ project (2023-2026, FPS HFCSE), in which metal impurities in a selection of FA will be analyzed and their contribution to the dietary metal exposure in Belgium will be estimated.

SC-ADD – [redacted] is a senior scientist with strong expertise in chemical analysis and in establishing fit-for-purpose sampling schemes. She coordinated several research projects related to FA, such as MULTIMADD (2018-2021), Propylene Glycol in Drinks (2020), FREEGLUTAMATE (RT20/3), MULTI-EXP-ADD (2021), and NITRITEFOOD (2022).

SC-TE – [redacted] is a senior scientist, with more than 15 years of experience in trace element analysis in the food chain. She is a member of the EFSA working group "Specifications of FA - impurities" supporting the "Scientific Panel on FA and Flavourings". The service holds the Belgian National Reference Laboratory (NRL) for trace elements and

nanomaterials in food and feed, and has a flexible scope accreditation (ISO 17025) for the analysis of elements and their species in foods.

We have also conducted or are currently conducting several research projects directly linked with the IMPOFAD proposal. The list and a brief description is given here below:

MULTI-EXP-ADD: Prioritisation and exposure assessment of FA for the Belgian population, December 2020-June 2022, FPS HFCSE. This project, conducted by Sciensano, highlighted that the potential exposure to metal impurities via FA could be substantial for the Belgian population. (Involved partners: SC-ADD, SC-RHIA and SC-TE).

Metalfood@: Metal impurities in FA and their contribution to dietary metal exposure in Belgium. Project accepted for funding by the FPS HFCSE. The objectives of this project are to quantify metal impurity levels in relevant FA and to estimate their contribution to the total dietary exposure to these metals. Overlap with the IMPOFAD project will be avoided by selecting different FA. (Involved partners: SC-RHIA, SC-TE and SC-ADD).

Related projects associated with the analytical methodologies:
FLUOREX/PFASFORWARD (PFAS in food) (Involved partners: SC-OCA, SC-RHIA and UL-MSLAB) and **TEQFOOD** (dioxins and beyond in food) (Involved partners: SC-RHIA and UL-MSLAB).

3.6 Use of the expected research results

The results obtained in the framework of the project will be communicated to the FPS Health, Food Chain Safety and Environment, as well as to the policy makers concerning the future European regulation. Furthermore, the results could be used by the FASFC to support the future strategy of the monitoring program. The relevant data will also be provided to EFSA using the SSD2 format, which will allow EFSA to update its exposure and risk assessment for food contaminants and food process contaminants in additives and compound foods. The impact of thermal processing on the formation of MCPDs and GEs will allow recommendations to be formulate for the manufacture of compound foods.

The research results and outcomes will be published in peer-reviewed scientific journals (with a preference for open access) and presented in (inter)national scientific symposia.

3.7 Risks

The risks associated with the proposal are very limited as the members of this consortium have long-standing expertise in the field of food contaminants, food process contaminants and risk assessment. The consortium has complementary skills to answer the research questions. The matrices to be analysed (i.e. FA) are somewhat unusual for most of the research teams, but the analytical instruments made available to the project are state-of-the-art, with the required sensitivity and specificity to achieve the analytical goals. If necessary, advice could always be requested at the NRL-EURL network.

A known analytical issue needs to be point out regarding the determination of MOAH in β-carotene. β-carotene is one of the main interferents for MOAH quantification, which cannot be completely removed using epoxidation without affecting the MOAH fraction. Nevertheless, the UL-ACL unit is validating an alternative method to remove β-carotene from the MOAH fraction based on an LC purification, which, so far, has shown promising results to overpass this risk.

The FA will be acquired through preliminary agreements with industrial using or supplying them.

Given the large number of impurities that will be analysed, total dietary exposure data, specific for the Belgian population, may not be available for all of them. In such cases, the exposure through food additive intake will be calculated, but calculation of the potential contribution to total dietary exposure will not be possible.

4 SPECIFIC INFORMATION

4.1 Scientific and operational methodology of the proposed research (about 15 pages)

WP 0: Coordination and project management

Coordination: UL-MSLAB
Partners: all

At the operational level, WP0 is designed to ensure that the project progresses in accordance with the work plan, in terms of its progress, milestones, deliverables and planned resources. At the organizational level, WP0 aims to achieve maximum efficiency of the infrastructure put in place between the different partners to support the project with particular attention to financial, logistic and coordination issues. The management of its different aspects is translated in practice by the organization of project meetings (kick-off, annual and final) to communicate information on the progress of the project to the Steering Committee and the Contractual Research to discuss any input to ensure a good exploitation of the results and resources. Teleconferencing systems will be the preferred communication tool between partners as they allow for short-term planning of meetings and reduce travel time and costs. This WP will also include maintenance of information carried over from the project after it is completed.

Milestones and Deliverables

- Approved meeting minutes
- Annual and final reports
- Scheduling of partner meetings
- Establishment of a contact person for project activities after the project ends.

WP 1: Assessment of the contamination of impurities in oil- or fat-derived food additives and the related exposure

Coordination: UL-MSLAB
Partners: all

The first WP is dedicated to the measurement of impurities levels in oil- or fat-derived FA. The association of the analytical expertise and capabilities of all the members of the consortium allow us to analyse about 280 impurities from 10 families summarised in Table 1 (task 1.2) in FA. A specific sampling plan (task 1.1) has been constructed for the 14 requested FA : E570 fatty acids, E306 tocopherol-rich extract, E322 lecithin and **hydrolysed lecithin from animal and vegetal origins**, E442 ammonium phosphatides, E470b magnesium salts of fatty acids, E471 mono- and diglycerides of fatty acids, E472a mono- and diglycerides of fatty acids, esterified with acetic acid, E472b mono- and diglycerides of fatty acids esterified with lactic acid, E472e mono- and diglycerides of fatty acids esterified with monoacetyl and diacetyl tartaric acid, E473 sucrose esters of fatty acids, E475 polyglycerol esters of fatty acids, E476 polyglycerol polyricinoleate, E481 sodium stearoyl-2-lactylate, and E492 sorbitan tristearate. It also includes the 15th additive specially for baby food, the E304(i) ascorbyl palmitate, and a 16th additive that is the most used fat soluble colours extracted from plant, **E160a** β-caroten. Finally, the contribution to the intake will be evaluated with the latest food consumption survey for different age groups of the Belgian population (task 1.3).

Table 1: List of impurities

PCDD/Fs & PCBs	Fatty acids & trans-fatty acids	Pesticides		
2, 3, 7, 8 - TetraCDD	decanoic acid (capirc acid)	2-phenylphenol	Trifluralin	Spinosad (sum of spinosyn A and spinosyn D)
1, 2, 3, 7, 8 - PentaCDD	dodecanoic acid (lauric acid)	Acrinathrin	Abamectin (sum of avermectin B1a, avermectin B1b)	Spirodiclofen
1, 2, 3, 4, 7, 8 - HexaCDD	Tridecanoic acid	Aldrin and Dieldrin		Tebuconazole
1, 2, 3, 6, 7, 8 - HexaCDD	Tetradecanoic acid (myristic acid)	Anthraquinone	Ametoctradin	Tebufenozide
1, 2, 3, 7, 8, 9 - HexaCDD	n-Hexadecanoic acid (palmitic acid)	Atrazine	Azoxystrobin	Tebufenpyrad
1, 2, 3, 4, 6, 7, 8 - HeptaCDD	Heptadecanoic acid	Azinphos-ethyl	Bifenox	Teflubenzuron
OctaCDD	Octadecanoic acid (stearic acid)	Azinphos-methyl	Bitertanol (sum of isomers)	Terbuthylazine
2, 3, 7, 8 - TetraCDF	eicosanoic acid	Benfluralin	Bixafen	Tetraconazole
1, 2, 3, 7, 8 - PentaCDF	docosanoic acid (behenic acid)	Bifenthrin (sum of isomers)	Boscalid	Thiamethoxam
2, 3, 4, 7, 8 - PentaCDF	tetracosanoic acid (lignoceric acid)	Bromophos-ethyl	Bromuconazole (sum of diasteroisomers)	Thiodicarb
1, 2, 3, 4, 7, 8 - HexaCDF	cis-9-hexadecenoic acid (palmitoleic acid)	Bromopropylate	Bupirimate	Thiophanate-methyl
1, 2, 3, 6, 7, 8 - HexaCDF	cis-10-Heptadecenoic acid	Chlordane (sum of cis- and trans-chlordane)	Carbaryl	Tridemorph
1, 2, 3, 7, 8, 9 - HexaCDF	trans-9-octadecenoic acid (elaidic acid)	Chlorfenapyr	Carbendazim	Trifloxystrobin
2, 3, 4, 6, 7, 8 - HexaCDF	cis-9-octadecenoic acid (oleic acid)	Chlorfenvinphos	Carbofuran sum included 3-OH	Triflumizole (sum of triflumizole and metabolite FM-6-1(N-(4-chloro-2-trifluoromethylphenyl)-n-propoxyacetamidine)
1, 2, 3, 4, 6, 7, 8 - HeptaCDF	cis-11-octadecenoic acid (cis-vaccenic acid)	Chlorobenzilate	Carboxine	
1, 2, 3, 4, 7, 8, 9 - HeptaCDF	13-docosenoic acid (erucic acid)	Chlorpyrifos	Chlorantraniliprole	
OctaCDF	cis-11-octadecenoic acid (linoleic acid)	Chlorpyrifos-methyl	Chloroxuron	
PCB 77	trans-9,trans-12-octadecadienoic acid	Cyfluthrin (sum of isomers)	Chlorpropham	Triflumuron
PCB 81	cis-9,trans-11-octadecadienoic acid (rumenic acid)	Cypermethrin (sum of isomers)	Clethodim	Fenthion (sum of fenthion and its oxigen analogue, their sulfoxides and sulfone)
PCB 126	trans-9,trans-11-octadecadienoic acid	Cyproconazole	Dichlorvos	
PCB 169	cis, cis, cis-9,12,15-octadecatrienoic acid (α-linolenic acid)	Cyprodinil	Difenoconazole	Triflumuron
PCB 105	cis-11,14-Eicosadienoic acid	DDT (sum of p,p´-DDT, o,p´-DDT, p-p´-DDE and p,p´-TDE (DDD))	Diflubenzuron	
PCB 114	cis-5,8,11,14-eicosatetraenoic acid (Arachidonic acid)		Dimethoate	
PCB 118	(Z,Z,Z)-6,9,12-octadecatrienoic acid	Deltamethrin (cis-deltamethrin)	Dimetomorph	
PCB 123	cis-6,9,12,15-octadecatetraenoic acid (stearidonic acid)	Diazinon	Disulfoton (sum of disulfoton, disulfoton sulfoxide and disulfoton sulfone)	
PCB 156	11c,14c,17c-Eicosatrienoic Acid	Endosulfan (sum of alpha- and beta-isomers and endosulfan-sulphate)	Emamectin B1a	
PCB 157	cis-5,8,11,14,17-eicosapentaenoic acid (EPA)	Endrin	Epoxiconazole	
PCB 167	7c,10,13c,16c,19c-Docosapentaenoic Acid (DPA)	Etofenprox	Ethirimol	
PCB 189	4c,7c,10c,13c,16c,19c-Docosahexaenoic Acid (DHA)	Fenazaquin	Ethoxyquin	
PCB 28	**MCPDs & GEs**	Fenitrothion	Famoxadone	
PCB 52	S 3-monochloropropanediol fatty acid esters (3-MCPDEs)	Fenvalerate (any ratio of constituent isomers (RR, SS, RS & SR) including esfenvalerate)	Fenhexamid	
PCB 101	S 2-monochloropropanediol fatty acid esters (2-MCPDEs)		Fenpropimorph (sum of isomers)	
PCB 138	S glycidyl fatty acid esters (GEs)	Flucythrinate (sum of isomers)	Fenpyrazamine	
PCB 153	3-monochloropropanediol (3-MCPD)	Fluquinconazole	Fenpyroximate	
PCB 180	2-monochloropropanediol (2-MCPD)	Flusilazole	Fipronil (sum fipronil + sulfone metabolite)	
Fat oxidation products	**PFASs**	Fluvalinate-tau (sum of isomers)	Fluazifop	
Malondialdehyde (MDA)	Acide perfluorobutanoique (PFBA)	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Fluazinam	
4-Hydroxy-2-hexenal (4HHE)	Acide perfluoropentanoïque (PFPeA)	Hexachlorobenzene	Flubendiamide	
Crotonaldehyde (CRT)	Acide perfluorohexanoïque (PFHxA)	Hexachlorocyclohexane (HCH), alpha-isomer	Fludioxonil	
Benzaldehyde (BNZ)	Acide perfluoroheptanoïque (PFHpA)	Hexachlorocyclohexane (HCH), beta-isomer	Flufenoxuron	
4-Hydroxy-2-Nonenal (4HNE)	Acide perfluorooctanoïque (PFOA)	Iprodione	Fluopyram	
Hexanal (HXL)	Acide perfluorononanoïque (PFNA)	Lambda-cyhalothrin	Fluxapyroxad	
2,4-Nonadienal	Acide perfluorodecanoïque (PFDA)	Lindane (HCH gamma-isomer)	Formetanate	
2,4-Decadienal	Acide perfluoroundecanoïque (PFUnDA)	Lufenuron	Hexythiazox	
Trans-2-nonenal	Acide perfluorododecanoïque (PFDoDA)	Malathion	Imazalil	
Mycotoxins	Acide perfluorotridecanoïque (PFTrDA)	Methoxychlor	Imidacloprid	
Zearaleonone (ZEN)	Acide perfluorotetradecanoïque (PFTeDA)	Nitrofen	Indoxacarb	
Deoxynivalenol (DON)	Acide Perfluorobutanesulfonique (PFBS)	Parathion	Malaoxon	
Ochratoxin A (OTA)	Acide perfluoropentanesulfonique (PFPeS)	Penconazole	Matrine	
Aflatoxin B1 (AFB1)	Acide Perfluorohexanesulfonique (PFHxS)	Pendimethalin	Metconazole (sum of isomers)	
Aflatoxin B2 (AFB2)	Acide perfluoroheptanesulfonique (PFHpS)	Permethrin	Methomyl	
15 + 1 EU PAHs	Acide Perfluorooctanesulfonique (PFOS)	Picoxystrobin	Methoxyfenozide	
Napthalene	Acide perfluorononanesulfonique (PFNS)	Pirimiphos-methyl	Metrafenone	
Acenapthene	Acide Perfluorodecanesulfonique (PFDS)	Procymidone	Novaluron	
Acenaphthylene	Acide perfluoroundecanesulfonique (PFUnDS)	Profenofos	Pencycuron	
Fluorene	Acide perfluorododecanesulfonique (PFDoDS)	Propiconazole	Penflufen	
Phenanthrene	Acide perfluoro tridecanesulfonique (PFTrDS)	Propoxur	Phentoate	
Anthracene	2,2,3-Trifluor-3-[1,1,2,2,3,3-hexafluor-3-(trifluormethoxy) propoxy]-propionic acid (DONA, the acid form of ADONA)	Propyzamide	Phorate (sum of phorate and its oxigen analogue, their sulfoxides and sulfone)	
Fluoranthene		Pyrazophos	Phosmet (sum of phosmet and phosmet oxon)	
Pyrene		Quinoxyfen	Phoxim	
Cyclopenta[c,d]pyrene	9-Chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS, the main component of the acid form of F53B)	Quintozene (sum of quintozene and pentachloro-aniline)	Prochloraz (sum of prochloraz, BTS 44595 (M201-04) and BTS 44596 (M201-03))	
Benzo[c]fluorene		Resmethrin (sum of isomers)	Propargite	
5-methylchrysene		Tecnazene	Proquinazid	
Benzo[j]fluoranthene	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS, the minor component of the acid form of F53B)	Tefluthrin (sum of isomers)	Prothioconazole: prothioconazole-desthio	
Dibenzo[a,l]pyrene		Tolclofos-methyl	Pyraclostrobin	
Dibenzo[a,e]pyrene	2,3,3,3-tetrafluor-2-(heptafluorpropoxy)-propanoic acid (HFPO-DA, the acid form of GenX)	Tolylfluanid (sum of tolylfluanid and dimethylaminosulfotoluidide)	Pyridaben	
Dibenzo[a,i]pyrene		Triadimefon	Pyriproxyfen	
Dibenzo[a,h]pyrene		Triazophos	Quinalphos	
Metals	**MOHs**		Spinetoram	
Lead (Pb)	Linear and branched alkanes and cycloalkanes (MOSH)			
Cadmium (Cd)	Alkylated and non-alkylated polycyclic aromatic hydrocarbons (MOAH)			
Arsenic (As)				

Task 1.1: Sampling design and collection (SC-ADD – Dr. [redacted])

This task aims to provide food additive (FA) samples commercially available in Belgium for chemical analysis described in Task 1.2. The analytical results will be used for exposure estimates in Task 1.3. Therefore, ensuring that the selection and collection of samples accurately represent the Belgian market is essential. To this end, the first step would be to examine the supply channels from producers to the users/customers. However, all aspects of the FA supply chain remain an underdeveloped study area with low information visibility. To address this gap, we will establish the global landscape of the Belgian FA market. From a preview of Belgian FA market analysis (see Figure 1), many companies (international and local) are involved in the marketing of additives, although around 18% of the market share are companies grouped under "others". The full report will detail this specific group.

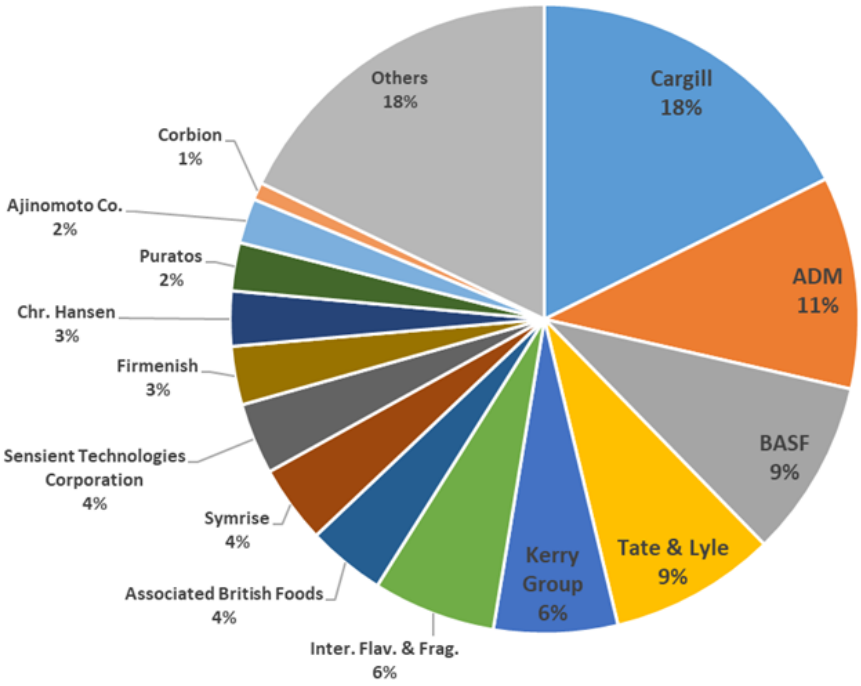


Figure 1: Belgium FA market:Company share for 2022.

Five companies dominate the market (more than 50%): Cargill, ADM, BASF, Tate & Lyle and Kerry Group, so their products will be well represented in the final sampling.

Additionally, we will verify the complete coverage of manufacturers and distribution channels by making contact with Associations representing specific FA sectors. Among others, ELMA (European Lecithin Manufacturers Association), EFEMA (European Food Emulsifiers Manufacturers Association), NATCOL (Natural Food Colours Association), and EU-SFI (EU Specialty Food Ingredients) already have on their website a list of manufacturers.

Then, the portfolio of companies offering the targeted additives will be examined. This step is critical for collecting samples and interpreting the analytical results because commercial FA can be marketed in their pure form, like lecithins or formulations incorporating other ingredients (including carrier FA). For instance, BASF's Lucarotin® is a β-carotene (E160a) formula that contains corn oil and other additives (E1450, E306, and E341) to enhance dispersibility in cold water for beverages or dairy products.

The next step of task 1.1 will be the collection of samples with a reasonable size (below 0.5 kg). Our direct and collaborating contacts (UL-SAF) with the agri-food industry (end-users) and key manufacturers in Belgium will allow us to have access to commercially available samples. While putting together this proposal, we have already had initial talks with our contacts and reached a preliminary agreement to obtain some samples. Additionally, the Prof. [redacted] from UL-SAF is in an excellent position to acquire "test samples" of FA from manufacturers or suppliers, which will help expand the range of samples available for analysis.

Sample collection is the critical challenge of this project, which will study 136 food additive samples. To efficiently allocate time and resources to address all questions described above, and distribute the number of samples per FA, we have established a priority scheme based on the following criteria and summarised in Table 2.

The usage frequencies in food products

As part of our previous project (MULTI-EXP-ADD), we conducted a comprehensive market survey of food products that were available in 2020 from six major grocery stores. From this survey, we obtained a dataset of 36601 unique food products (FABEL). Our analysis of the frequencies of identified FA showed that the top 51 most frequently used FA accounted for 82% of the total occurrences. Hence, these 51 FA are the most frequently used in Belgium. We extracted from this study, the rank and number of food products containing the FA of interest and results are reported in Table 2. Only four FA of this project are included in the list of the top 51: E322, E471, E160a and E476.

Authorisation to be used as "carrier"

As per Annex III of the European Regulation 1333/2008, carriers are authorised for some FA, flavourings, enzymes, and nutrients, then carried over to the final food product. However, labelling FA is not mandatory if used as carriers. This means that the frequency of use of such FA during a label survey might be underestimated. Therefore, it's necessary to consider this factor when prioritising the scheme. After referring to Annex III, the approved carriers from our target list can be found in Table 2.

Sources and types of the food additive

FA can be derived from plants, animals, or minerals, or they can be synthetic. They are available in various physical forms, including liquid, viscous, powder, and granular. Lecithin (E 322) is the most commonly used additive from the list of additives studied. It is available in different forms, such as powder or oil-based, and is derived from various plant sources, including soybeans, sunflowers, and rapeseed. Companies like Cargill, ADM, and Lecico widely manufacture it. Therefore, it is essential to consider the availability of additives when constructing a representative sample list. Table 2, also contains this information retrieved from Regulation Commission Regulation (EU) No 231/2012 and EFSA opinions.

The priority scheme for distributing food additive samples will be based on the criteria described above.

FA most frequently used (in the top 51 list) are selected as priority 1 (13 samples per additive).

Then, FA not comprised in the top 51 list but are authorised as carriers and/or with the most variable sources and forms will be given priority 2 (8 samples per additive). Priority 3 (5 samples per additive) will be given to the remaining FA, including E304(i), not reported in the FABEL data set. In total, 136 samples are accounted for, and 14 additional samples can be added. These extra samples are very useful in case additional insight for certain FA is necessary and offer a certain level of flexibility to the sampling.

Table 2: Targeted FA list with corresponding information for prioritisation

FA	Num of occurrences	Frequencies in %	Rank	Priority	Nb. Of samples	Carrier	Physical form	Sources
E322	1516	9,45	9	P1	13	Y	Lecithins: liquid or viscous semi-liquid or powder Hydrolysed lecithins: viscous liquid or paste	The main source of lecithins is soya bean oil. Other plant sources include oil from cottonseeds, corn, sunflower seeds and rapeseed, together with animal sources such as egg yolk
E471	1110	6,92	13	P1	13	Y	Mixture	Source materials can be, among others, coconut, palm, palm kernel, soya, rapeseed (Canola), sunflower, cottonseed, corn, olive, tallow and lard.
E160a	992	6,18	15	P1	13		Powder, oil-based	E 160 a (i) β-carotene, E 160 a (ii) plant carotenes, E 160 a (iii) β-carotene from Blakeslea trispora, E 160 a (iv) algal carotenes.
E476	228	1,42	49	P1	13	Y	Highly viscous liquid	Mixture of products formed by the esterification of polyglycerols with condensed castor oil fatty acids
E306	217	1,35	52	P3	5		Viscous oil	Product obtained by the vacuum steam distillation of edible vegetable oil products, comprising concentrated tocopherols and tocotrienols Contains tocopherols such as d-α-, d-β-, d-γ- and d-δ-tocopherols
E570	149	0,93	70	P2	8	Y	Liquid or solid	Obtained from oils and fats
E481	132	0,82	74	P3	5		Powder or brittle solid	Mixture of the sodium salts of stearoyl lactylic acids and its polymers
E472b	83	0,52	91	P3	5		Mobile liquids to waxy solids of variable consistency	Esters of glycerol with lactic acid and fatty acids occurring in food fats and oils
E472a	82	0,51	92	P2	8	Y	Liquids to solids	Esters of glycerol with acetic and fatty acids occurring in food fats and oils
E473	77	0,48	96	P2	8	Y	Stiff gels, soft solids or powders	Essentially the mono-, di- and triesters of sucrose with fatty acids occurring in food fats and oils.
E475	75	0,47	97	P2	8	Y	Oily to very viscous liquids, hard and waxy solids	Polyglycerol esters of fatty acids are produced by the esterification of polyglycerol with food fats and oils or with fatty acids occurring in foods fats and oils.
E442	74	0,46	98	P2	8		Unctuous semi-solid to oily liquid	Mixture of the ammonium compounds of phosphatidic acids derived from edible fat and oil.
E470b	67	0,42	105	P2	8	Y	Powders, flakes or semi-solids	Magnesium salts of fatty acids occurring in foods oils and fats, these salts being obtained either from edible fats and oils or from distilled food fatty acids.
E492	60	0,37	109	P2	8		Beads or flakes or hard, waxy solid	Mostly vegetable sourced.
E472c	41	0,26	127	P2	8	Y	Liquids to waxy solids or semi-solids	Esters of glycerol with citric acid and fatty acids occurring in food oils and fats.
304 (i)	ND	ND	ND	P3	5		Powder	Ester made by the reaction of the primary alcohol group of ascorbicacid (vitamin C) with the carboxylic acid group of palmitic acid.

Task 1.2: Analysis of impurities in food additives (All partners)

For this specific task, all the expertise needed to carry out the most exhaustive characterisation of impurities in FA has been gathered within the IMPOFAD consortium. The research teams involved have extensive experience in their field (see publications, international networks) and, above all, state-of-the-art instruments to carry out the analyses and achieve the sensitivity and specificity required for these analyses in complex matrices. For all analytical methods, suitable internal quality criteria (calibration, blanks, internal standards and certified reference materials) will be applied to evaluate the analysis sequence.

No analysis will be subcontracted as part of the IMPOFAD project, which is a guarantee of control and total mastery of the results produced within the consortium. All the required skills are brought together in this group of partners.

3-MCPDEs & GEs (UL-MSLAB – Prof. [redacted])

3-MPCDEs & GEs will be measured by GC-MS after their separation from the free 3-MCPD & glycidol using the isotope dilution technique for the quantitative aspect with deuterated analogues. In brief, MPCDEs & GEs are extracted from the matrix and separated from free 3-MCPD & glycidol by liquid-liquid extraction. Then GEs are converted in 3-MBPDEs and transesterified along with the 3-MPCDEs in 3-MBPD and 3-MCPD. Finally, they are derivatised to be analysed by GC-MS in Selected Ion Monitoring (SIM) mode. This method has been validated according to EU regulation 333/2007 and its amendments for infant formula, chocolate spread and compound foods like waffles, pancakes, pastries, … and meet the required performances for the official control. For FA, Limits of Quantification (LOQs) will be reassessed and are expected to be between 1 and 10 µg/kg wet weight. Nevertheless, this method performance has been proven through the successful participation to proficiency tests (PTs) organized by ISO17043 accredited companies, and are routinely checked by the inclusion of Quality Control samples (QC) within the samples series.

MOHs & PAHs (UL-ACL – Prof. [redacted])

The analysis of MOAH and PAHs is highly related, as the PAHs are easily co-extracted and purified with MOAH. Therefore, a common sample preparation is proposed. The extraction is performed by solid- or liquid-liquid extraction, or when necessary, performing a preliminary saponification step, following the method reported in the ISO/DIS 20122, for which the UL-ACL laboratory has been part of the interlaboratory trials for its optimization. Alternatively, a simpler method based on chromatographic purification for which the validation is ongoing will be used. Purification from naturally present olefin (expected for many samples) will be performed following the epoxidation procedure suggested in the aforementioned ISO method only when highly necessary, as the procedure has been shown to impact also the recovery of MOAH with a higher number of aromatic rings, which are the ones of more toxicological concern. Therefore, when not necessary, the purification achievable by exploiting the chromatographic selectivity will be preferred, both based on the validated comprehensive multidimensional GC (GC×GC) method ([redacted] et al., 2023) and on a more powerful LC separation under development in our lab.

The final extract will be used for the analysis of both MOAH and PAHs, but also MOSH, to increase the reliability of the results by verifying the petrogenic origin based on the first plausibility check reported by the Joint Research Center (JRC) Guidance for the analysis of mineral oils.

MOSH and MOAH will be determined using LC-GC×GC-FID/MS, to also distinguish between the number of MOAH rings, and in particular rings 1-2 and rings 3 and above (different toxicological concerns). Furthermore, the use of GC×GC-MS can provide additional information relating to the presence of markers to support the study of the origin of

contamination. The method proposed reaches LOQs in the 0.1-1 mg/kg range according to the specific matrix.

The analysis of PAHs will be performed simultaneously with the MOAH but also verified by analyzing the same fraction by LC coupled with a fluorimetric detector, or if necessary, by GC-MS or MS/MS ([redacted] et al. 2008). Both methods can reach LOQ significantly below the 0.9 µg/kg required by the European regulation Reg 836/2011. The monitoring will not be limited to the 4 PAHs but extended to all the 15+1 PAHs required in Recommendation 2005/108/EC.

The UL-ACL laboratory has broad experience in determining both PAHs and MOSH&MOAH in different food commodities and fats and oils. Nevertheless, the methods have never been tested in all the FA herein included. Therefore, they will be all verified for recovery, LOQ, and linearity, using suitable spiking material (i.e.; standard PAHs and deuterated ones; and the solution provided during the interlaboratory trial conducted by the JRC for the verification of the performance of the MOAH analysis). The verification will follow the Eurachem Guidance.

PFASs (SC-OCA – Dr. [redacted])

Other potential contaminants in FA are per- and polyfluoroalkyl substances (PFAS). The project will benefit from the methods developed in the framework of PFASFORWARD (RT 23/07). The methods available in Sciensano are not limited to the 4 PFAS evaluated by EFSA, but the other PFAS listed in Commission Recommendation (EU) 2022/1431 will be included, i.e. 4-EFSA-PFAS, other carboxylate-PFAS (C_4-C_{14}), sulfonate-PFAS (C_4-C_{13}), and PFAS substitutes (DONA, F53B minor and major forms and HFPO-DA). The analytical method consists of a solid/liquid extraction followed by a two-step purification using solid-phase extraction (SPE) on Envicarb cartridges in combination with weak anion exchange cartridges. Analyses will be performed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). The methods are validated according to the EURL guidance document (EURL, 2022), and they are ISO 17025 accredited for most food matrices. The methods available at Sciensano are sufficiently sensitive to reach at least the required LOQ indicated in the Commission Recommendation (EU) 2022/1431 or in the Commission Regulation (EU) 2023/915, i.e. 0.005 ng/g for plant products or 0.1 ng/g for complex matrices such as liver or eggs. The existing methods will be optimized further to include the matrices covered in the project with the lowest possible LOQs. Simultaneously, it will be investigated if the number of PFAS can be increased, extending the method to neutral compounds (FOSA, fluorotelomer alcohols) and positively charged compounds (capstone A and B), without hampering the LOQs of negatively charged compounds.

The methods will be validated according to the EURL Guidance document (EURL, 2022) and Commission Regulation (EU) 2023/915. Furthermore, SC-OCA has participated successfully in several PTs related to PFAS in different food and feed matrices since 2015.

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 10 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 no contamination occurred during the extraction procedure or in the LC-HRMS system. The contribution of blank levels will be assessed as ≤ 50 % 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 is 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 fall outside this range, samples will be re-analyzed.

PCDD/Fs & PCBs (UL-MSLAB – Prof. [redacted])

The 17 WHO PCDD/Fs, 12 WHO DL-PCBs and 6 NDL-PCBs will be analysed by a congener-specific analytical protocol validated according to the EU regulation 2017/644 and included in our ISO17025 accreditation scope for barely 20 years. In brief, the analytes are extracted through ASE before a multicolumn chromatographic purification and an analysis by GC-HRMS set in Selected Ion Monitoring (SIM) mode. The quantitative aspect is achieved through the isotope dilution technique using $^{13}C_{12}$ labelled analogues of every congener. This method, known as the "gold standard", displays performances compliant with the EU regulation 2017/644 and has the low LOQs required to detect the low levels expected in FA (LOQs for the sums of PCDD/Fs, DL-PCBs and NDL-PCBs are respectively 0.11, 0.09 pg WHO-TEQ/g sample and 2.3 ng/g sample) with good accuracy and precision leading to expanded uncertainties of 19.6, 23.9 and 11.0 %, respectively. In addition, performances are checked by the inclusion of Quality Control samples (QC) in the series and by the successful participation to PTs provided by ISO17043 accredited companies.

Heavy metals (SC-TE – Dr. [redacted])

The toxic elements Lead (Pb), Cadmium (Cd) and Arsenic (As), will be determined by the Unit Trace Elements. The analysis of these elements in foodstuff by ICP-MS (Induced Coupled Plasma Mass Spectrometry) is covered by ISO 17025 accreditation. The validation of the analysis methods in foodstuff is conform to the performance criteria specified in Commission Regulation (EC) No 333/2007. The analysis methods are expected to be transferable to the analysis of the FA in the current project proposal. The validated scope will be extended by a separate validation (determination of LOQ, precision and trueness) for oil- or fat-derived FA. Exact quantification limits are expected to be in the range 1-10 µg/kg, whereas current limits are in the range 100-5000 µg/kg for the toxic elements, so LOQs are expected to be fit-for-purpose.

Fat soluble mycotoxins (SC-OCA – Dr. [redacted])

During this project, several mycotoxins, that may be present in oil- or fat-derived FA, will be analyzed. The project will benefit from the method previously developed and validated by the partner SC-OCA for mycotoxins in vegetable oil using LC–MS/MS. This method is used for confirmation and quantification of mycotoxins due to its high specificity and sensitivity. This existing method will be further optimised to include the matrices covered by the project with the lowest possible LOQs. The European Commission has only regulated maximum levels (EU regulation 2023/915) of zearalenone (ZEN) in corn oils. However, the presence of deoxynivalenol (DON) and ochratoxin A (OTA) in vegetable oils ([redacted] et al. 2013, Qian et al. 2015) has also already been reported over the last decade. In this context, ZEN but also other fat-soluble mycotoxins such as OTA or aflatoxins, will also be analysed.

For the analysis, the mycotoxins contained in the sample are extracted using a modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. A solid-liquid extraction is followed by an induced $MgSO_4$/NaCl phase separation., which concentrates the mycotoxins in the organic phase. After centrifugation, filtration and the addition of internal standards, the organic phase is analysed without further purification. In addition, quality control procedures, including the use of certified reference materials and running blank/spiked samples to assess recovery, are essential to monitor the performance of the analytical process and will be used.

Fat soluble pesticides (SC-OCA – Dr. [redacted])

In this project, the pesticide residues present in oil- or fat-derived FA will be analyzed. Out of 659 pesticide residues defined in the EU pesticide database, 159 pesticide residues are indicated as fat-soluble. The partner SC-OCA already disposes of multi-residue methods for analyzing 61 fat-soluble pesticide residues by GC-MS/MS and 61 by LC-MS/MS. These methods also cover 24 pesticide residues (i.e. 21 by GC and 3 by LC) covered by the European multi-annual control program (EU Regulation 2023/731) for pesticide residues to be analyzed in fat. Furthermore, the FASFC defined a list of major pesticides to be analyzed in oil samples and 63 (out of 65) pesticide residues are included in the methods available at Sciensano allowing us to quantify about 150 fat soluble pesticides.

Regarding the analysis, the non-polar pesticide residues will be extracted using an optimized procedure based on the methodology developed by the EURL-animal origin (EURL-AO). The method is based on a modified QuEChERS with specific salt, allowing the extraction of the pesticide residues with acetonitrile, followed by a purification of the supernatant with a dispersive solid-phase extraction (d-SPE) using the enhanced matrix removal sorbent (EMR-sorbent). For the polar pesticides, the EURL-AO method is based on acetonitrile extraction followed by purification in two steps (i) a freezing out and (ii) a d-SPE with ODS-C18/PSA sorbent.

A quality control plan will be established to ensure the daily accuracy and reliability of the results. Acceptability criteria will be based on the SANTE document dedicated to validating and quality control for analyzing pesticides in food (EU Guidance document 2021). The sensitivity and stability of the system will be verified using a solution at the LOQ level (i.e. 0.010 mg/kg for most pesticide residues) at the beginning and end of each run. A signal-to-noise ratio > 6 for the quantification and the confirmation transitions for all pesticides will ensure no sensitivity loss. All concentrations of the calibration levels will be back-calculated using the regression equation of the calibration curve. A deviation lower than ± 20 % illustrated an accurate pesticide quantification. The absence of pesticide contamination (<limit of detection, LOD) and/or carry-over in the chromatographic systems will be assessed by analyzing a procedural blank for each analytical batch.

Finally, SC-OCA has participated with an extended pesticide scope in EU-PTs, including native rapeseed oil (2022), rapeseed oil (2020), and pork fat (2016), with good results for LC (polar) and GC (non-polar) amenable pesticides.

Fatty acid oxidation products (UL-LADA – Prof. [redacted])

Nine aldehydes that can be formed after polyunsaturated fatty acid oxidation were chosen to evaluate the lipid oxidation of samples: malondialdehyde (MDA), 4-Hydroxy-2-nonenal (4-HNE), 4-Hydroxy-2-hexenal (4-HHE), crotonaldehyde, benzaldehyde, hexanal, trasn-2-nonenal, 2,4-nonadienal and 2,4-decadienal. The aldehyde measurement will be performed according to Douny et al. (2016). Briefly, 2 g of sample, added with BHT and methylmalondialdehyde, methylcrotonaldehyde, benzaldehyde-^{13}C, hexanal-D_{12} and 4-HNE-D_3 as internal standards, will be extracted two times with trichloroacetic acid 5%/ethanol 50/50 (v/v). Dinitrophenylhydrazone derivatives will be prepared by adding a 2,4-dinitrophenyl-hydrazine solution (0.05 M in acetonitrile/acetic acid 9:1 (v/v)) and incubating for 2 h at 60 °C. Separation and detection of aldehydes as dinitrophenylhydrazone derivatives will be performed using a ThermoFinnigan Surveyor MS pump and autosampler and a ThermoFinnigan LCQ DecaXP ion trap mass spectrometer, equipped with an electrospray source. Separation will be achieved on an Atlantis T3 C18 column (3 µm, 2.1 x 150 mm), with an Atlantis guard column T3 C18 (3 µm, 2.1 x 10 mm), both from Waters Corporation (Milford, MA, USA). The solvent flow will be 0.25 mL/min, column temperature will be set at 40°C and the injection volume

will be 20 µL. The mobile phase will be acetonitrile (solvent A) and acetic acid solution at pH=3.55 (solvent B). The gradient elution conditions will be from 40 to 65% of solvent A in 8.5 min and from 65 to 100% in 4 min; then, conditions will be held for 7.5 min and the contribution of solvent A will be decreased to 40% over 2 min and maintained for another 6 min reconditioning. The analysis with the mass spectrometer will be performed in MS/MS mode, with positive ionization for MDA and MeMDA and negative ionization for the other compounds.

Fatty acid and trans-fatty acid, including erucic acid (UL-LADA – Prof. [redacted])

The fatty acid profile, including erucic acid and the trans-fatty acid content of the samples will be determined by analysis of the fatty acid methyl esters (FAME) by GC-MS, according to Douny et al. (2015), with modifications. The method involves the saponification and methylation of the fat previously extracted from samples by the Folch method (Folch et al., 1957), in the presence of an internal standard (nonadecanoic acid, C19:0), followed by two extractions with hexane. Twenty-nine fatty acid methyl esters will be separated on a Focus GC gas chromatograph (Thermo Fisher Scientific) on a CP-Sil88 column for FAME (Varian, 100 m × 0.25 mm, 0.2 mm) and analyzed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). The GC parameters will be as follows: inlet at 250 °C, splitless injection, helium as carrier gas at 1.5 ml/min; temperature program − 55 °C for 1 min, followed by an increase of 5 °C/min to 180 °C, then 10 °C/min to 200 °C, 200 °C for 15 min, then a rise of 10 °C/min to 225 °C, and 225 °C for 14 min; the total run time was 59.50 min; injection volume was 1 µl. The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards. The mass spectrum conditions were as follows: transfer line at 250 °C; ion source at 220 °C; collision energy at 35 eV, positive ionization mode. Fatty acid methyl esters will be detected using SIM mode and different ions will be monitored for each analyzed fatty acid, which allowed detecting and quantifying m/z 74 and 143 for saturated fatty acids (SFA) and 79 and 91 for monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively.

Task 1.3: Exposure to impurities through food additive intake (SC-RHIA – Dr. N. Waegeneers)

To estimate the potential contribution of the intake of FA impurities to the total exposure to these impurities/contaminants via food, the exposure through FA intake has to be determined. Hereto, the intake of each of the considered FA has to be known. As it is beyond the scope of the IMPOFAD project to perform FA intake assessments, we will rely on previous estimates performed by EFSA. Intake estimates, specific for Belgium, are available for 14 of the selected FA for toddlers (1-3 years), children (3-9 years), adolescents (10-17 years), adults (18-64 years) and elderly (65 years and older). For E 481 (sodium stearoyl-2-lactylate), E 160a (β-carotene) and for infants (all FA), intake data specific for Belgium are not available and EU mean data will be used.

The average exposure to impurities through FA intake could be calculated by multiplying, for each impurity, the mean intake of each FA with the mean impurity concentration in that additive, and summing these exposures per impurity (deterministic approach). However, this assumes that "the average consumer" consumes each FA at an average level, which is very unlikely and may quickly lead to unrealistic high impurity exposures. Furthermore, it is difficult to determine high-level exposures this way. An option could be to calculate the "average exposure" as explained above but replacing -for the additive that contributes most to this average exposure- the mean FA intake with the high-level FA intake. Again, the assumptions behind this exposure calculation are not very likely to happen.

As an alternative to this approach, we propose a refined approach that determines the exposure to impurities through FA intake via probabilistic modelling. Probabilistic modelling techniques allow more realistic estimates of exposure by computing the full range of potential exposure levels that may occur in a population rather than single "worst-case" exposures. The probabilistic exposure assessment will be carried out through Monte Carlo simulations. For each impurity, a random concentration of the impurity in the FA (between the lowest and highest measured concentrations) and a random intake of that FA (from a probability distribution derived from EFSA evaluations) will be multiplied by other, and the obtained exposures will be summed over the different FA. This represents the exposure of one individual to the impurity/contaminant due to FA intake. This calculation will be repeated multiple times to obtain a population of exposed individuals. Mean, median and high-level exposure data can be derived from this population. The assessments will be performed for each age population. The difference between the probabilistic approach and the deterministic approach is that the variability in food additive intake for the different FA and the variability in impurity concentrations within a food additive can be explicitly taken into account.

The exposure to impurities through FA intake will be assessed for 25 impurities/contaminants. To estimate the potential contribution of these impurities in FA to the total exposure to these impurities/contaminants via food, the generated exposure data will be compared to the total dietary exposure to the impurity/contaminant. Total dietary exposure data will be obtained from EFSA opinions, unless more recent data specific for Belgium are available. Such data are e.g. generated in the projects RF21/6350 Fluorex (PFAS), RT22/06 TEQFOOD (DL-PCBs) and RF23/23 Metalfood@ (metals) that are all carried out by Sciensano and Uliège.

Milestones and Deliverables for WP1

- Additive list containing all samples to be purchased
- Sampling campaign plan
- Quantitative measurements of about 280 impurities out of 10 families of impurities (see list in Table 1) in the 16 types of FA, for a total of 136 + 14 samples.
- Estimates of the potential contribution of the intake of FA impurities to the total exposure for adults, infants and children

WP 2: Assessment of the contamination by MCPDs and GEs in compound food under discussion at the European Level

Coordination: UL-MSLAB
Partners: SC-ADD

Task 2.1: Sampling design and collection (SC-ADD – Dr. [redacted])

Occurrence data for MCPDs and GEs will be generated for the fifteen foodstuffs under discussion at EU level for maximum limits. These food products have been classified in Table 4 according to Regulation 1333/2008 as the use of food additives is suspected to be a possible source of contamination. Additionally, a sub-classification based on Regulation 609/2013 and FoodEx2 descriptions for foods for infants and children was added to detail the number of samples allocated. The survey of food items targeted will occur in Belgium's most relevant grocery (e.g. Colruyt, Delhaize) and specialised shops (e.g. bakeries, pharmacies and drugstores). The individual items will be analysed without pooling to prevent dilution effects and loss of correlation between concentration and food composition in the final composite sample. As the major factors involved in forming MCPDs and GEs (precursors and food processing methods) are not fully understood, and results from WP1 are unpredictable, it is not easy to foresee the results of such a survey before its implementation. However, some guidance

can be provided regarding the total number of samples to be analyzed and their distribution in various food categories, while allowing for flexibility in case of necessary adaptations.

First, the food products are clustered into three groups according to the sampling strategies.

Group 1: Common foods with the targeted FAs (100 samples)

This group gathers all the foods from the "15 under discussion" where FAs are commonly used. In the food industry, a vast variety of food products and new foods are available, each with different forms and numerous brands. Since it is not possible to analyse all food items, a selection process based on information from the FABEL dataset will be used. This selection aims to identify foods with the highest co-occurrences of FAs that have been identified to contribute most to the contamination in WP1. For instance, E 322, the most frequently used FA from this project, co-occurs mostly with E471 and E160a, as shown in Table 3. This means, for instance, that if any of these 3 additives are contaminated with MCPDs and GEs, it will be easy to identify food samples that contain them and select the ones that belong to the Group 1 food categories for analysis.

This exercise can also be done, to look for samples that only contain 1 contaminated FA of interest. Therefore, a customised list of food items will be constructed using WP1 and the FABEL dataset results.

Table 3: Co-occurences of lecithins with the other targeted FAs from FABEL dataset

Co-occurrences for E322 within the FABEL dataset		
Co-occurring FA	Number of co-occurrence	(%)
E471	302	19.9
E160a	204	13.5
E476	75	4.9
E472a	41	2.7
E472b	31	2.0
E160b	30	2.0
E475	29	1.9
E492	25	1.6
E306	24	1.6
E472c	19	1.3
E481	19	1.3
E442	4	0.3
E470b	4	0.3
E473	3	0.2

Then, the number of samples attributed to the different food categories from Group 1 are directly linked to the available items on the market and have to cover specialised shops like bakeries and supermarkets.

Consequently, sampling resources will be dedicated to providing hand-picked food items to generate occurrence levels and more insights into possible contamination sources.

Group 2: Foods for infants and toddlers (50 samples)

There are fewer brands on the market that represent this group compared to group 1. As part of the NITRITEFOOD project, we have identified 35 brands offering targeted foods in supermarkets, pharmacies, and online shops. We will be sampling all of these brands as listed in Table 4. Food items will be chosen based on their composition, including the type of oil used and FAs. For instance, Nestlé's "Cerelac" cereal product contains palm, rapeseed, coco, and sunflower oils but no targeted FA, while Hipp's "bolognese" prepared meal contains

tocopherol-rich extract as an antioxidant. Additionally, the number of samples per sub-category will also depend on the availability of the products on the market. Cereals with added high proteins are very specific and may not be available in all brands' catalogues, unlike prepared meals. Table 4 takes these factors into account when earmarking the number of samples.

Group 3: Foods with particular sampling requirements (30 samples)

Food supplements are selected based on their composition and intended health benefits. Omega-3 fatty acids are often marketed as key ingredients in supplements that aim to promote normal muscle, heart, liver, and brain function. Examples of these supplements include fish oil, krill oil, cod liver oil, and algal oil (a vegetarian option). Fatty acids can also be included in supplements as minor ingredients to enhance their effects in combination with other elements such as vitamins, oligo-elements, and pigments like lutein and zeaxanthin, which are known to support good eye function. Eight samples are foreseen to cover this category and will cover different brands and health features.

For the last food category, french fries, potatoes and vegetable crisps, previous publications have demonstrated that migration of MCPDs and GEs from the frying oils is the main contamination route; as a result, all the samples were contaminated (Nguyen et al. 2022, Pantalone et al. 2023). Interestingly, the variation in levels was coming from either the product composition (e.g. salts) or type of vegetables (beetroot crisps had 7 times higher amount of 3-MCPDEs than the mean value of 212 µg/kg) while the same type of crisps contained the lowest levels of GEs (Nguyen et al. 2022). Following a statistical test with the analytical results, no correlation was found between GEs and MCPDs. Considering these results and the growing popularity of these potato alternative crisps, we have allocated 22 samples for this group. Therefore, we have allocated 10 samples out of 22 to cover exclusively these vegetable crisps. Then, 5 samples are given to french fries (ready-to-eat) and 7 samples of potato crisps with products with (e.g. Springles with E471) or without targeted FAs.

So far, 180 samples have been distributed in the different food categories considered in the project. An additional 20 ad hoc samples are proposed. They will be allocated later in the project for further investigations or when the survey has identified interesting samples exceeding the proposed number per category.

The expiration date will be given special care because the analysis must be performed before this deadline. Consequently, the sampling will have to be done during different campaigns in accordance with the laboratory analysis planning.

Table 4: Number of samples to be analysed per relevant food category

Food Categories 1333/2008 (FCS)		**Number of samples**
GROUP 1		**100**
FCS Nb.	**Name**	
2.2.2.	Other fat and oil emulsions including spreads	
	Margarine and similar products	10
5.2.	Other confectionnary (sandwich spreads such as chocolate based)	8
6.3.	Breakfast cereals	9
7.2.	Fine bakery wares	
	Biscuits	20
	Pastries and cake	20
12.2.2	Seasonings and condiments	8
12.6.	Sauces (only mayonnaise and vegetable oil based sauces)	12
15.1	Potato-, cereal-, flour- or starch-based snacks (only crackers)	8
18	Processed foods (only instant noodles)	5

GROUP 2		50
FCS Nb.	**Name**	
13.1.3.	Processed cereal-based foods and baby foods for infants and young children	
	Simple cereals*	6
	Cereals with an added high protein food*	4
	Pastas*	5
	Rusks and biscuits*	15
	Ready-to- eat meals (vegetable, meat, fish, fruit based) #	15
	Mixed meals (dairy or fruits + biscuits)#	5
GROUP 3		**30**
FCS Nb.	**Name**	
17	Food supplements	8
4.2.6	Processed potato products (only french fries)	5
15.1.	Potato-, cereal-, flour- or starch-based snacks (excluding crackers see Group 1)	
	Vegetable crisps	10
	Potato crisps	7

Task 2.2: Analysis of MCPDs and GEs in compound foods (UL-MSLAB – Prof. [redacted])
The analyses will be performed by the method described in the first paragraph of the task 1.2. In addition, compound food need to be freeze-dried, grinned and mixed before the extraction to ensure the homogeneity of the samples. This method has been fully validated according to the EU regulation 333/2007 and its amendments requirements and has LOQs of 6.4 µg/kg wet weight for 3-MCPDEs and 8.9 µg/kg wet weight for GEs with associated expanded uncertainties of 15 and 21%, respectively. Its trueness has also been proven through the successful participation to several PTs organized by ISO17043 accredited companies, and will be daily checked by the inclusion of QCs within the sample's series.

Milestones and Deliverables for WP2

- Compound food list containing all samples to be purchased
- Sampling campaign plan
- Quantitative measurements of MCPDs and GE in the 15 prioritized categories recommend by EU following the sampling design and corresponding to 180 (to 200) analyses in total.

WP 3: MCPDs and GEs formation during the manufacturing of the compound food

Coordination: UL-SAF
Partners: UL-MSLAB

The thermal processing of food containing lipids -in the presence of carbohydrates- can contribute to the formation of toxic compounds or alter their level (Sadowska-Rociek et al., 2019). The addition of certain additives, such as lecithin, in the compound food formulation can enhance the formation of processing contaminants like MCPDs and GEs (Belkova et al., 2021). Based on this, the impact of the formulation and selected processing parameters will be evaluated in compound foods. Due to the limited available budget and in order to remain consistent with the analytical working plan (knowing that all the produced samples will be analysed by the analytical partners), two types of critical matrices will be considered to investigate the impact of the process: biscuits/cookies and extruded snacks (either sweet or salty). However, all the analyses of WP3 will be performed **in duplicates** to enhance the reliability of the results.

WP3 is made of two main Tasks (3.1. and 3.2.): the first one will be devoted to the study of the impact of the formulation and the second one to the impact of the processing parameters on the level of MCPDs and GEs in the compound food products. Beside this, a third task (3.3.) will

be devoted to the preparation of selected compound foods that will be used for a validation step. Along with those 3 tasks, CART will provide the analytical support required for the MCPDs and GEs measurements.

Task 3.1: Impact of the formulation (UL-SAF – Prof. [redacted])

According to the literature (Belkova et al. 2019, Sadowska-Rociek et al. 2021, Kadir et al. 2022), the main experimental parameters to be considered are the type of fat, the amount of fat, the presence of diacylglycerols (DAG), known as possible GE precursors, the presence of selected additives (such as lecithin, type and amount) and the use of fat alternatives/fibers (type and amount).

Different formulations of biscuits and extruded snacks will be produced to investigate their impact on MCPDs and GEs production during their manufacture. To this end, references (controls) will be first produced using fixed processing parameters:

A. For biscuits/cookies: two types (formulations/products) will be considered.
B. For extruded snacks: one single formulation (including 5% of oil) will be considered

The produced models (3 references) will be analysed during WP2 (see above) to report the levels of MCPDs and GEs obtained after the application of the classical processes (baking / extrusion). Afterwards, the 3 formulations will be systematically modified in order to determine the impact of each ingredient or additive on the formation of unwanted contaminants.

A. Biscuits/cookies: (18 samples, including the references)
    1. The type of fat (a shortening made of a blend of palm and rapeseed oil) will be kept constant but its level will be modified: one higher and one lower compared to the reference (→2 samples/product)
    2. The amount of DAG present in the fat will be increased (two levels) (→2 samples/product)
    3. Addition of lecithin will be considered (two levels) (→2 samples/product)
    4. A formulation including a fat substitute (ex inulin) will be considered (→2 samples/product)
B. Snacks (7 samples including the references):
    1. The type of fat (rapeseed oil) will be kept constant but its level will be modified: one higher (10%) and one lower (0%) compared to the reference (→2 samples)
    2. The amount of DAG present in the oil will be increased (two levels) (→2 samples/product)
    3. Addition of lecithin will be considered (two levels) (→2 samples/product)

Task 3.2: Impact of the processing parameters (UL-SAF – Prof. [redacted])

Only extruded snacks will be considered for task 3.2. Two formulations will be selected from task 3.1.B: one leading to the formation of the lowest amount of contaminants (=Ref Low) and one leading to the formation of the highest amount of contaminants (=Ref High). The main experimental parameters to be considered are the temperature, the residence time, and other processing parameters related to heat effects (ex: screw rotating speed).

A. The impact of the curing temperature and of the screw rotation speed which condition the residence time of the product in the extruder (but also the pressure levels in the barrel) will first be studied on the two selected formulations. Three temperature levels (130, 150 and 170°C) and two screw speeds (200 and 250 rpm) will be considered for each formulation (Ref Low and Ref High). (→ 12 samples)
B. Further, a three-variable Ben-Boxen statistical design will be established to allow one of the formulation parameters (e.g. quantity of oil, lecithin or DAG) to be added. This will allow to draw cross-conclusions regarding the impact of the processes and

formulations. This step can also allow the establishment of predictive models which will allow to estimate the quantities of undesirable contaminants produced according to the operating parameters and the formulation. (→ 30 samples)

Task 3.3: Production of food samples for a validation step (UL-SAF – Prof. [redacted])

The last task of WP3 will be devoted to a validation step. Indeed, some key compound foods selected from the list of WP2 will be produced at lab scale. Among them, emulsions (like margarines, mayonnaise, dressings, spreads…) will be of particular interest as, beside the oil phase, several additives (for instance, emulsifiers) are needed to formulate such compound foods. (→ 10 samples)

The produced samples will then be analyzed by the UL-MSLAB. This will allow to compare the actual levels with the predicted (calculated) levels based on the results from WP1.

Milestones and Deliverables for WP3

- Biscuit/cookies: identify formulations that minimize the production of unwanted products and report the impact of each investigated ingredient.
- Extruded snacks: Establish prediction models to estimate the quantities of MCPDs and GEs formation produced during the extrusion process depending on the operating parameters and the composition of the snacks.
- A total of 50 + 84 + 20 = 154 samples will be performed.

WP 4: Dissemination and Valorization

Coordination: UL-MSLAB
Partners: all

Task 4.1: EFSA standardized reporting and General communication (press, web site, …) (All partners)

Results will be reported using the EFSA standardized model for transmission of chemical occurrence data from different data providers (SSD). 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 and will need to be filled for the 10 families of contaminants from the WP1 and for the samples from the WP2.

In addition, results dissemination to a broad audience, mainly policy makers and competent authorities will be considered with the agreement of the FPS. Wide range of communication media, like institutional websites or press releases (for the professional or general public) will be discussed with the FPS before publication.

Task 4.2: Scientific communication (Open access publication and Congress) (All partners)

Results will be published in peer-reviewed scientific journals with a focus on the Open access to increase the visibility and the dissemination of the scientific output. In addition, results will be presented at national and international scientific symposia.

Milestones and Deliverables for WP4

- Report of the results in EFSA format
- Workshop on analytical methods for FASFC
- Preliminary results presented at national and international conferences
- Peer-reviewed publications

Chronogram

	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**	
WP 0	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**18750**
WP 1															**243000**
T 1.1		x	x												7500
T 1.2	x	x	x	x	x	x	x	x							213500
T 1.3								x	x						22000
WP 2															**42500**
T 2.1				x	x										12500
T 2.2					x	x	x	x	x	x	x				30000
WP 3															**67000**
T 3.1						x	x	x	x	x	x	x			22000
T 3.2								x	x	x	x	x			35000
T 3.3												x	x	x	10000
WP 4															**28750**
T 4.1					x	x	x	x	x	x	x	x	x	x	18750
T 4.2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	10000

4.2 Structure and organisation of the research (about 2 pages)

At the PM level, the total budget shows a ratio of 42% for the staff. This amount is sound in view of the analytical costs associated with WP1 and WP2 in particular. Note that the analytical budget where no personnel costs are mentioned in paragraph 5.2 implicitly includes them (i.e. for the analysis of metals, MOSH, MOAH, PAHs, fatty acids and fatty acids oxidation products).

Code	Task description	Contracting institution(s)	Required personnel (qualifications)	PM
WP 0	**Coordination and project management**	**ULiege**	**PhD**	**1.5**
WP 1	**Assessment of the contamination of impurities in oil- or fat-derived food additives and the related exposure**	**ULiege & Sciensano**		**15**
T 1.1	Sampling design and collection	Sciensano		
T 1.2	Analysis of impurities in food additives	ULiege & Sciensano	PhD, MSc & Bachelor	12
T 1.3	Exposure to impurities through food additive intake	Sciensano	MSc.	3
WP 2	**Assessment of the contamination by MCPDs and GEs in compound food under discussion at European Level**	**ULiege & Sciensano**		**3**
T 2.1	Sampling design and collection	Sciensano		

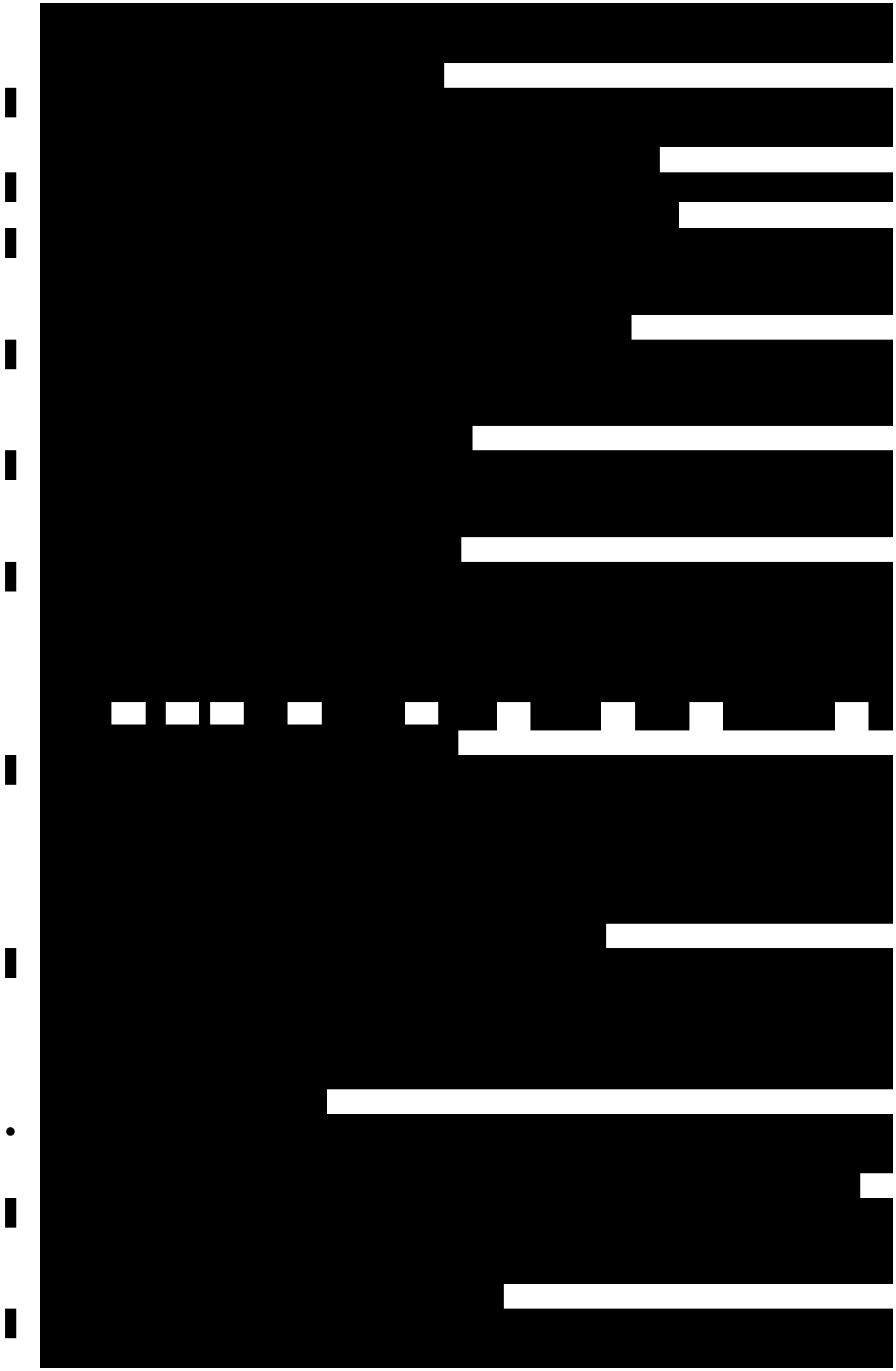
T 2.2	Analysis of MCPDs and GEs in compound foods	ULiege	PhD & Bachelor	3
WP 3	**MCPDs and GEs formation during the manufacturing of the compound food**	**ULiege**		**3**
T 3.1	Impact of the formulation	ULiege	PhD	1
T 3.2	Impact of processing parameters	ULiege	PhD	1.5
T 3.3	Production of samples for the validation	ULiege	PhD	0.5
WP 4	**Dissemination and Valorization**	**All partners**		**1.5**
T 4.1	EFSA standardized reporting and general communication (press, web site, …)	All partners	PhD	1.5
T 4.2	Scientific communication (open access publications and congress)	All partners		

4.3 Collaboration, complementarity and/or parallel applications

We are not planning any cooperation or are aware of whether complementarity exists with research groups that do not request a financial contribution from the FPS Health for this project but receive research grants from other bodies to conduct their own research.

This project has not been submitted to any other organisation.

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



5 BUDGETARY INFORMATION

5.1 Budgetary overview table

Type of cost	*[Coordinator]*	*[Promotor 2]*	*[Promotor 3]*	*[Promotor 4]*	*[Promotor 5]*	*[Promotor 6]*	*[Promotor 7]*	*[Promotor 8]*	**Total per item**
Staffing	€ 120,000	€ 0	€ 0	€ 0	€ 28,930	€ 18,330	€ 0	€ 0	€ 167,260
Operational	€ 57,000	€ 42,000	€ 39,000	€ 22,000	€ 23,177	€ 1,833	€ 17,000	€ 14,000	€ 216,010
General	€ 12,000	€ 0	€ 0	€ 0	€ 2,893	€ 1,833	€ 0	€ 0	€ 16,726
Total per partner	€ 189,000	€ 42,000	€ 39,000	€ 22,000	€ 55,000	€ 21,996	€ 17,000	€ 14,000	**€ 399,996**
Own contribution	€ 0	€ 0	€ 0	€ 0	€ 0	€ 0	€ 0	€ 0	€ 0
% own contribution	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
FPS Grant	€ 189,000	€ 42,000	€ 39,000	€ 22,000	€ 55,000	€ 21,996	€ 17,000	€ 14,000	**€ 399,996**
% FPS Grant	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%

5.2 Detailed budget proposal

4.1.	**Staffing costs**						**€ 167,260**
		year	seniority in years	number of person-months	budget in euros		
4.1.1.	Lab of						120,000
4.1.1.1.	N.					60,000	
	Pay scale	2024	*17*	*0.5*	4,804		
		2025	*18*	*2*	20,067		
		2026	*19*	*2*	20,917		
		2027	*20*	*1.4*	14,212		
4.1.1.2.	N.					60,000	
	Pay scale	2025	*16*	*6*	36,150		
		2026	*17*	*3.8*	23,850		
4.1.2.	Lab of						0
4.1.3.	Lab of						0
4.1.4.	Lab of						0
4.1.5.	Lab of						28,930
4.1.5.1.	N.					28,930	

	Pay scale [redacted]	2026	*6*	*5*	28,930			
4.1.6.	Lab of [redacted]						18,330	
4.1.6.1.	N.					18,330		
	Pay scale SW1	2026	*2*	*3*	18,330			
4.1.7.	Lab of [redacted]						0	
4.1.8.	Lab of [redacted]						0	
4.2.	**Operational costs**							**€ 216,010**
4.2.1.	Lab of [redacted]						57,000	
4.2.1.1.	Standard operational costs (flat-rate)					18000		
4.2.1.2.	Specific operational costs					39000		
4.2.1.2.1.	*Analysis of PCDD/Fs & PCBs*				*19000*			
4.2.1.2.2.	*Analysis of MCPDs and Ges*				*20000*			
4.2.2.	Lab of [redacted]						42,000	
4.2.2.1.	Standard operational costs (flat-rate)					0		
4.2.2.2.	Specific operational costs					42000		
4.2.2.2.1.	*Anlaysis of MOH and PAHs by LC-GCxGC-FID/MS*				*40000*			
4.2.2.2.2.	*Dissemination*				*2000*			
4.2.3.	Lab of [redacted]						39,000	
4.2.3.1.	Standard operational costs (flat-rate)					0		
4.2.3.2.	Specific operational costs					39000		
4.2.3.2.1.	*Production of extruded snacks*				*18000*			
4.2.3.2.2.	*Production of model biscuits*				*11000*			
4.2.3.2.3.	*Production of food emulsions*				*8000*			
4.2.3.2.4.	*Dissemination*				*2,000*			
4.2.4.	Lab of [redacted]						22,000	
4.2.4.1.	Standard operational costs (flat-rate)					0		
4.2.4.2.	Specific operational costs					22000		
4.2.4.2.1.	*Analysis of fatty acids*				*10000*			
4.2.4.2.2.	*Analysis of fatty acids oxidation products*				*10000*			
4.2.4.2.3.	*Dissemination*				*2000*			
4.2.5.	Lab of [redacted]						23,177	
4.2.5.1.	Standard operational costs (flat-rate)					2893		
4.2.5.2.	Specific operational costs					20284		
4.2.5.2.1.	*Analysis of PFAS*				*5284*			
4.2.5.2.2.	*Analysis of pesticides*				*5000*			
4.2.5.2.3.	*Anlaysis of mycotoxins*				*10000*			
4.2.6.	Lab of [redacted]						1,833	
4.2.6.1.	Standard operational costs (flat-rate)					1833		
4.2.6.2.	Specific operational costs					0		
4.2.7.	Lab of [redacted]						17,000	
4.2.7.1.	Standard operational costs (flat-rate)					0		
4.2.7.2.	Specific operational costs					17000		
4.2.7.2.1.	*Sampling strategy et sampling design*				*15000*			
4.2.7.2.2.	*Dissemination*				*2000*			
4.2.8.	Lab of [redacted]						14,000	
4.2.8.1.	Standard operational costs (flat-rate)					0		
4.2.8.2.	Specific operational costs					14000		
4.2.8.2.1.	*Analysis of heavy metals*				*12000*			
4.2.8.2.2.	*Dissemination*				*2000*			
4.3.	**General costs**							**€ 16,726**
4.3.1.	Lab of [redacted]						12,000	
4.3.1.1.	Overheads (flat-rate)					12000		
4.3.2.	Lab of [redacted]						0	
4.3.2.1.	Overheads (flat-rate)					0		
4.3.3.	Lab of [redacted]						0	
4.3.3.1.	Overheads (flat-rate)					0		
4.3.4.	Lab of [redacted]						0	
4.3.4.1.	Overheads (flat-rate)					0		
4.3.5.	Lab of [redacted]						2,893	
4.3.5.1.	Overheads (flat-rate)					2893		

4.3.6.	Lab of [redacted]		1,833
4.3.6.1.	Overheads (flat-rate)	1833	
4.3.7.	Lab of [redacted]		0
4.3.7.1.	Overheads (flat-rate)	0	
4.3.8.	Lab of [redacted]		0
4.3.8.1.	Overheads (flat-rate)	0	

Indicate own contributions with *

TOTAL **€ 399,996**

6 ADMINISTRATIVE INFORMATION

6.1 Proposal for a guidance committee

Title – First name – Name	Institution	E-mail
Dr. [redacted]	FPS Public Health	[redacted]
Mme [redacted]	FPS Public Health	[redacted]
Mr [redacted]	FPS Public Health	[redacted]
Mr [redacted]	FAVV-AFSCA	[redacted]
Mme [redacted]	FAVV-AFSCA	[redacted]
Prof. [redacted]	UAntwerpen	[redacted]
Dr. [redacted]	VITO	[redacted]
Prof. [redacted]	UGent	[redacted]

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

Institution coordinator and promotors 2,3 & 4	**Located at**	**Name representative institution coordinator and promotors 2,3 & 4**	**Position**
ULiege	Liege	Prof. Dr. [redacted]	Rector
		Name coordinator	**Position**
		Prof. Dr. Ir. [redacted]	Full Professor
Institution promoter 5, 6, 7 & 8		**Name representative institution promoter 5, 6, 7 & 8**	**Position**
Sciensano	Brussels	Dr. [redacted]	Director

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

Company registration number : [redacted]
IBAN : [redacted]
BIC : GKCCBEBB
Name and address of the account holder : BELFIUS – Liège

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On behalf of the consortium, [redacted]

[redacted]

September 21st 2023