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AGRO4AGRI

Deliverable 2.1 Scientific SSbD evaluation for materials and production processes

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EXECUTIVE SUMMARY

In late 2022, the European Commission (EC) published a recommendation on the implementation of the Safe and Sustainable by Design (SSbD) framework for chemicals and materials across different phases of the innovation journey (such as design, preparation, experimentation, and prototyping), during which choices are made regarding the progression, discontinuation, or modification of the innovative approach. The recommendation of the European Commission launches a testing period and a voluntary reporting mechanism, and the Commission plans to revise the framework based on feedback from stakeholders such as industry, academia, the research community, and support from European Member States. The recommendation from the European Commission underlines that both safety and sustainability should be addressed in the design phase and not considered as an afterthought, e.g., when a material or product has been developed and is about to be used in society.

Following a Safe and Sustainable by Design (SSbD) approach, AGRO4AGRI will explore 4 different materials and several approaches to reach the controlled-delivery fertiliser targets, and 2 approaches as slow and controlled release mechanisms. Technologies explored in AGRO4AGRI by core and participants partners are Nanocellulose derivative (NCL); Mesoporous Silica Nanoparticle (MSNs); Hydrophobic Deep Eutectic Solvents (HDESs); Super Absorbing Polymers (SAPs); Nanoclays (NCs); RNA interference (RNAi); double-stranded RNA (dsRNA); Plant Biostimulant (PBs) for enhanced Nitrogen Use Efficiency (NUE).

In this deliverable we focus on Stage 1 of the EC SSbD framework, and we report on the completion of Task 2.2 that revolves around establishing and matching principles for (re)design of agrochemical solutions according to the SSbD framework. The task has two subtasks, namely subtask 2.2.1 that aims to define goal, scope, and system boundaries and subtask 2.2.2. Evaluation of production of the new agrochemical solutions against the SSbD principles.

For each AGRO4AGRI solutions, we completed the four steps of the SSbD framework:

1) definition of SSbD system elements;

2) setting system boundaries;

3) description of the goal(s) of innovation; and

4) the preliminary identification of potential hotspots along the life cycle of the innovation.

The starting point of the definition of the system to be assessed will depend on the organisation's position in the life cycle of the chemical/material. When it comes to the technologies explored in AGRO4AGRI, the organisation's position in the life cycle of the chemical/material is at the very early stages of research and development when it comes to the production processes as well as the product applications. At this point in time, it is also not known to a full extent what chemicals and materials might be used in the development of the technologies. However, to establish an early engagement with the value-chain, the framework Early4AdMa developed by the OECD in 2023 was used to probe and outline already identified potential candidates for each of the technologies. Additionally, the Early4AdMa framework was utilized to identify pivotal knowledge gaps that would have to be addressed in the upcoming WPs (e.g. WP7 – Environmental, socio-economic and sustainability assessment) and can be used to support decisions on selection of materials and processes in WP2 (task 2.4). In general, Early4AdMa is an early awareness and action system that through 65 questions help to systematically identify potential effects related to four topics 1) human health, 2) environment, 3) sustainability and 4) regulatory preparedness.

Regarding setting system boundaries which is the step 2 of the SSbD framework, AGRO4AGRI focuses on all conventional sustainability/SSbD imbedded aspects, including environmental sustainability and socio-economic assessment. A critique that has been raised regarding conventional existing assessment schemes, and thus mainly focusing on induced and (generally) disregarding avoided impacts (e.g. avoided human toxicological impacts related to (avoided) indirect land use change (iLUC)) and thus eroding the actual sustainability coverage. Therefore, a need was identified here to also explore how the more elusive, yet equally relevant, indirect impacts can be accounted for as part of/in addition to the SSbD assessment framework. This need will be addressed in future work in WP7. The definition of the SSbD system furthermore requires the description of the goal(s) of innovation subject to the SSbD evaluation. The goals of each of the different technologies explored in AGRO4AGRI varies and include modifying the nanocellulose and biochar to interact more effectively with the active ingredients in fertilizers. When it comes to the goal of the innovation, the SSbD framework recommended by the European Commission covers three levels of the term 'by design':

- Molecular design, to design new chemicals and materials based on their chemical structure;
- Process design, to make the production process safer and more sustainable, both for chemicals and materials being developed and for existing chemicals and materials;
- Product design, where the results of the SSbD assessment support the selection of the chemicals or materials to meet the functional demands of the final product in which they are used.

All the different AGRO4AGRI solutions are found to include elements of all 3 levels of design. For instance, molecular design is crucial in developing materials that can interact at the chemical level with fertilizers and other substances, ensuring that these interactions are conducive to the slow and controlled release of nutrients. A lot of effort will also be made regarding process design, namely in designing the methods and conditions under which AGRO4AGRI solutions are synthesized and modified in order to make the production of these solutions scalable, cost-effective, and environmentally sustainable. This includes optimization of synthesis parameters (such as temperature, pH, and reactant concentrations) and the development of functionalization techniques that modify the surface properties of the materials to enhance their performance as delivery systems. At the product design level, the results from molecular and process design are implemented to produce AGRO4AGRI solutions that meet the functional requirements of specific agricultural applications. This includes ensuring that, for instance, nanocarriers can deliver nutrients in a controlled manner to maximize their efficiency and minimize environmental impact.

Once the goal(s) of innovation is described, it triggers a series of questions in the SSbD framework, for example: What are the principles on which the (re)design is going to be based? After having reviewed principles for chemicals and materials in general as well as SSbD principles for nanomaterials in specific, we have decided to focus on 3 overall principles of SSbD design. These are:

- Avoid substances, mixtures and materials that are classified as e.g., CMRs, reproductive toxicants according to the European Classification, Labelling and Packaging (CLP) Regulation known as the CLP Regulation;
- Avoid substances that fulfil the criteria for being Persistent (P), Bioaccumulative (B), Toxic (T) or Mobile (M);
- Avoid unintended exposure to humans and environment.

Depending on the chemical composition and nanoparticle morphology, nanoclays are organized into various groups such as montmorillonite, bentonite, sepiolite kaolinite, hectorite, and halloysite. Different hazard notifications have been provided by companies to the European Chemicals Agency in accordance to the Regulation on Classification, Labelling and Packaging ranging from no hazards to H315 (Causes skin irritation), H318 (Causes serious eye damage), H335 (May cause respiratory irritation) and H350 (May cause cancer, state route of exposure if conclusively proven that no other route applies). Biochar is notified as a flammable solid (H228), whereas hazards associated with the synthesis of biochar include: The heat source of the kiln, the release of toxic and flammable gases when extracting biochar from the reactor and possible leakage of toxic and flammable gases which could lead to an explosion. HDESs and SAPs were chosen for their ability to modify the release properties of fertilizer carriers and candidates for materials to be explored in AGRO4AGRI. Deep eutectic solvents with different hydrophobicity properties include: Betaine, menthol, thymol and hexanoic acid. No hazards have been reported on betaine and decanoic acid whereas ECHA Registration data indicates that menthol is slightly toxic and skin and eyes irritation (H315 and H319). Thymol is reported Acute Toxicity 4 (H302), Skin corrosion 1B (H314) and ecotoxicity for aquatic chronic 2 (H411). Finally, Hexanoic acid has been reported to be slightly ecotoxic in freshwater fish. There are several different chemicals and materials involved in the development of SAPs and some of these have CLP classifications such as cellulose (Respiratory hazards (H335) and acute toxicity if its contacted to skin or swallowed (H312 and H302 respectively), starch (Eye Irritation 2 (H319, H320), Acute toxicity by inhalation (H332, H335), Toxicity to aquatic life (H411)), chitosan (Skin and eye irritation (H315 and H319), Affects to organs (lungs if it is inhaled (dry powder) and carrageenan (Eye Irritation 2 (H319) and suspect to be carcinogenic Category 2 (H351)). Due to the hazard properties of carrageenan, this material will be

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not used moving forward in AGRO4AGRI. In general, NFC, NCHs and NFs are believed to be biodegradable, non-toxic, and sourced from renewable materials, making them environmentally friendly options for agricultural applications. The chemicals and materials used in the synthesis of NFC, NCHs and NFs include NaOH, H_2O_2, HCl, oxygen, sodium chlorite and liquid nitrogen. Collectively, these chemicals and materials have been classified as causing severe skin burns and eye damage (NaOH, H_2O_2, HCl, sodium chlorite) in addition to may be corrosive to metals (NaOH, HCl), may cause fire or explosion (strong oxidiser) (H_2O_2), harmful if swallowed (H_2O_2) and harmful/toxic if inhaled (H_2O_2, HCl), may cause respiratory irritation (HCl), harmful to aquatic life with long lasting effects (H_2O_2, Sodium chlorite), may damage fertility or the unborn child (HCl), may cause damage to organs through prolonged or repeated exposure (HCl, sodium chlorite) and contains gas under pressure and may explode if heated HCl, may cause or intensify fire (oxidiser) (oxygen), fatal in contact with skin (sodium chlorite), toxic if swallowed (sodium chlorite), very toxic to aquatic life (sodium chlorite), may cause fire or explosion (strong oxidiser) (sodium chlorite) and may explode if heated and contains refrigerated gas and may cause cryogenic burns or injury (liquid nitrogen).

All the delivery systems developed in AGRO4AGRI on NCs, NCLs, MSN and biochar will be combined with fertiliser raw materials. Raw materials for controlled release fertilisers that will be explored include urea and ammonium nitrate as nitrogen sources, monoammonium phosphate and phosphoric acid as phosphorous sources and potassium chloride and potassium sulfate as potassium sources. Collectively, these raw materials have been classified as may intensify fire (oxidiser) (ammonium nitrate) and causes serious eye irritation (ammonium nitrate, phosphoric acid, potassium sulfate), may cause respiratory irritation (ammonium nitrate) and causes skin irritation (ammonium nitrate, phosphoric acid, potassium sulfate), harmful if swallowed (phosphoric acid) and may be corrosive to metals (phosphoric acid)).

For Target-specific nematicides based on RNAi, encapsulation technologies for controlled release of dsRNA and PB for enhance NUE no information on CLP was available.

The Early4AdMa framework was successfully applied for the initial agrochemical technologies (MSN, NC and biochar) highlighting marked uncertainty related to the categories of safety assessment of human health and environment. Uptake and transport across biological membranes was the most frequently highlighted issue and should be prioritized in further assessment of the technologies.

In conclusion, the preliminary hotspot analysis reveals that there is a lack of data and information on the hazards of the envisioned AGRO4AGRI solutions in - and of themselves, which underlines the importance of generating this information in WP7 and utilizing the information for the final selection of materials and processes in WP2 (task 2.4). The hotspot analysis also reveals that several of the potential chemicals and materials involved in the development of HDES and SAPs and synthesis of NFC, NCHs and NFs have many different hazard classifications in the EU. Identifying the least hazardous chemicals and materials in the development and synthesis of these AGRO4AGRI solutions will be a paramount importance in WP3, 4 and 5. The preliminary hotspot identification underscored the necessity for ongoing assessment and optimization throughout the product lifecycle.

Overall, the application of the SSbD and Early4AdMa frameworks provided a robust foundation for developing sustainable agrochemical solutions, with a strong focus on safety, functionality, and environmental impact. Future work will continue to refine these approaches, addressing identified knowledge gaps and indirect impacts to achieve comprehensive sustainability in agrochemical innovation. This will enable AGRO4AGRI to plan and define the materials and processes eventually be employed in the development of controlled delivery fertilisers, PB and precision-plant nematicides under Task 2.4 of WP2.

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AGRO4AGRI's details

Project name	FOSTERING THE ADVANCED USE OF AGROCHEMICALS FOR A SUSTAINABLE AGRICULTURE
Project acronym	AGRO4AGRI
Grant Agreement number	101130890
Duration and dates	48 months (1 May 2024 – 30 April 2028)
Call and topic	HORIZON-CL4-2023-RESILIENCE-01-34: Advanced (nano and bio-based) materials for Sustainable Agriculture
Granting authority	European Health and Digital Executive Agency (HADEA), under the powers delegated by the European Commission
Official project website	TBU (M6)

The AGRO4AGRI consortium

Nº	NAME	ROLE	COUNTRY
1	AINIA (AINIA)	Coordinator	Spain
2	FUNDACION CENTRO TECNOLOGICO DE COMPONENTES (CTC)	Beneficiary	Spain
3	SYDDANSK UNIVERSITET (SDU)	Beneficiary	Denmark
4	DANMARKS TEKNISKE UNIVERSITET (DTU)	Beneficiary	Denmark
5	FUNDACION GRUPO CAJAMAR (FGC)	Beneficiary	Spain
6	PROEFCENTRUM HOOGSTRATEN (PCH)	Beneficiary	Belgium
7	F. INICIATIVAS, CONSULTADORIA E GESTAO, UNIPESSOAL, LDA (FIG)	Beneficiary	Portugal
7.1	F. INICIATIVAS ESPANA I MAS D MAS I SLU (FI GROUP)	Affiliated Entity	Spain
8	SIPCAM OXON SPA (SIPCAM)	Beneficiary	Italy
9	INSTITUT FUR HOHERE STUDIEN - INSTITUTE FOR ADVANCED STUDIES (HIS)	Beneficiary	Austria
10	SYSPRO AUTOMATION SL (SYSPRO)	Beneficiary	Spain
11	MIRAT FERTILIZANTES SL (MIRAT)	Beneficiary	Spain
12	OPTIMAT LIMITED (OPTIMAT)	Associated partner	United Kingdom

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Project's summary

Agrochemicals are chemical products used in agriculture such as fertilizers, plant-biostimulants or pesticides. The application of fertilizers in synergistic combination with biostimulants provides the nutrients required for enhancing the crops yield, while pesticides are used to reduce the risk of loss from plant diseases and weeds on agricultural production. Today, the agricultural sector faces several challenges, namely the loss and leaching of fertilisers, the large amounts of pesticides used, the bioaccumulation and bioconcentration of them and the high dependency on water availability.

In this context, nano and biotechnology strategies have recently gained more interest in the agricultural sector compared to conventional agricultural techniques.

AGRO4AGRI seeks to provide ground-breaking and Safe and Sustainable by Design solutions for plant nutrition and protection consisting of nano and biobased controlled delivery fertilisers and plant biostimulants, and target-specific biopesticides based on RNAi technology, both for enhanced agrochemicals use efficiency. AGRO4AGRI involves R&D and validation stages, aiming to minimize in the long term the use of agrochemicals in agriculture in more than 50% to be aligned with the Farm to Fork Strategy, among other EU initiatives. Further project developments include the evaluation of safety, social and economic impacts, activities to promote society and policy makers engagement to bring wider impacts and better fulfil EU targets and position Europe at the forefront of the agroindustry.

Document details

Deliverable type	(Document, report / Demonstrator, pilot, prototype / Websites, patent filings, ideas, etc / Data Management Plan / Other)
Deliverable nº	D2.1
Deliverable title	Scientific SSbD evaluation for material and production processes
Lead beneficiary	DTU
Work package and task	WP 2 Task 2.2
Document version	1.2
Contractual delivery date	M2
Actual delivery date	M2
Dissemination Level	Public
Purpose	The purpose of the deliverable is to establish and match principles for (re)design AGRO4AGRI agrochemical solutions according to the SSbD framework and enable the use of these principles for AGRO4AGRI. This includes defining goal, scope, and system boundaries and the evaluation of production of the new agrochemical solutions developed in AGRO4AGRI up against the SSbD principles.

Document's abstract

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In late 2022, the EC recommended the SSbD framework to integrate safety and sustainability into the design phase of chemical and material innovation. This initiative introduces a testing period and voluntary reporting mechanism, seeking feedback from stakeholders, including industry, academia, and research communities. The AGRO4AGRI project follows this framework, focusing on Stage 1 to establish and match principles for redesigning agrochemical solutions. Specifically, this deliverable aimed to implement the Safe and Sustainable by Design (SSbD) framework recommended by the European Commission (EC) for chemicals and materials, focusing on its application within the AGRO4AGRI project. The project explores various materials and approaches to achieve controlled-release fertilizer targets, such as Nanocellulose derivatives (NCL), Mesoporous Silica Nanoparticles (MSNs), Hydrophobic Deep Eutectic Solvents (HDESs), Super Absorbing Polymers (SAPs), Nanoclays (NCs), RNA interference (RNAi), double-stranded RNA (dsRNA), and Plant Biostimulants (PBs) to enhance Nitrogen Use Efficiency (NUE).

The analysis performed in Task 2.2 of the AGRO4AGRI project involves defining the goal, scope, and system boundaries, and evaluating new agrochemical solutions against SSbD principles. The four steps of the SSbD framework were completed for each solution: 1) defining SSbD system elements, 2) setting system boundaries, 3) describing innovation goals, and 4) preliminarily identifying potential hotspots along the innovation lifecycle. The Early4AdMa framework was also applied to probe and outline potential candidates and knowledge gaps in the development of these technologies.

A key finding highlighted the critique of conventional sustainability assessments, which often neglect avoided impacts like human toxicological impacts related to indirect land use change (iLUC). This identified a need to explore these indirect impacts further, to be addressed in upcoming work packages (WP7). It is found that the SSbD framework can effectively guide the initial stages of innovation in AGRO4AGRI, emphasizing the integration of safety and sustainability from the outset. The exploration included molecular, process, and product design levels, ensuring comprehensive consideration of chemical interactions, production processes, and final product functionality. The study identified three primary SSbD principles: avoiding CMRs and reproductive toxicants, substances classified as PBT or Mobile, and unintended human and environmental exposure. In conclusion, the preliminary hotspot analysis reveals that there is a lack of data and information on the hazards of the envisioned AGRO4AGRI solutions in - and of themselves, which underlines the importance of generating this information in WP7. The hotspot analysis also reveals that several of the potential chemicals and materials involved in the development of NCs, biochar, HDES and SAPs and synthesis of NFC, NCHs and NFs have many different hazard classifications in the EU. Identifying the least hazardous chemicals and materials in the development and synthesis of these AGRO4AGRI solutions will be a paramount importance in WP3, 4 and 5. The preliminary hotspot identification underscored the necessity for ongoing assessment and optimization throughout the product lifecycle.

Overall, the application of the SSbD and Early4AdMa frameworks provided a robust foundation for developing sustainable agrochemical solutions, with a strong focus on safety, functionality, and environmental impact. Future work will continue to refine these approaches, addressing identified knowledge gaps and indirect impacts to achieve comprehensive sustainability in agrochemical innovation. This will enable AGRO4AGRI to plan and define the materials and processes eventually be employed in the development of controlled delivery fertilisers, PB and precision-plant nematicides under Task 2.4 of WP2.

Document's revision history

The following table describes the main changes done in the document since it was created.

REVISION	DATE	DESCRIPTION	AUTHOR (PARTNER)
V.0.1	10-06-2024	First draft of SSbD framework (chapter 1-3) and outline of chapter 4-7	DTU

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V.0.2	11-06_2024	Revisions of SSbD framework (chapter 1-3)	SDU
V.0.3	22-06-2024	First draft of chapter on AGRO4AGRI solutions (chapter 4)	AINIA, PCH, CTC, MIRAT, SIPCAM
V.0.4	26-06-2024	Second draft of chapter 1-7	DTU
V.1.1	27-06-2024	Quality control	AINIA
V.1.2	28-06-2024	Final revisions	DTU

Terminology and acronyms

TERM/ACRONYM	EXPLANATION
ASAR	Active substance adsorption ratio
BRL	Business Readiness Level
dsRNA	Double-stranded RNA
EC	European Commission
FAIR	Findable, Accessible, Interoperable and Reusable
HDES	Hydrophobic Deep Eutectic Solvent
IP	Intellectual Property
KER	Key Exploitable Results
KPI	Key Performance Indicator
LCA	Life Cycle Assessment
MSN	Mesoporous Silica Nanoparticle
NC	Nanocellulose derivative
NCH	Nanocellulose hydrogel
NCL	Nanoclay
NF	Nanocellulose foam
NFC	Nanofiber of cellulose
NUE	Nitrogen Use Efficiency
ORE	Open Research Europe

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PB	Plant Biostimulant
PPP	Plant Protection Products
RNAi	RNA interference
SAP	Super Absorbing Polymer
SEIA	Social, economic and sustainability impact assessment
SO	Specific objective
SSH	Social Science and Humanities
SSbD	Safe and Sustainable by Design
TRL	Technology Readiness Level
USP	Unique Selling Proposition

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1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

In late 2022, the European Commission (EC) published a recommendation on the implementation of the Safe and Sustainable by Design (SSbD) framework for chemicals and materials across different phases of the innovation journey (such as design, preparation, experimentation, and prototyping), during which choices are made regarding the progression, discontinuation, or modification of the innovative approach. The recommendation of the European Commission launches a testing period and a voluntary reporting mechanism, and the Commission plans to revise the framework based on feedback from stakeholders such as industry, academia, the research community, and support from European Member States. The recommendation from the European Commission underlines that both safety and sustainability should be addressed in the design phase and not considered as an afterthought, e.g., when a material or product has been developed and is about to be used in society.

The EC SSbD framework consists of two stages. Stage 1 entails a series of guiding (re)design principles whereas Stage 2 focusses on assessment and documentation of safety and sustainability of an existing chemical or new material.

Following a Safe and Sustainable by Design (SSbD) approach, AGRO4GRI will explore 4 different materials and several approaches to reach the controlled-delivery fertiliser targets, and 2 approaches as slow and controlled release mechanisms (see Table 1).

TECHNOLOGIES	CORE PARTNER/PARTICIPANT PARTNER	RELATED WPs
NCLs and MSNs	CTC/MIRAT, PCH, FGC, SDU, DTU, IHS	2,3,5,6,7
Biochar	CTC/MIRAT, PCH, FGC, SDU, DTU, IHS	2,3,5,6,7
HDESs and SAPs	CTC/AINIA, PCH, FGC, SDU, DTU, IHS	2,3,5,6,7
NCs derivatives	AINIA/SYSPRO, MIRAT, PCH, FGC, SDU, DTU, IHS	2,3,5,6,7
Target-specific pesticides based on RNAi	AINIA/SIPCAM, PCH, FGC, SDU, DTU, IHS	2,4,5,6,7
Encapsulation technologies for controlled release of dsRNA	AINIA/SIPCAM, PCH, FGC, SDU, DTU, IHS	2,4,5,6,7
PBs for enhanced NUE	SIPCAM/ PCH, FGC, SDU, DTU, IHS	2,5,6,7

Table 1. Technologies explored in AGRO4AGRI by core and participants partners. NCL = Nanocellulose derivative; MSNs = Mesoporous Silica Nanoparticle; HDESs = Hydrophobic Deep Eutectic Solvent; SAPs = Super Absorbing Polymer; NCs = Nanoclay; RNAi = RNA interference; dsRNA = double-stranded RNA; PBs = Plant Biostimulant; NUE = Nitrogen Use Efficiency.

1.2 Objectives

In this deliverable we focus on Stage 1 of the EC SSbD framework and we report on the completion of Task 2.2 that evolves around establishing and matching principles for (re)design agrochemical solutions according to the SSbD framework. The task has two subtasks, namely subtask 2.2.1 that aims to define goal, scope, and system

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boundaries and subtask 2.2.2. Evaluation of production of the new agrochemical solutions against the SSbD principles.

2. Subtask 2.2.1 Definition of goal, scope, and system boundaries

The purpose of this subtask is to provide guidance on which SSbD principles to consider in AGRO4AGRI at the (re)design stage to maximise the possibilities of a successful safety and sustainability assessment outcome. These principles include principles that apply to chemicals and materials in general as well as principles that have been proposed for nanomaterials in specific. Before going into detail with the design principles that could be and will be used in AGRO4AGRI, the goal, scope and system boundaries of the AGRO4AGRI have to be defined according to the SSbD framework of the European Commission (Abbate et al. 2024). The SSbD framework of the European Commission consists of four steps:

1) definition of SSbD system elements (see section 2.1);

2) setting system boundaries (see section 2.2);

3) description of the goal(s) of innovation (see section 2.3); and

4) the preliminary identification of potential hotspots along the life cycle of the innovation. Step 4 of the SSbD framework of the European Commission corresponds to subtask 2.2.2 of AGRO4AGRI on evaluation of production of the new agrochemical solutions against the SSbD principles and will therefore be addressed in a chapter of its own (see chapter 3).

2.1 AGRO4AGRI definition of SSbD system elements

According to the EC Joint Reseach Centre's recent methodological guidance on SSbD of chemicals and materials (Abbate et al. 2024), it is key to define the system under study by, for instance, describing the chemical/material under assessment (chemical/material), its function (final product/application) and considering the life cycle, including relevant processes and products (value chain) (see Figure 1).

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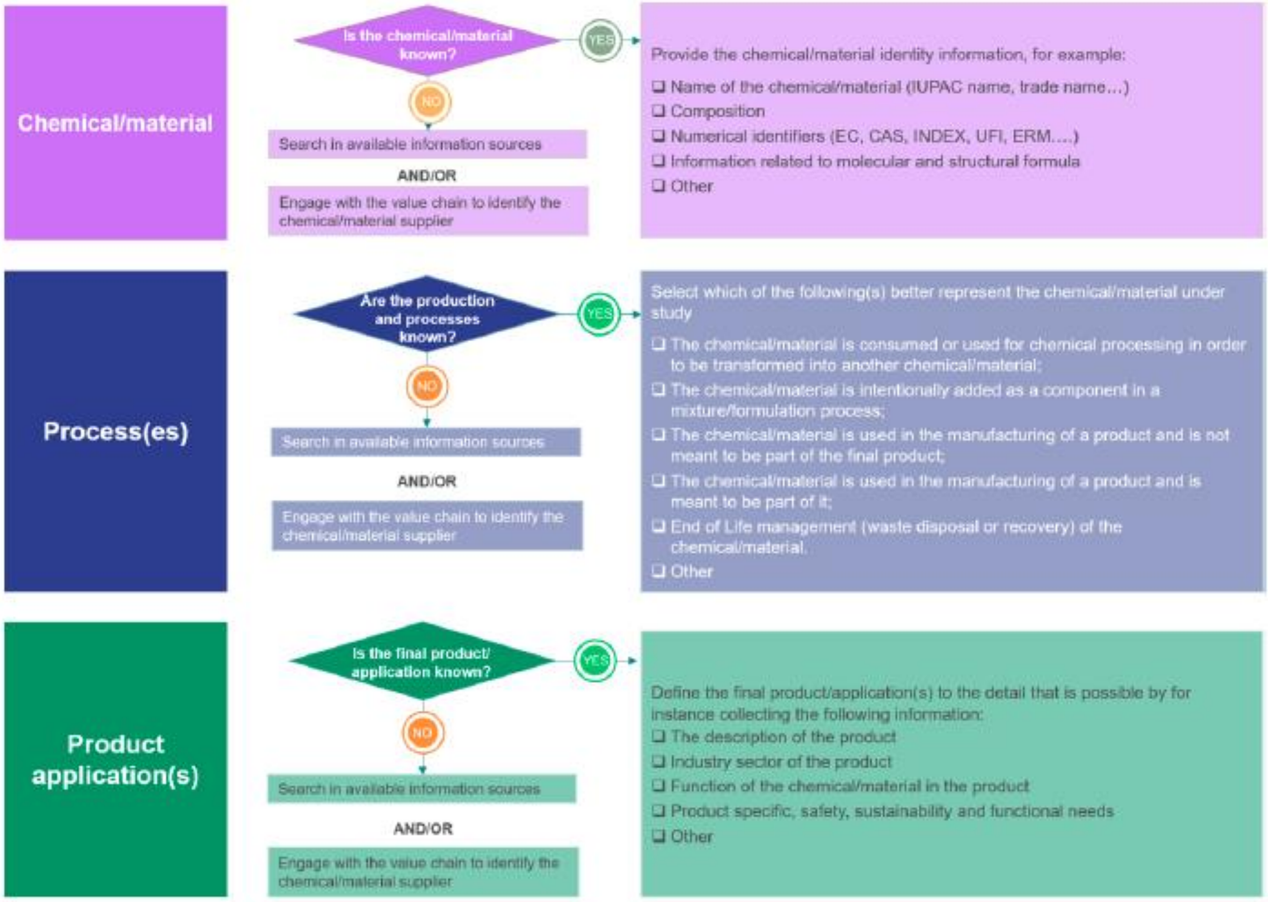

Figure 1 SSbD system elements (chemical/material, process and product) and information to define them (From Abbate et al. 2024).

The starting point of the definition of the system to be assessed will depend on the organisation's position in the life cycle of the chemical/material. When it comes to the technologies explored in AGRO4AGRI, the organisation's position in the life cycle of the chemical/material is at the very early stages of research and development when it comes to the production processes as well as the product applications. At this point in time, it is also not known to a full extent what chemicals and materials might be used in the development of the technologies. However, to establish an early engagement with the value-chain, as suggested by the EC JRC (see figure 1), the framework Early4AdMa (OECD, 2023) was used to probe and outline already identified potential candidates for each of the technologies (Table 1). Additionally, the Early4AdMa framework was utilized to identify pivotal knowledge gaps that would have to be addressed in the upcoming WPs (e.g. WP7 – Environmental, socio-economic and sustainability assessment). In general, Early4AdMa is an early awareness and action system that through 65 questions help to systematically identify potential effects related to four topics 1) human health, 2) environment, 3) sustainability and 4) regulatory preparedness. The latter having a strong focus related to advanced materials where it is still uncertain whether the current regulation or test methodologies will be applicable. Consequently, Early4AdMa provides a tool for anticipatory risk governance to facilitate development of SSbD approaches. It should be noted that technological development within nano-and advanced materials is rapidly expanding and changing, thus the framework should be used dynamically as new properties and potential risks of advanced materials are identified. Applying Early4AdMa to the agrochemicals within AGRO4AGRI (Table 1) identifies potential knowledge gaps to help to prioritize actions to address the knowledge gaps and provides additional case-studies and much needed feedback for further development of the framework.

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2.2 Setting AGRO4AGRI system boundaries

There are different manners in which the system boundaries can be defined (see Figure 2). The different elements of the SSbD framework Stage 2, which focuses on assessment of SSbD, has different boundaries in a of itself e.g., the hazard assessment is aimed at the chemicals/materials used; the human health and safety aspects focuses on production and processing and the human health and environmental aspects focuses on the final application.

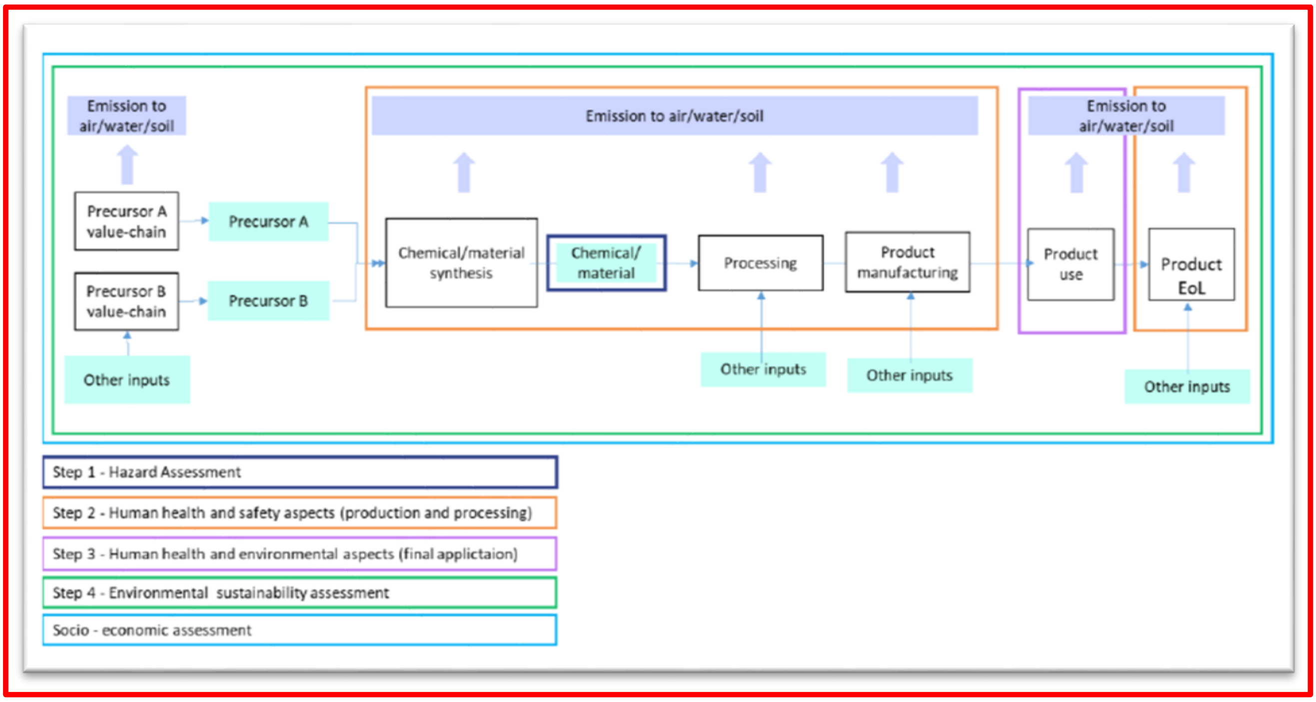

Figure 2 Representation of the alignment between the chemical/material life cycle and the progressive required broadening of the SSbD assessment steps and boundaries (boxes in white are processes stages, coloured boxes are inputs and outputs of the processes). [EoL: end of life] (From Abbate et al. 2024). Red line indicates the boundaries of AGR04AGRI.

AGRO4AGRI focuses on all conventional sustainability/SSbD imbedded aspects, including environmental sustainability and socio-economic assessment. A critique that has been raised regarding conventional existing assessment schemes, and thus mainly focusing on induced and (generally) disregarding avoided impacts (e.g. avoided human toxicological impacts related to (avoided) indirect land use change (iLUC)) and thus eroding the actual sustainability coverage. Therefore, in addition to the conventional impacts directly induced and/or avoided within the system boundaries presented in Figure 2, WP 7 of AGRO4AGRI will also explore how the more elusive, yet equally relevant, indirect impacts can be accounted for as part of/in addition to the SSbD assessment framework. Furthermore, WP 2 Task 2.3 will WP7 will investigate how these indirect impacts can be used to make SSbD-derived decision support more ethically defendable. The more elusive indirect impacts considered include human and eco-toxicological effects, biodiversity changes, as well as climate change-induced impacts that occur when materials and chemicals developed as part of AGRO4AGRI induce changes in global demands for crops. As a concrete however hypothetical example of these elusive indirect impacts, we envision a situation where a material developed in AGRO4AGRI increases the European barley yield by, for instance, 2%. In this hypothetical example, the material developed is known to induce both human and eco-toxicological impacts in the region where it is applied, while also increasing the yield. The yield increase induced by the material developed in AGRO4AGRI will make EU less dependent on crops imported from outside the EU, hence lowering the demand for e.g. corn, which is mainly imported from Brazil in South America. By lowering the demand for corn from Brazil, the export options for Brazilian agriculture will decrease, thereby reducing Brazilian applications of pesticides (bearing in mind that the pesticides used in Brazil in relation to corn production are quite different from those used in the EU in relation to barley production) and the need for agricultural land/deforestation. Consequently, lowering the demand for corn production in Brazil will reduce impacts on biodiversity, ecosystems, human health, and climate in regions outside the EU, while increasing ecosystem and human health impacts within the EU. By

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neglecting these indirect impacts (such as the impacts induced/avoided in Brazil in the hypothetical example), it could be argued that the SSbD assessment framework has a neo-colonial bias and thus a dubious ethical foundation. AGRO4AGRI will explore how these indirect impacts and thus how an "expanded set of system boundaries" can be utilized to visualize "impact export" outside EU (i.e. export out of EU and out of sight to more economically challenged parts of the world) with the sole aim to avoid impacts internally in the EU. Subsequently if will be explored how this information on indirect impacts can be used to provide additional quantitative and qualitative/ethical dimensions to the decision support derivable from the SSbD framework.

2.3 AGRI4AGRO goals of technological innovation

The definition of the SSbD system furthermore requires the description of the goal(s) of innovation subject to the SSbD evaluation. The goals of each of the different technologies explored in AGRO4AGRI varies and include modifying the nanocellulose and biochar to interact more effectively with the active ingredients in fertilizers (see Table 2). When it comes to the goal of the innovation, the SSbD framework recommended by the European Commission covers three levels of the term 'by design':

- Molecular design, to design new chemicals and materials based on their chemical structure;
- Process design, to make the production process safer and more sustainable, both for chemicals and materials being developed and for existing chemicals and materials;
- Product design, where the results of the SSbD assessment support the selection of the chemicals or materials to meet the functional demands of the final product in which they are used.

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TECHNOLOGIES	GOAL WITH INNOVATION	LEVEL OF "DESIGN **Molecular/Process/Product**		
Nanoclay and Mesoporous Silica Nanoparticle (CTC/ MIRAT, PCH, FGC, SDU, DTU, HIS)	By employing NCLs and MSNs as carriers, the project aims to increase the efficiency of fertilizers by ensuring that nutrients are delivered directly to the plant roots in a controlled manner, reducing nutrient loss and increasing uptake efficiency. The development focuses on enabling a slow and controlled release of nutrients, which is responsive to environmental triggers such as changes in moisture, temperature, or soil pH. This targeted release helps to provide nutrients when plants are most able to absorb them, enhancing overall usage efficiency.	X	X	X
Biochar (CTC/ MIRAT, PCH, FGC, SDU, DTU, HIS)	By using biochar as a carrier, the project aims to optimize fertilizer delivery through a slow and controlled release mechanism. This involves optimizing the pyrolytic production of biochar to tailor its physical and chemical properties, such as porosity and phytonutrient composition, to meet specific agricultural requirements as well as the development of biochar-based fertilizer systems that respond to environmental triggers (like changes in moisture or temperature).	X	X	X
Hydrophobic Deep Eutectic Solvent; SAPs = Super Absorbing Polymer (CTC/AINIA, PCH, FGC, SDU, DTU, HIS)	Both HDESs and SAPs are being developed to act as components of a slow and controlled release system of nutrients i.e., fertilizers. This system is designed to release nutrients in response to specific environmental stimuli such as changes in moisture or temperature. This targeted release improves nutrient uptake efficiency by crops and reduces nutrient losses to the environment and increases the persistence of fertilizers in the soil.	X	X	X
Nanocellulose derivatives (AINIA/ SUSPRO, MIRAT, PCH, FGC, SDU, DTU, HIS)	By using NCs as bio-based carriers, the project aims to improve the delivery of fertilizers. This involves modifying the nanocellulose to interact more effectively with the active ingredients in fertilizers, which helps in achieving a controlled release. This controlled release mechanism is particularly important as it allows the fertilizer to be released in response to specific external stimuli such as changes in water content.	X	X	X
Target-specific pesticides based on RNA interference	The goal of developing target-specific nematicides based on RNA interference (RNAi) for agricultural purposes in the project is to enhance plant health and crop yields by effectively controlling nematode pests in a precise and environmentally sustainable manner. By knocking down essential genes in the	X	X	X

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(AINIA/ SIPCAM, PCH, FGC, SDU, DTU, HIS)	nematode genome, these nematicides prevent the pests from maturing and reproducing, thereby reducing their populations and the associated crop damage. These nematicides are designed to be highly specific, targeting only the nematodes and minimizing unintended impacts on non-target organisms.			
Encapsulation technologies for controlled release of double-stranded RNA (AINIA/ SIPCAM, PCH, FGC, SDU, DTU, HIS)	The goal of developing methods for encapsulation of dsRNA as this is critical for the effective application of RNAi-based pesticides. By developing encapsulation systems and designing a pH-triggered release system, this project will ensure that dsRNA reaches the intestinal tract of nematodes and can exert its effect. This involves creating systems that release dsRNA in acidic conditions, which match the internal pH of nematode intestines.	X	X	X
Plant Biostimulants for enhanced Nitrogen Use Efficiency (SIPCAM / PCH, FGC, SDU, DTU, HIS)	By improving NUE, the biostimulants developed is this project will help plants utilize nitrogen more effectively, which is crucial for protein synthesis and overall growth. The biostimulants are designed to enhance root development and canopy growth whereby improving water and nutrient uptake, robust plant growth and increased photosynthetic capacity. This involves studying the synergistic effects of combining these biostimulants with controlled-release fertilizers.	X	X	X

Table 2. Goals of different technologies explored in AGRO4AGRI

3. Identification AGRO4AGRI (re)design principles

Once the goal(s) of innovation is described, it triggers a series of questions according to the EC JRC, for example: What are the principles on which the (re)design is going to be based? What are the needs to improve/ensure the safety, sustainability, and functionality within the SSbD system? Which elements of these considerations will be addressed and assessed, and in what degree of detail? (Abbate et al. 2024). The implementation of the design principles is to be accompanied with a selection of indicators and actions that describe the performance and implementation of the selected design principles (see Figure 3).

It is important to understand that although several different SSbD design principles are mentioned in both the EC Recommendation (European Commission, 2022a) as well as in the EC JRC (Abbate et al. 2024), the choice of principles to be pursued has to be adapted to the needs of the specific sector under consideration and the particular application.

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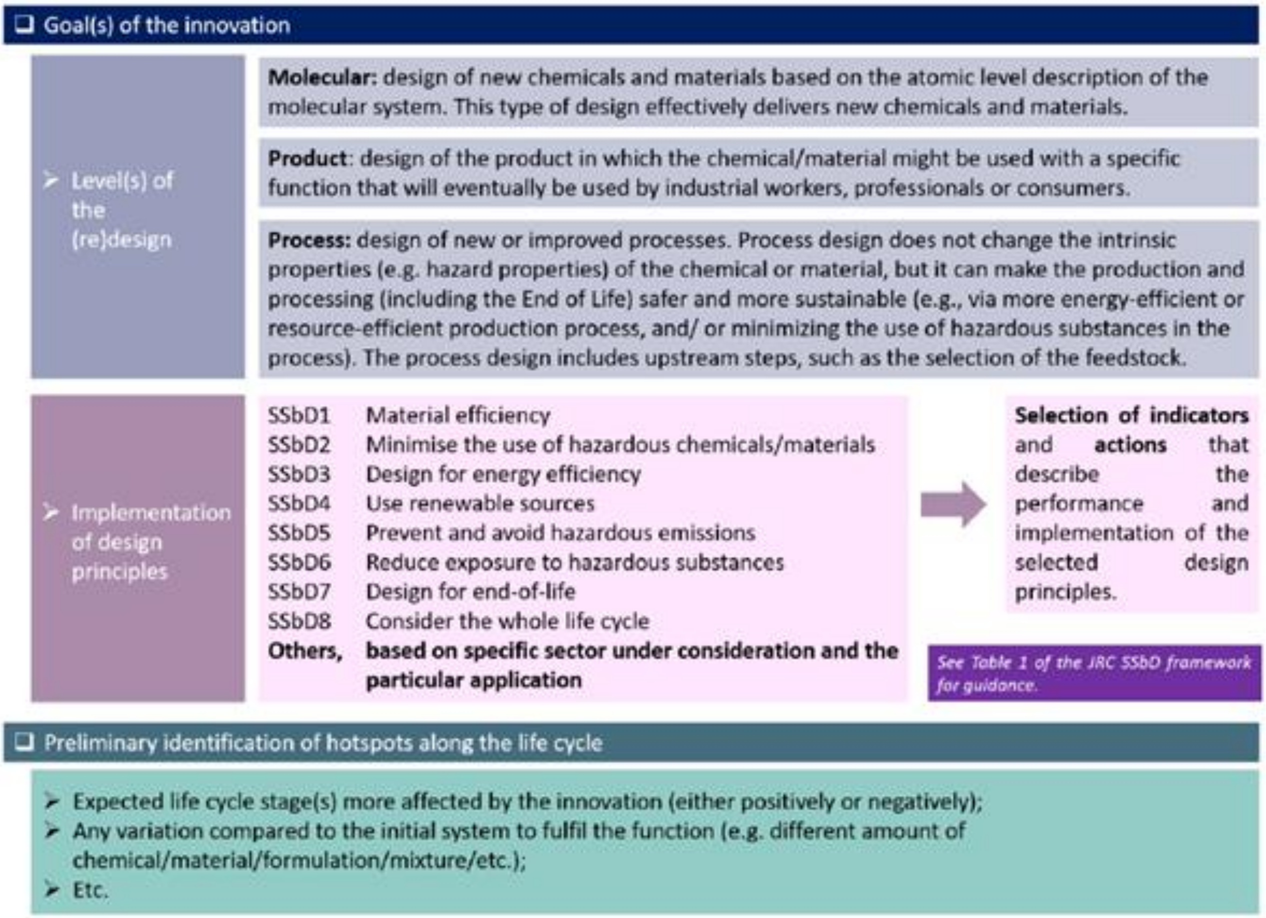

Figure 3. Information to collect for the (re)design definition according to the EC JRC (From Abbate et al. 2024).

3.1 General design principles for SSbD

The link between safety and sustainability of chemicals is not always clear-cut, although it seems generally accepted that the use of hazardous chemicals that are persistent and dispersive across a wide spatial range is not sustainable (UBA 2009) (see Figure 34).

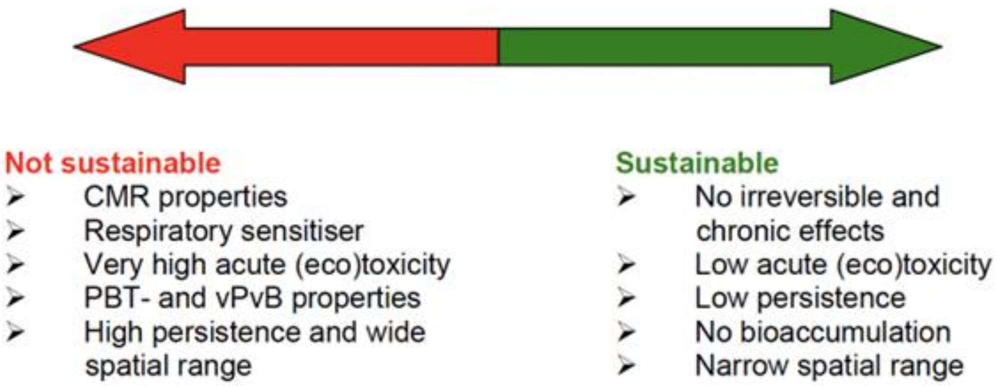

Figure 3 Characteristics of inherently safe chemicals (From UBA 2009).

In the SSbD framework recommended by the European Commission, a range of general design principles for SSbD are mentioned and listed. These fall into the following categories: Material efficiency; Minimise the use of hazardous chemicals or materials; Design for energy efficiency; Use renewable sources; Prevent and avoid hazardous emissions; Design for end of life; and consider the whole life cycle (see Table 3).

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Green chemistry (Anastas & Warner 1998)	Green engineering (Anastas and Zimmerman 2003)	Sustainable chemistry (UBA 2009)	Sustainable chemistry (UBA 2016)	Circular chemistry (Keijer et al. 2019)
Prevention. It is better to prevent waste formation than to treat it after it is formed. Atom economy. Design synthetic methods to maximize incorporation of all material used into final product. Less hazard. Synthetic methods should, where practicable, use or generate materials of low human toxicity and environmental impact. Safer chemicals. Chemical product design should preserve efficacy whilst reducing toxicity. Safer solvents. Avoid auxiliary materials - solvents, extractants - if possible, or otherwise make them innocuous. Energy efficiency. Energy requirements should be minimized: conduct synthesis at ambient temperature and pressure. Renewable feedstocks. Raw materials should, where practicable, be renewable. Reduce derivatives. Unnecessary derivatization should	Designers need to strive to ensure that all material and energy inputs and outputs are as inherently nonhaz-ardous as possible. It is better to prevent waste than to treat or clean up waste after it is formed. Separation and purification operations should be designed to minimize energy consumption and materials use. Products, processes, and systems should be designed to maximize mass, energy, space, and time efficiency. Products, processes, and systems should be "output pulled" rather than "input pushed" through the use of energy and materials. Embedded entropy and complexity must be viewed as an investment when making design choices on recycle, reuse, or beneficial disposition. Targeted durability, not immortality, should be a design goal. Design for unnecessary capacity or capability	Qualitative development: Use of harmless substances, or where this is impossible, substances involving a low risk for humans and the environment, and manufacturing of long-life products in a resource-saving manner. Quantitative development: Reduction of the consumption of natural resources, which should be renewable wherever possible, avoidance or minimization of emission or introduction of chemicals or pollutants into the environment. Such measures will help to save costs. Comprehensive life cycle assessment: Analysis of raw material production, manufacture, processing, use and disposal of chemicals and discarded products in order to reduce the consumption of resources and energy and to avoid	If possible, only use substances (as such, in mixtures or in articles) which are not mentioned on lists of problematic substances. Using problematic substances assess the different uses and potential users of the substance as such. If the substance cannot be exchanged you have to take responsibility for the consequences of its use. Never only evaluate the substance in isolation but think through the entire lifecycle. As much as possible use substances which are not dangerous to human health (in particular none, which are classified as carcinogenic, mutagenic or reprotoxic), which are easily degraded, don't bioaccumulate and don't widely disperse in the environment. Don't use substances, which require a high degree of risk management according to the	1. Collect and use waste. 2. Maximize atom circulation. 3. Optimize resource efficiency. 4. Strive for energy persistence. 5. Enhance process efficiency. 6. No out-of-plant toxicity. 7. Target optimal design. 8. Assess sustainability. 9. Apply ladder of circularity. 10. Sell service, not product. 11. Reject lock-in. 12. Unify industry and provide coherent policy framework.

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be avoided where possible. Smart catalysis. Selectively catalyzed processes are superior to stoichiometric processes Degradable design. Chemical products should be designed to be degradable to innocuous products when disposed of and not be environmentally persistent. Real-time analysis for pollution prevention. Monitor processes in real time to avoid excursions leading to the formation of hazardous materials. Hazard and accident prevention. Materials used in a chemical process should be chosen to minimize hazard and risk for chemical accidents, such as releases, explosions, and fires.	(e.g., "one size fits all") solutions should be considered a design flaw. Material diversity in multicomponent products should be minimized to promote disassembly and value retention. Design of products, processes, and systems must include integration and interconnectivity with available energy and materials flows. Products, processes, and systems should be designed for performance in a commercial "afterlife". Material and energy inputs should be renewable rather than depleting.	the use of dangerous substances. Action instead of reaction: Avoidance, already at the stage of development and prior to marketing, of chemicals that endanger the environment and human health during their life cycle and make excessive use of the environment as a source or sink; reduction of damage costs and the associated economic risks for enterprises and remediation costs to be covered by the state; Economic innovation: Sustainable chemicals, products and production methods produce confidence in industrial users, private consumers and customers from the public sector and thus, result in competitive advantages.	easy-to-use workplace control scheme for hazardous substances or the COSHH approach Prefer substances which are available in excess or made from renewable resources to substances which are scarce and produced from fossil raw materials. Avoid long-distance transports at any stage of the supply chain, in particular for substances which you use in high amounts. Pay attention to a low energy and water consumption of substances you use in large amounts as well as to a low generation of wastes in manufacturing and use. Assess whether your suppliers conform to high environmental and social standards. Select substances considering the transparency of the supply chain and the commitment of its actors to sustainability. Furthermore, products should not be put on the market for which a societal benefit and a benefit for	

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			consumers can not be identified.	

Table 3. General design principles for SSbD

3.2 SSbD principles for NMs in specific

When it comes specifically to nanomaterials, a range of SSbD design principles have also been proposed that might be relevant to consider as for the AGRO4AGRO innovations.

Already back in 2009, Morose suggested five principles of "Design for Safer Nanotechnology" (see Figure 5). Size, surface and structure refers to changing the size, surface, or structure of the nanoparticle so that the desired product functionality is preserved, but the hazard and/or exposure potential of the nanoparticle is diminished. Similarly, functionalization and encapsulation refer to functionalization and encapsulation of the nanoparticle in a manner such that the desired product properties are preserved, but the hazard and/or exposure potential of the nanoparticle is reduced or eliminated. Using alternative material (nano or bulk) refers to exploring the use of alternatives to replace the nanoparticle identified as hazardous. In situations, where the nanoparticle hazard cannot be reduced or eliminated while maintaining the desired product functionality, the continued use of the hazardous nanoparticle may be necessary, but it should be investigated if there is a possibility of using smaller quantities of the hazardous nanoparticle in the product (Morose 2009).

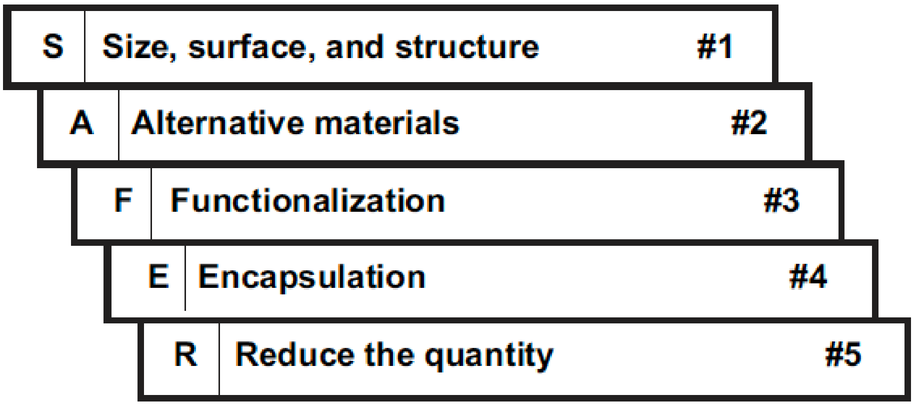

Figure 5. The five principles of safer nanotechnology (From Morone 2009).

Along the same lines as Morone (2009), Hansen et al. (2012) proposed that historical early warning signs of health and environmental risks could be used in the initial R & D process by nanomaterial researchers and developers to develop and screen for nanomaterials that are "safe by design". These early warning signs were signs such as novelty, persistency, whether materials are readily dispersed in the environment, and whether they bioaccumulate or lead to potentially irreversible action. For instance, by changing the number of functional groups on C60, the solubility of C60 and hence the dispersibility can be altered. Similarly, nZVI could be engineered with coatings that could reduce potential (eco) toxicity or better control environmental migration (Hansen et al. 2012).

More recently, Markiewicz et al. (2018) have proposed a series of so-called "green design principles" for nanomaterials (NMs):

- Non-active and non-soluble NMs should be preferred over the (re)active or soluble ones wherever the reactivity is not the actual desired function of the NMs. Finding appropriate replacements might not be easy or even possible if the application requires e.g. antimicrobial activity from ions. In such cases, a green design approach might alternatively be to ensure that dissolution and ion release occurs slowly, only under the specified conditions during application and not afterwards.

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- Increase size as larger particles tend to be more prone to destabilisation (even in the presence of natural organic matter (NOM)), have lower specific surface area and therefore dissolve slower/to lower extent, generate less formation of reactive oxygen species and present less surface for interaction with NOM;
- Avoid cationic stabilisers as they generally increase interactions with living organisms and thus toxicity. This interaction is mitigated by NOM as it has a higher affinity for positively charged particles and can reverse the charge of NMs, at least temporarily. However, following internalisation by organisms, acidic lysosomal compartments may degrade the NOM corona, re-exposing the underlying cationic surface.
- Protect the core of the NM from dissolution by using core/shell structures or by doping e.g., ZnS shell protects the core of QDs and decreases leaching of ions. The presence of NOM can enhance dissolution of NMs but the protective shell should hinder that to a large extent.
- Decrease photoactivity whenever possible e.g. decrease photoactivity of ZnO in antimicrobial applications (e.g. by using a core/shell structure or by doping), use UV-active rutile instead of visible light-active anatase in paints where the only function of the TiO2 is to provide a white colour etc.
- Minimising exposure be prevention of release into the environment. Specifically, at the production stage, care should be taken to maximise recovery from process/waste streams to save resources and minimise the release of NMs (Markiewicz et al. 2018).

Several European research projects of relevance to Safety by design have been funded under H2020 nanosafety projects under the calls NMBP-15 – "Safe by design, from science to regulation: metrics and main sectors" and NMBP-16 – "Safe by design, from science to regulation: multi-component nanomaterials" (see Falk et al. 2021 for a review of all the different projects). For instance, the NanoReg2 project specified three design pillars (Sánchez Jiménez et al., 2020; Soeteman-Hernandez et al., 2019).

- Safe(r) material/product: minimising, in the R&D phase, possible hazardous properties of the NM or NEP while maintaining function;
- Safe(r) production: ensuring industrial safety during the production of NM and NEP, more specifically occupational, environmental and process safety aspects; and
- Safe(r) use and end-of-life: minimising exposure and associated adverse effects through the entire use life, recycling and disposal of the NM or NEP (Tavernaro et al. 2021).

Building on EU projects such as NanoReg2, CaLIBRAte and GRACIOUS, HARMLESS proposed a range of different design principles during production and manufacturing, use and end-of-life. These principles include identification of any legal restrictions applying to the nanomaterial enable product (NEP) or chemical components of the NEP (REACH and sector specific); identification of alternatives to chemical components of concern; ensure none of the intended use of the NEP (or NEP components) is restricted (no SVHC, ED, MPT) and quantification of environmental release and nano-specific human health risks of workers and consumers (and general population) due to NM release during use and end-of-life. It also includes the requirements to test a new material or product on physicochemical and hazard endpoints mentioned in REACH in a cost-effective manner and management and minimization of emissions of non-nano emissions and wastes from the production process (Adam et al. 2023). The design principles are to be applied at different stages of the innovation e.g., product idea and screening, lab scale and pilot scale.

3.3 Selection of AGRO4AGRI (re)design principles

According to the EC JRC SSbD methodological guidance (Abbate et al. 2024), the implementation of the design principles must be accompanied with a selection of indicators and actions that describe the performance and implementation of the selected design principles. Based on our analysis of existing (re)design principles for chemicals and nanomaterials and multifunctional materials, we have decided to adopt 3 overall design principles in AGRO4AGRI. These are:

1. Avoid substances, mixtures and materials that are classified as e.g., CMRs, reproductive toxicants according to the European Classification, Labelling and Packaging (CLP) Regulation known as the CLP Regulation;

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2. Avoid substances that fulfil the criteria for being Persistent (P), Bioaccumalte (B), Toxic (T) or Mobile (M);
3. Avoid unintended exposure to humans and environment.

3.3.1 AGRO4AGRI Design principles # 1: Avoid substances, mixtures and materials that are CLP classified

The first design principles that will be operationalized in AGRO4AGRI is the design that substances and materials that are classified according to the CLP Regulation will not be used at different stages of the innovation e.g., product idea and screening, lab scale and pilot scale. The principle revolves around whether the chemical and material is known or suspected to cause serious detrimental effects according to Regulation (EC) No 1272/2008 also known as the CLP Regulation (ECHA 2024).

A fundamental aspect of the CLP Regulation is the 'self-classification' conducted by the manufacturer, importer, or downstream user. This process requires them to determine the hazards associated with a substance or mixture and to align this information with the criteria established in the CLP. The classification depends on the inherent hazardous properties of the substance or mixture, without considering the probability of exposure or risk factors. (ECHA 2024).

CLP classifications include the most serious health and environmental outcomes where the chemical or material is either known or strongly suspected to cause severe and potentially irreversible harm. To determine a self-classification, the classifier must collect all available information and assess its adequacy and reliability. This information must then be measured against the classification criteria to determine the appropriate classification. Manufacturers, importers, and downstream users are required to stay updated with new scientific or technical developments and decide if a re-evaluation of the self-classification of the substance or mixture they market is necessary (ECHA 2024). Consequently, the use of CLP information ensures that previous knowledge is considered and enables broader identification of potential hazards associated with the chemicals, mixtures and materials than if novel technology eventually developed in AGRO4AGRI is reviewed alone.

3.3.2 AGRO4AGRI Design principles # 2: Avoid substances that fulfil the criteria for being Persistent (P), Bioaccumulative (B), Toxic (T) or Mobile (M)

Substances that are P, B and T or P, M and T are of special concern and will be avoid in the development of novel technologies in AGRO4AGRI. PBT assessment is required under EU's chemical regulation for all substances manufactured or imported in amounts of 10 tonnes or more per year, unless exemptions apply. Under the Biocidal Product Regulation in the EU, all biocidal active substances also have to undergo a formal PBT assessment. The criteria for P, B, T and M can be found in Table 4. Substances that have been assess as PBT or are under assessment can be found at the website of the European Chemicals Agency via https://echa.europa.eu/pbt (ECHA 2024b).

Persistence	(a) the degradation half-life in marine water is higher than 60 days; (b) the degradation half-life in fresh or estuarine water is higher than 40 days; (c) the degradation half-life in marine sediment is higher than 180 days; (d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days; (e) the degradation half-life in soil is higher than 120 days.
Bioaccumulation	A substance shall be considered to fulfil the bioaccumulation criterion (B) where the bioconcentration factor in aquatic species is higher than 2000

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Toxicity	a) the long-term no-observed effect concentration (NOEC) or ECx (e.g. EC10) for marine or freshwater organisms is less than 0,01 mg/l; (b) the substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B, or 2) according to Sections 3.5, 3.6 or 3.7; (c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification as specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Section 3.9; (d) the substance meets the criteria for classification as endocrine disruptor (category 1) for human health or the environment according to Sections 3.11 or 4.2
Mobility	A substance shall be considered to fulfil the mobility criterion (M) when the log Koc is less than 3. For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log Koc value for pH between 4 and 9 is less than 3.

Table 4: PBT and M criteria according to Annex XIII to the REACH Regulation and Delegated Regulation amending CLP Regulation

3.3.3 AGRO4AGRI Design principles # 3: Avoid unintended exposure to humans and environment

Several of the different proposed safety by design principles call for minimization of exposure based on the assumption that if there is no exposure, then there will not be a risk as risk is dependent on the inherent hazardous properties and exposure to a given chemical, mixture and material (Morose 2009, Markiewicz et al. 2018). During the initial R&D processes, the actual assessment of the overall exposure potential can be hampered by both lack of information and lack of access to information about which and how much of a given chemical, mixture or materials are to be used at different stages of the innovation. Hansen et al. (2008) has proposed a framework to aid exposure assessment in consumer products that will be adopted in AGRO4AGRI. The framework is based on categorising consumer products that have been claimed to entail nanomaterials, but can be expanded to other categories of chemicals, mixtures, materials and products as well. In essence the categorization is placed on the location of the chemical, mixture or material in the process and product and grouping these into three different exposure categories:

- expected to cause exposure
- may cause exposure
- no expected exposure to the consumer

Chemical, mixture or materials that would typically fall under the first category are products with 'suspended in liquids" or "airborne" whereas chemical, mixture or material that are "surface-bound" and "suspended in solids" would fall into the second and third category, respectively (see Figure 6).

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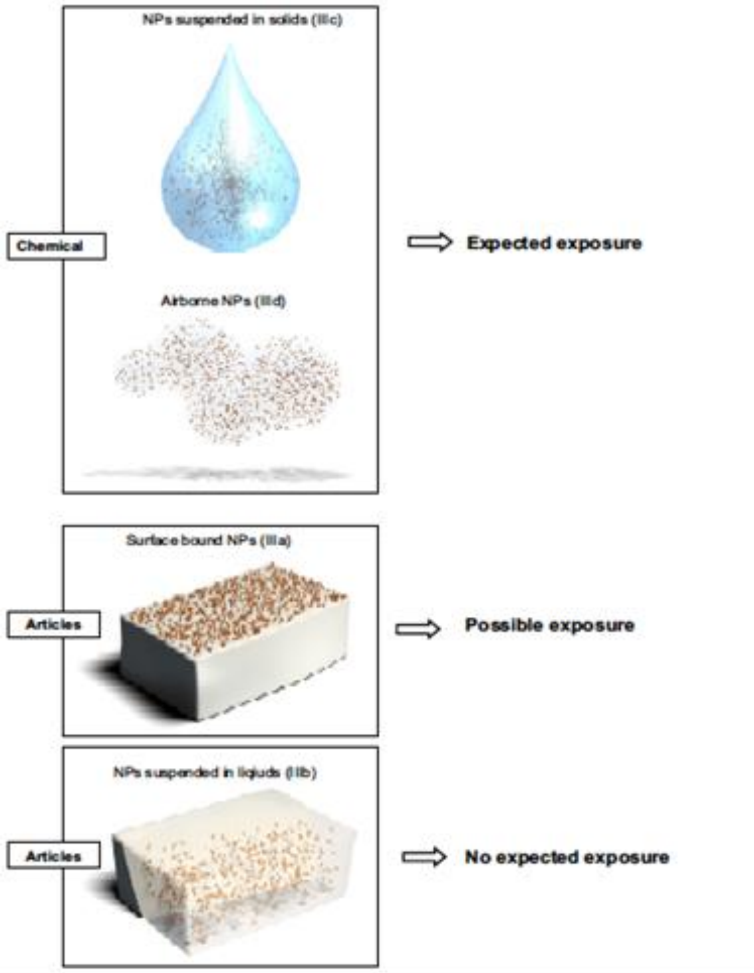

Figure 6. Different exposure categories depending on the location of the nanomaterial (From Hansen et al. 2008).

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4. Subtask 2.2.2 SSbD evaluation of production of the new agrochemical solutions

4.1 Preliminary identification of potential hotspots along the life cycle

The definition of the SSbD system is completed by the preliminary identification of potential hotspots along the life cycle. The identification process requires first a detailed understanding of the life cycle and second an evaluation of the chemicals/materials involved, the operations and in which these chemicals and materials are involved in and finally, any risk management measures that might normally be in place.

In order to SSbD evaluate the production of new agrochemical solutions developed in Agro4Agri, each step of their synthesis was first mapped and subsequently evaluated up against the SSbD principles mentioned by the Commission and operationalized in subtask 2.1. Using the methodology outlined by Lakhe et al. (2019), our focus will be both on synthesis at the lab scale (grams of the new agrochemicals) as well as aspect the process scaleup and large-scale production (kilogram quantities) as some hazards might not be so relevant at the lab scale compared to when scaled up.

4.2 Synthesis of Nanoclays (NCLs) and mesoporous silica (MSNs)

4.2.1 Goal of the synthesis of Nanoclays (NCLs) and mesoporous silica (MSNs)

The overarching goal with synthesis of Nanoclays (NCLs) and mesoporous silica (MSNs) is to optimize fertilizer usage and improve agricultural productivity while reducing environmental impact.

4.2.2 Synthesis at the lab scale development of NCLs

The synthesis of NCLs will be first completed at the lab scale in AGRO4AGRI and involves several key steps and methodologies (see Figure 7):

Step 1. Extraction of Raw Clay: Mining and purification/selection
Step 2. Mechanical milling, Modification and Exfoliation
Step 3. Functionalization

Specifically, step 2 involves grinding bulk clay material into fine particles using balls in a rotating cylinder (ball milling) and raw clays are treated with organic or inorganic agents to intercalate or insert molecules between the clay layers (intercalation). Common intercalating agents include quaternary ammonium salts. The intercalated clay is then swollen in a suitable solvent, which helps to increase the distance between the clay layers (swelling/expansion). It also involves exfoliation in the form of mechanical, thermal or chemical processes that are applied to exfoliate the clay into individual nanometer-thick layers. This can be done using techniques like high-shear mixing, ultrasonication, High temperature treatment or chemical exfoliation. Step 3 on functionalization involves the modification of the surface of the nano clay particles to enhance chemical compatibility with other substances (in AGRO4AGRI it will be with fertilisers). This is typically done by grafting functional groups onto the clay surface.

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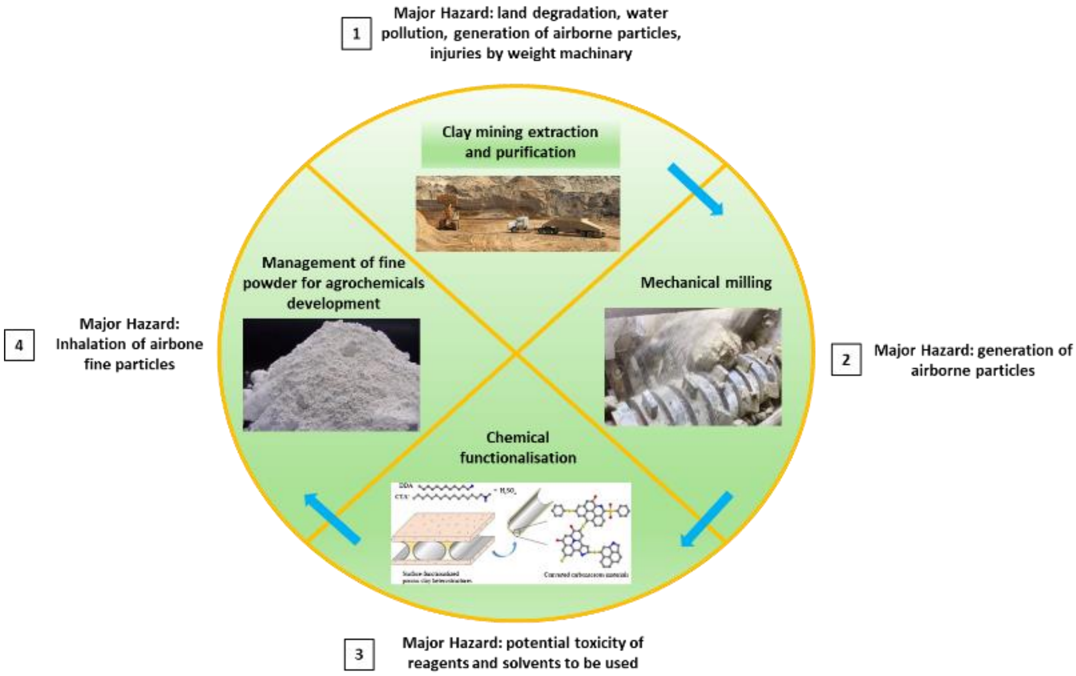

Figure 7. Diagram of nanoclays production for the new agrochemical development

4.2.3 Synthesis at the lab scale development of MSNs

In order to prepare MSNs, there are several mechanisms, highlighting the formation of silicate structure around the liquid crystal phase and the self-assembly of inorganic precursors and the organic surfactant takes place, which results in an ordered array (see Figure 8).

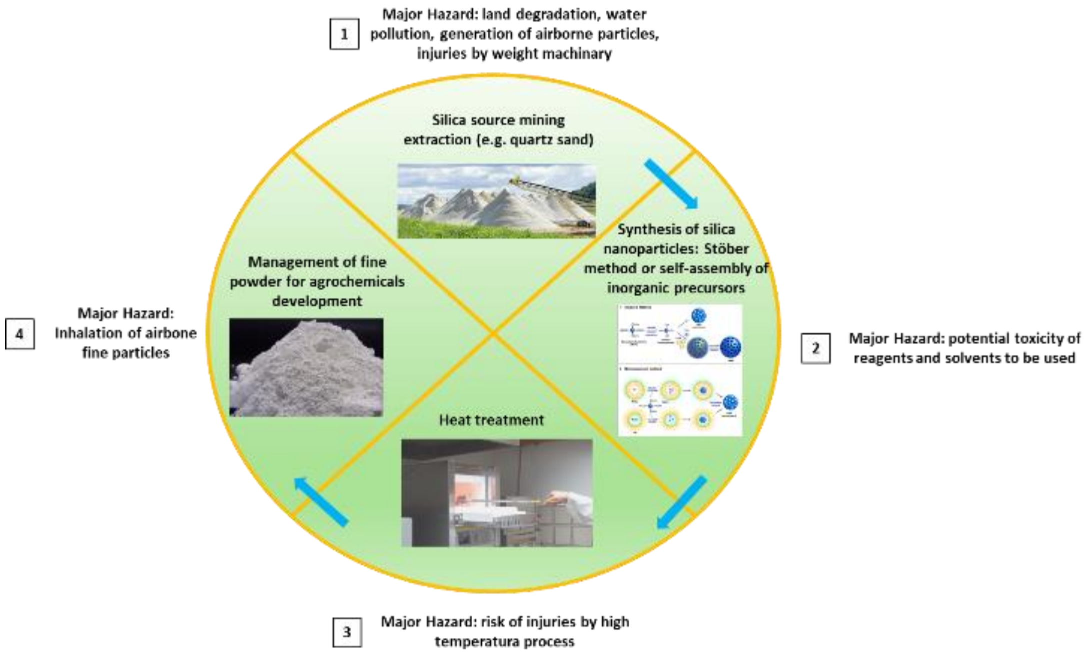

Figure 8. Diagram of MSNs production for the new agrochemical development.

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In general, the techniques for extracting silica particles can be classified into two, which are top-down or physical approach and bottom-up or chemical approach as illustrated in Figure 9. The top-down approach is defined by applying special size reduction techniques to reduce the dimension of the original size. Meanwhile, the popular method for producing MSNs from the atomic or molecular scale is the bottom-up approach. Lithography and milling are some examples of silica nanoparticles synthesis using the top-down approach. Chemical vapour deposition (CVD), hydrothermal synthesis, microemulsion technique, sonochemical technique, co-precipitation, and sol-gel technique are the examples of silica nanoparticles synthesis using the bottom-up approach[5].

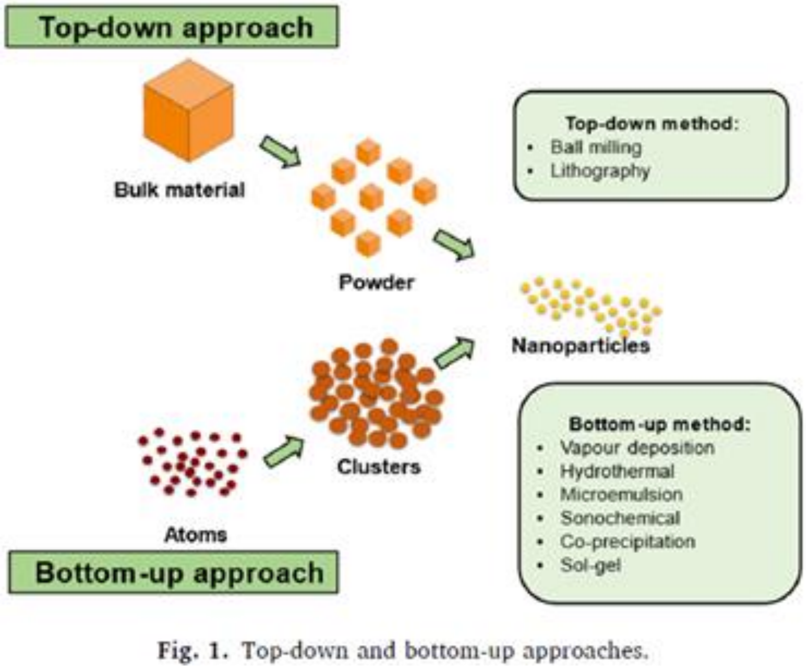

Figure 9. The top-down and bottom-up techniques for extracting silica particles

4.2.4 AGRO4AGRI R&D on NCLs and MSNs

In AGRO4AGRI, various types of NCLs (like sepiolite) and MSNs (such as MCM-41 and silica fume) will first be evaluated by analyzing their physicochemical characteristics to select the best options considering factors such as market availability, cost, and specific material properties that align with agricultural needs. In order to enhance the functionality of NCLs and MSNs, chemical surface modification strategies are implemented. This functionalization is aimed at improving the adsorption capacity of the materials, which is crucial for their efficiency as carriers in fertilizer delivery systems. The integration of fertilizers provided by a MIRAT will be integrated into the nanostructured materials using infusion methods supported by controlled temperature and pressure conditions. This step is critical to ensuring that the fertilizers are properly incorporated into the nanocarriers. AGRO4AGRI will furthermore explores the use of Hydrophobic Deep Eutectic Solvents (HDES) and Super Absorbing Polymers (SAPs) to achieve a slow and controlled release of fertilizers. These substances aid in modifying the release properties of the carriers, tailoring them to release nutrients in response to specific environmental triggers. Once the nanocarriers are developed, they undergo various characterization tests to measure the efficiency of nutrient absorption and release. These tests help determine the desorption rate of active substances and evaluate the stability and effectiveness of the release mechanisms under different conditions. Finally, the ultimate validation of NCLs and MSNs as effective delivery systems for fertilizers takes place through field trials. These trials test the practical application of the nanocarriers in real agricultural settings, assessing their performance in enhancing crop growth and nutrient utilization.

4.2.5 Levels of SSbD design

The development of NCLs and MSNs in the AGRO4AGRI project encompasses all three levels of design—molecular design, process design, and product design:

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Molecular Design:

- **Objective**: At this level, the focus is on designing the molecular structure of NCLs and MSNs to achieve specific properties beneficial for their use as carriers in agricultural applications. This includes tailoring their pore size, surface area, and chemical functionalities to optimize their ability to adsorb, retain, and release nutrients and other agrochemicals effectively.
- **Applications**: Molecular design is crucial in developing materials that can interact at the chemical level with fertilizers and other substances, ensuring that these interactions are conducive to the slow and controlled release of nutrients.

Process Design:

- **Objective**: This involves designing the methods and conditions under which NCLs and MSNs are synthesized and modified. The process design aims to make the production of these nanomaterials scalable, cost-effective, and environmentally sustainable.
- **Applications**: Process design includes the optimization of synthesis parameters (such as temperature, pH, and reactant concentrations) and the development of functionalization techniques that modify the surface properties of the nanomaterials to enhance their performance as delivery systems.

Product Design:

- **Objective**: At the product design level, the results from molecular and process design are implemented to produce NCLs and MSNs that meet the functional requirements of specific agricultural applications. This includes ensuring that these nanocarriers can deliver nutrients in a controlled manner to maximize their efficiency and minimize environmental impact.
- **Applications**: The nanomaterials are designed to be part of a larger system that may include integration with other agricultural products or techniques, such as combination with biochar or biostimulants, to create multifunctional agricultural inputs that improve crop yield and soil health.

4.2.6 REACH and/or CLP classification of NCLs and MSNs

Nanoclay is a natural or synthetic fine grained solid with particle size of 10–100 nm and their layers that are on the nanoscale scale. The fundamental components of clay minerals are alumina, silica and water, iron, magnesium, alkalis, and alkaline earth, and varying amounts of non-clay-mineral particles like quartz and calcite. NCLs possess layered silicate structure and well-defined layered geometry. Individual layers are composed of sheets of $(SiO4)^{4-}$ tetrahedra or $[AlO_3(OH)_3]_6$ octahedra. Depending on the chemical composition and nanoparticle morphology, nanoclays are organized into various groups such as montmorillonite, bentonite, sepiolite kaolinite, hectorite, and halloysite (Kalpana et al., 2022).

According to ECHA (European Chemicals Agency), the following nanoclays (more relevant to be use in the project) are evaluated from regulatory point of view:

- Montmorillonite (CAS no.: 1318-93-0). Considering the majority of notifications provided by companies to ECHA in CLP (Classification, Labelling and Packaging) notifications no hazards have been classified. ECHA Registration data for Montmorillonite listed the following hazard statement: H315 (Causes skin irritation), H318 (Causes serious eye damage), H335 (May cause respiratory irritation).
- Bentonite (CAS no.: 1302-78-9). According to the classification provided by companies to ECHA in CLP notifications this substance causes serious eye irritation, causes skin irritation and may cause respiratory irritation. Besides, this substance is approved in the EEA and/or Switzerland for use in biocidal products more favourable for the environment, human or animal health. Registration data for Bentonite listed hazard statement, highlighting: H315 (Causes skin irritation), H318 (Causes serious eye damage), H319 (Causes serious eye damage), H335 (May cause respiratory irritation).
- Sepiolite (CAS no.: 63800-37-3). According to the majority of notifications provided by companies to ECHA in CLP notifications no hazards have been classified.

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- Amorphous silica (CAS no.: 112945-52-5). The following hazard statements are listed: H315 (Causes skin irritation), H319 (Causes serious eye irritation), H335 (May cause respiratory irritation), H350 (May cause cancer, state route of exposure if conclusively proven that no other route applies).

4.3 Synthesis of Biochar (Carbonous material)

4.3.1 Goal of the synthesis of biochar

Biochar (CAS 16291-96-6) is a carbon-rich material obtained from the thermo-chemical decomposition of organic matter (biomass) at temperatures generally ranging from 300-700 ˚C and in the absence of oxygen (pyrolysis). The primary goal is to create a biochar-based delivery system that can improve the efficiency of fertilizer use while contributing to soil health and reducing environmental impact. Biochar is recognized for its large surface area, high porosity, and various functional groups. These characteristics make it highly suitable for soil amendment applications by improving soil fertility, aeration, and water retention, and by reducing the availability of potentially toxic elements.

By leveraging the natural properties of biochar, AGRO4AGRI aims to provide farmers with a sustainable option that supports both crop production and ecological balance.

4.3.2 Synthesis at the lab scale Lab scale development of biochar

The synthesis of biochar within AGRO4AGRI involves several steps (see Figure 10):

Step 1. Feedstock Selection: The first step involves selecting appropriate biomaterials for the pyrolysis process. This selection is based on the type of organic matter available, which can include agricultural waste, forestry residues, and other organic materials. Mainly, the materials used for the synthesis of biochar in AGRO4AGRI will be agro-forestry waste and food waste, specifically spent coffee waste.
Step 2. Pyrolysis Process Optimization: The project focuses on optimizing the conditions of the pyrolysis process to tailor the physicochemical properties of the biochar to specific agricultural needs. Factors such as temperature, duration, and the nature of the feedstock are adjusted to achieve the desired quality and characteristics of the biochar. In order to optimise the production of biochar, it is necessary to study the process variables. These variables are: raw material, pyrolysis temperature, heating rate, reactor type, residence time and carrier gas.

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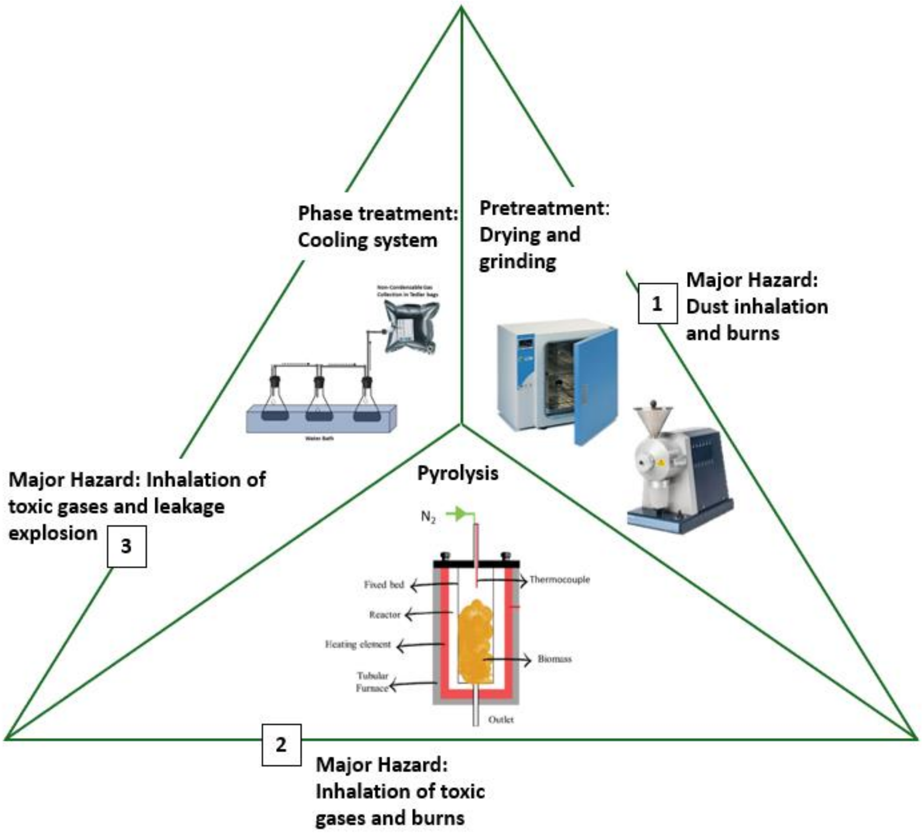

Figure 10: Synthesis of biochar within AGRO4AGRI

Regarding step 2 the following steps in the pyrolysis process will be followed based on:

1. Pretreatment of raw material (Initially it is planned to use agro-forestry biomass and spent coffee waste). At this stage, the raw material will be dried and crushed to obtain the optimum particle size for biochar production. In the case of lignocellulosic biomass, the particle should be between 2 and 10 mm. One of the hazards associated with this stage is the possibility of inhalation of dust from the crushing of the raw material. Another hazard is the heat source for drying, which can lead to burns.
2. Pyrolysis: Pyrolysis is the thermal decomposition of organic matter. Depending on the temperature and residence time, there are: slow, intermediate and fast pyrolysis. It will work in slow pyrolysis with temperatures between 300-600 °C and residence times between 30 min and 1h, although it could be increased to days. The reactor will be a fixed-bed reactor and the carrier gas, N_2.
3. Phase treatment: Three products are obtained in pyrolysis which are biochar, bio-oil and bio-gas. The biochar remains in the reactor whereas bio-oil and biogas through a cooling system. The bio-oil is collected in the cooling traps and, the bio-gas after passing through the cooling system, exits to the outside through a pipe.

The whole process will be connected and sealed to prevent the input and output of gases.

4.3.3 AGRO4AGRI R&D on biochar

The properties of biochar can vary significantly based on the pyrolysis conditions, influencing its effectiveness in particular agricultural applications. AGRO4AGRI will explores different settings to identify the optimal conditions that maximize the beneficial properties of biochar. Biochar's role extends beyond soil amendment; it is also utilized as a carrier for slow and controlled release of fertilizers. This involves further processing to enhance its capacity to adsorb and gradually release nutrients in response to environmental conditions. After production, the biochar will be subjected to various tests to characterize its absorption capabilities, stability, and effectiveness in releasing nutrients. This phase is crucial to ensure that the biochar meets the stringent requirements for

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agricultural use. The final validation occurs through field trials, where the biochar's performance is assessed in real-life agricultural settings. These trials help determine its impact on crop yield, soil health, and overall environmental sustainability.

4.3.4 Levels of SSbD design

The development of biochar in the AGRO4AGRO project touches on all three levels of design—molecular design, process design, and product design—as described previously.

- **Molecular Design**: At this level, the chemical and physical properties of biochar are engineered to enhance its functionality as a soil amendment. This involves optimizing the porosity, surface area, and functional groups (like carboxyl or hydroxyl groups) of biochar to increase its efficacy in nutrient retention and water holding capacity.
- **Process Design**: The production of biochar involves the design of the pyrolysis process used to convert biomass into biochar. This includes deciding the temperature, heating rate, and duration of the pyrolysis to achieve the desired properties of the biochar. The process design aims to make the production of biochar more efficient, scalable, and environmentally friendly, often using residual biomass as a feedstock to enhance sustainability.
- **Product Design**: At the product design level, biochar is tailored for specific agricultural uses, such as enhancing soil fertility, improving water retention, and reducing the leaching of nutrients. The biochar product is designed to meet specific requirements of different agricultural systems, ensuring that it performs effectively in real-world applications where it can deliver measurable benefits to crop growth, soil health, and environmental sustainability.

4.3.5 REACH and/or CLP classification of biochar

According to the notifications provided by companies to ECHA in REACH registrations on biochar, no hazards have been classified. Additionally, the CLP identifies that this substance is a flammable solid (H228).

The hazards associated with the synthesis of biochar include:

1. The heat source of the kiln
2. The release of toxic and flammable gases when extracting biochar from the reactor and
3. Possible leakage of toxic and flammable gases which could lead to an explosion.

4.4 Synthesis of Hydrophobic Deep Eutectic Solvents (HDESs) and Super Absorbing Polymers (SAPs)

4.4.1 Goal of the synthesis of HDESs and SAPs

The synthesis and production of Hydrophobic Deep Eutectic Solvents (HDESs) and Super Absorbing Polymers (SAPs) within the AGRO4AGRI project are aimed at enhancing the controlled release mechanisms for fertilizers. The main goal is to enhance the efficiency and sustainability of fertilizer use in agriculture by slowing down mass transport from the fertilizers within the nanocarriers to the target (crop roots). By doing so, the project aims to reduce nutrient loss, decrease the frequency of fertilizer application, and minimize environmental impact.

4.4.2 Synthesis of Hydrophobic Deep Eutectic Solvents (HDESs) and Super Absorbing Polymers (SAPs)

The synthesis of HDESs involves combining eutectic mixtures that are designed to be hydrophobic but can be tuned to achieve partial hydrophilicity. This tuning allows the HDESs to release their contents slowly under specific conditions, such as changes in soil moisture or pH levels. This study of the synthesis or process associated to delivery systems based on Deep Eutectic Solvents will focus on optimizing water instability by modulation of hydrophobicity of Hydrogen bond compounds and their molar ratios. The approach proposed in this

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project is obtaining partial hydrophobic Deep Eutectic Solvents whose compounds have a reduced environmental impact and they are extracted from natural resources. Thus, through a dry mix of hydrophilic HBA and hydrophobic HBD powders it is possible to obtain binary mixtures (with different proportions of HBD different hydrophobic character depending on their chain length). This project will carry out different mixtures in order to control that hydrophobicity. Hydrophobicity is expressed as:

$\text{Log } K_{O/W(HBD)} + \text{Log } K_{O/W(HBA)} = \text{Log } P$

The higher value of Log P is measured for HDES mixture the higher degree of hydrophobicity is obtained. Then, the proportions of different compounds in HDES will modulate the stability of HDES in water. Those proportions were fixed according to previous studies found in literature[20,21,22,23]. The proportions selected for binary mixtures of HBA and HBD compounds are preliminary and include:

1. Menthol/ HBD (Alkyl caboxylic acid) with molar ratios of 2:1, 1:1 and 1:2
2. Thymol/ HBD (Alkyl carboxylic acid) with molar ratios of 2:1,1:1 and 1:2
3. Betaine/HBD (Hexanoic acid) with molar ratios of 2:1,1:1 and 1:2

The preparation of HDES involves a process of five steps (see Figure 11):

1. HBA and HBD powder mixture
2. Melting of solids until obtaining a homogeneous transparent solution
3. Impregnation of solid (NCLSs, BC, etc.)
4. Elimination of excess of HDES
5. Milling and post processing the mixture of nanoparticles and HDES

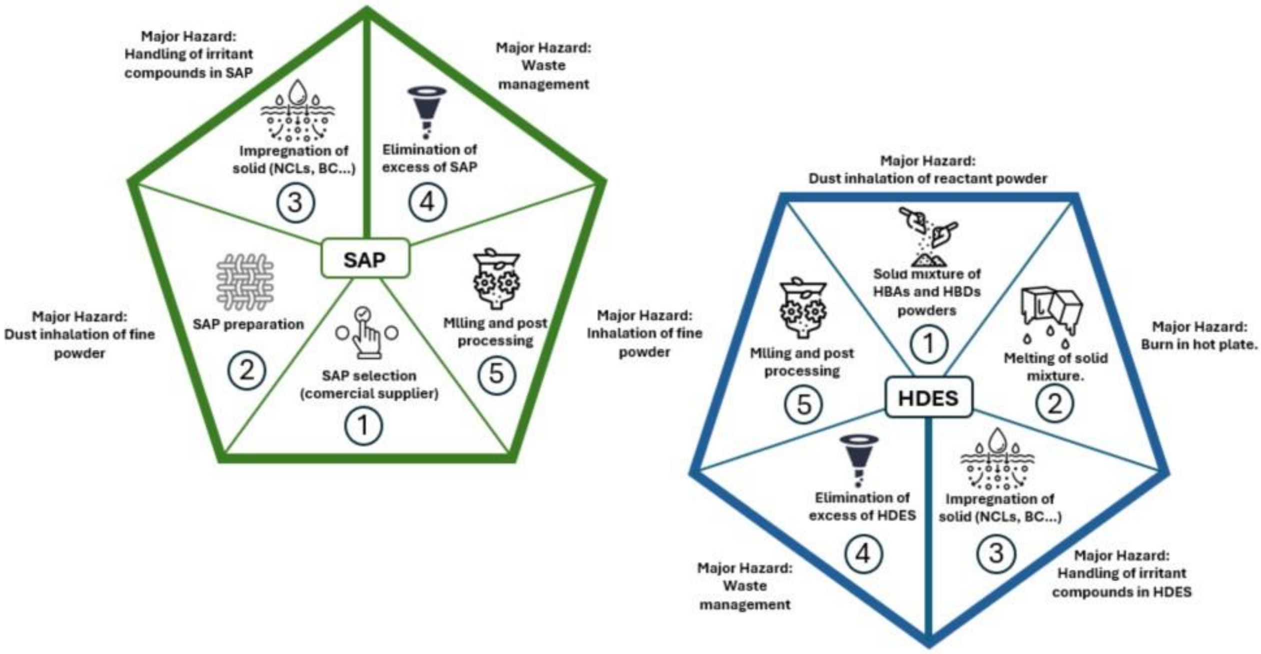

Figure 11: An overview of the reparation of SAP and HDES in AGRO4AGRI

SAPs are synthesized from biodegradable and environmentally friendly materials, primarily derived from natural sources. The production process focuses on achieving polymers with high water retention capabilities, which are critical for their function in agricultural applications.

4.4.3 AGRO4AGRI R&D on HDESs and SAPs

HDESs and SAPs were chosen for their ability to modify the release properties of fertilizer carriers. HDESs can adjust their hydrophobicity to control miscibility with water, while SAPs are known for their high-water absorption

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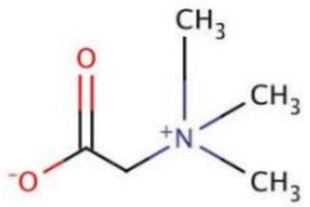
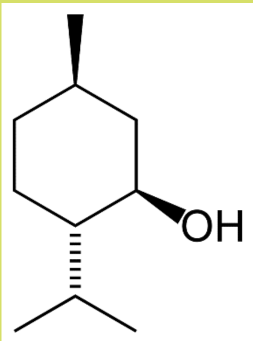
capabilities, making them ideal for maintaining moisture and releasing agrochemicals in response to environmental triggers. All chemicals employed for design of delivery systems based on hydrophobic deep eutectic solvents are well known and provide enough information like numerical indexation or CAS, composition, physicochemical information and safe and security information to be defined as SSbD system elements. In addition, both materials will be obtained from natural sources, and so they will have biodegradable characteristics and will be sustainable and safety, being these performance of great interest in agriculture of future. HDES are composed by two or more components divided by hydrogen bond donor materials (HBD) and hydrogen acceptor materials (HBA). AGRO4AGRI project has carried out a previous selection of several materials to obtain deep eutectic solvents with different hydrophobicity properties. The materials selected can be found in Table 5.

COLUMN HEAD	COLUMN HEAD	COLUMN HEAD
HBAs		Betaine (CAS 107-43-7): Quaternary ammonium salt obtained from sugar beet and other seeds. This is considered a non-essential amino acid with no persistence in organisms being metabolized by sarcosine to dimethylglycine in kidney and liver cells6. Is a well-known product employed as nutritional supplement or surfactant in shampoos and skin cleansers. According to its safety data sheet neither irritant effect nor toxicity (aquatic) are reported. This product is not listed in any carcinogenic category (according to IARC, NTP and OSHA-Ca).
		Menthol (CAS 2216-51-5): A saturated secondary alcohol found in mainly in oils extracted from mint foils. Substance used commonly for biocides applications, cleaning product and fragrances in cosmetics and personal care formulations. Menthol can be synthetically obtained from) was developed by the German company Haarmann & Reimer Friedel-Crafts-alkylation of fossil-based m-cresol with propene to p-thymol over alumina16 or acidic zeolites. This process is followed by hydrogenation process with cobalt or nickel supported catalyst. The product is a racemic mixture that can be further purified by isomerization downstream processes8. ECHA Registration data for Menthol listed hazards like low toxicity (LD50 3180mg/Kg) and no carcinogenic categories for this substance (according to IARC, NTP and OSHA-Ca). IT is reported a LC50 22.3mg/L after 96h and no ecotoxicity for long term due to biodegradability of menthol. No toxicity was found to plants, arthropods other microorganisms or microorganism in soil (according to ECHA CHEM database). There is no data for carcinogenic affect. However, skin

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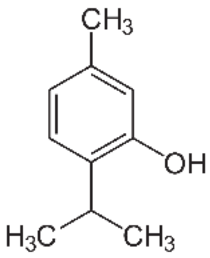
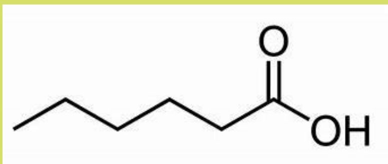
		and eyes irritation (H315 and H319) are reported9.
		Thymol (CAS 89-83-8): Aromatic compound extracted from natural sources like essential oils of thyme and oregano. Its industrial production could involve a batch reaction between propylene and m-cresol and a further distillation. It is widely used as component in antiseptic products due to its antibacterial and its antifungal effect and in some occasions is employed ad a stabilizer in pharmaceutical preparations or flavoring agent in food industry. Its synthesis and distillation. Restrictions about its use are reported by CLP regulation (1272/2008). It is reported Acute Toxicity. 4 (H302), Skin corrosion 1B (H314) and ecotoxicity for aquatic chronic 2 (H411) [3]. According to its safety data sheet low oral toxicity were reported (LD50=980mg/Kg) and is classified as irritant for skin. Ecotoxicity information reported in freshwater fish a value of LC50:38.8mg/L for short term experiments (96h). Meanwhile a 3h-EC50 of 39.6 mg/L to activated sludge was also reported as the toxicity of thymol to microorganisms in soil. However, EU Method C.4 -E (equivalent to OECD 301 D), test substance concentration 0.8 mg/L, demonstrated a 83% of biodegradation in 28 days; readily biodegradable (Currenta, 2010)
HBD's (Alkyl carboxylic acids)		Hexanoic acid (CAS 142-62-1). Short alkyl carboxylic acid obtained from chemical production or refinery. Hexnaoic acid is reported as preventive agent for infection control in tomato plants (Botrytis Cinerea fungus)10. For oral acute toxicity no signs of systemic toxicity were observed during the 14 days observation period (doses of LD50 >2000mg/Kg). Ecotoxical information LC50 88mg/L 96h and for long term in fresh water fishes LC50 6.4mg/L. No toxicity was found to plants, arthropods other microorganisms or microorganism in soil (according to ECHA CHEM database). There is no data for carcinogenic effects.

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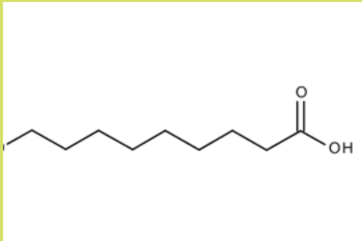
		Octanoic acid (CAS 124-07-2). Mid alkyl carboxylic acid obtained from chemical production or refinery. Oral acute toxicology LD50 >2000mg/Kg- Ecotoxicological (aquatic) test for fish mortality LC100>300mg/L; LC50>170mg/L. Moderate corrosive to skin (Scored 3 over 4) No toxicity found to plants, arthropods other macroorganisms or microorganism in soil (according to ECHA CHEM database)11. There is no data for carcinogenic affect.
		Decanoic acid or Capric acid (CAS 334-48-5). Long alkyl carboxylic acid obtained from chemical production or extracted from natural coconut oil. Oral acute toxicology LD50 >2000mg/Kg. Ecotoxical Aquatic LC50 20-31mg/L (48h). No toxicity was found to plants, arthropods other microorganisms or microorganism in soil (according to ECHA CHEM database). Noncorrosive effects observed in invitro transcutaneous electrical resistance test in human tissue samples.

Table 5. Candidates for materials to obtain deep eutectic solvents with different hydrophobicity properties.

SAPs are highly swollen, hydrophilic polymer networks capable of absorbing large amounts of water or saline solution, practically 10-1000 times of their original weight or volume[13]. Combining SAPs with fertilizer nanocarriers to prepare slow-release fertilizers system with water-absorbing and water-retaining functions, allows improving fertilizer utilization and saving water resources. Upon contact with soil solution, super absorbent polymers absorb water and swell into a gel-like state. The soluble fertilizer within the hydrogel experiences hindered diffusion due to the swollen gel, resulting in a slowed and controlled release rate of fertilizer nutrients. This obstruction effect improves fertilizer utilization efficiency. Under specific conditions, the adsorbed nutrients slowly diffuse from the hydrogel through the network system into the soil, facilitating crop absorption and utilization, and ultimately promoting plant growth. Synthetic polymers such as polycaprolactone, polyethylene, polyvinyl alcohol, and acrylate-based polymers are used to achieve slow release of fertilizers to improve soil conditions (as superabsorbent polymers, SAPs)[14]. The current polymers used commercially for this purpose are mainly acrylate-based SAPs. However, the persistence of synthetic polymers poses huge problems to the environment. These synthetic polymers may not be biodegradable, and environmental concerns relating to these materials' degradation could arise. Some examples could be the rising concerns over the bioaccumulation and environmental contamination of polyfluoroalkyl substances and micro/nanoplastics. For instance, polyethylene and polypropylene used in agriculture can persist in the soil for prolonged periods, causing negative impacts to the environment and living systems. The outlook toward synthetic polymers is therefore shifting toward natural polymers, such as polysaccharides. For this reason, in this project, the use of bio-based polymers is proposed. Such bio-based polymers can be either naturally derived from plants, animals, food waste or fabricated via biological processes such as by living microorganisms. The significant advantage of natural polymers is that they can be degraded by soil microorganisms resulting in environmentally non-toxic products. Different applications for each of these polymers in the food industry are tabulated in the following Table 6. In this project a first selection has been made for CRF (controlled release fertilizer) application.

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	Food				Agriculture		
	Stabilizer (Rayner et al., 2016)	Thickener (Lovegrove et al., 2017)	Gelling (Lovegrove et al., 2017)	Food Packaging (Grujić et al., 2017)	CRF (Akalin and Pulat, 2019; Jiao et al., 2018; Perez and Francois, 2016; Tang et al., 2017)	Pesticides (Neri-Badang and Chakraborty, 2019; Campos et al., 2015)	Water Retention (Thombare et al., 2018; Demitri et al., 2013)
Alginate	✓	✓	✓	✓		✓	✓
Carrageenan	✓	✓	✓	✓	✓		✓
Cellulose				✓	✓	✓	✓
Chitosan				✓	✓	✓	
Curdlan							
Cyclodextrin						✓	
Gelatin	✓		✓		✓		
Guar gum							✓
Pectin						✓	
Starch		✓		✓	✓		
Xanthan gum	✓	✓					

Table 6. Different applications for each of these polymers in the food industry

Both HDESs and SAPs are integrated into nanocarrier systems, such as those based on nanoclays and mesoporous silica, to enhance the controlled release of fertilizers. This integration involves encapsulating the nanocarriers with these materials to ensure that the fertilizers are protected from premature release. The project develops mechanisms where HDESs control the release of fertilizers by adjusting their hydrophobic properties, while SAPs provide a physical barrier that retains water and nutrients, releasing them gradually to the crops. The effectiveness of HDESs and SAPs in controlling fertilizer release is thoroughly tested through various characterization tests. These tests measure the adsorption and desorption rates, the stability of the release under different conditions, and the overall efficiency of the nutrient delivery to plants. Ultimately, the performance of these materials is validated in real-life agricultural settings, assessing their impact on crop growth, soil health, and environmental sustainability. These trials are crucial for demonstrating the practical benefits and feasibility of using HDESs and SAPs in commercial agriculture.

4.4.4 Levels of SSbD design

The development of Hydrophobic Deep Eutectic Solvents (HDESs) and Super Absorbing Polymers (SAPs) in the AGRO4AGRO project incorporates all three levels of design—molecular design, process design, and product design:

Molecular Design:

- **Objective**: This level focuses on designing the chemical structure and molecular interactions of HDESs and SAPs. For HDESs, this involves selecting and combining different components to form eutectic mixtures with desired hydrophobic properties that can be tuned for specific agricultural needs. SAPs are designed at the molecular level to achieve high water absorption and retention capabilities, crucial for their use in agricultural applications.

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- **Applications**: Molecular design is critical in tailoring the properties of these materials, such as solubility, stability, and responsiveness to environmental stimuli (like pH changes), which directly affect their functionality as delivery systems for agrochemicals.

Process Design:

- **Objective**: This level involves developing and optimizing the manufacturing processes for both HDESs and SAPs to ensure they are economically viable, scalable, and sustainable. Process design includes the methods of synthesis, conditions like temperature and pressure, and the protocols for combining ingredients to form the final products.
- **Applications**: Effective process design is essential to produce these materials at a commercial scale while maintaining their quality and functional properties. It also seeks to minimize energy consumption and reduce waste, aligning with environmental sustainability goals.

Product Design:

- **Objective**: At the product design level, HDESs and SAPs are formulated into products that meet specific agricultural requirements. This involves integrating them into delivery systems that can effectively release agrochemicals in a controlled manner. The design ensures that these materials function effectively under field conditions, optimizing the delivery and efficacy of active ingredients.
- **Applications**: The final products are designed to be part of comprehensive agricultural solutions, such as controlled-release fertilizers or targeted pesticide delivery systems, that improve crop productivity and environmental safety.

4.4.5 REACH and/or CLP classification of HDESs and SAPs

There are several different chemicals and materials involved in the development of SAPs:

- Cellulose (CAS 9004-34-6) based biomaterials could be used as raw materials for developing SAPs with high biodegradability, having high strength after absorbing water. Most biomasses such as corn stove, wheat straw, rice straw and bagasse which are referred to as lignocellulosic materials are rich in cellulose fibres. Respiratory hazards (H335) and acute toxicity if its contacted to skin or swallowed (H312 and H302 respectively.
- Starch (CAS 9005-25-8) is a hydrocarbon polymer which is composed of two different types of α-glucans, amylose and amylopectin, representing approximately 98–99 % of the dry mass forming semi-crystalline and amorphous layers, the crystallinity is usually between 15 and 45%[16]. Starch is a naturally available and abundant polysaccharide which is second in abundance only to cellulose in terms of the quantity available on Earth[17]. It is available in the foliage of most green crops and in the fruits, seeds, rootlets and tubers of the majority of plants, such as wheat, potatoes, corn, rice, lentil, barley, rye, beans, quinoa, peas, sorghum, cassava, sweet potatoes, avocados, taro, bananas peel, mangoes, pineapples, amaranthus.L, sago (palm starch), etc[18]. Starch is available in a variety of macroscopic morphologies including round, oval, angular, and lenticular, and the size of the granules commonly ranges among 1 to 100 μm[19]. Eye Irritation 2(H319, H320). Acute toxicity by inhalation (H332, H335). Toxicity to aquatic life is reported H411.
- Chitosan (CAS 9012-76-4) is one of the nature-derived polymers that has many implications in agriculture. With its inherent growth enhancement and antimicrobial properties, chitosan could be exploited in agriculture, either stand alone or as an encapsulation matrix for nutrients. Skin and eye irritation (H315 and H319 respectively). Affects to organs (lungs if it is inhaled (dry powder).
- No hazards have been reported to g*elatins* (CAS 9000-70-8).
- Carrageenan (CAS 9000-07-1) is classified as: Eye Irritation 2 (H319) and suspect to be carcinogenic Category 2 (H351). For this reason, this material will be not selected.

4.5 Nanocellulose derivatives (NCs): Nanofibres of cellulose (NFC), Nanocellulose hydrogel (NCHs) and Nanocellulose foams (NFs)

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4.5.1 Goal of the synthesis of NCs

The overarching goal is to leverage the advanced properties of nanocellulose materials to create more efficient and sustainable fertilizer delivery systems. By doing so, the project aims to enhance plant growth, optimize fertilizer usage, and reduce environmental impacts associated with conventional fertilizer applications. The synthesis and production of Nanofibres of Cellulose (NFC), Nanocellulose Hydrogel (NCHs), and Nanocellulose Foams (NFs) within the AGRO4AGRI project are designed to enhance the delivery systems for fertilizers through advanced biopolymer technologies. These cellulose derivatives are selected for their unique properties such as high surface area, hydrophilicity, and porosity.

4.5.2 Synthesis at the lab scale development of NCLs

NFCs are produced by extracting cellulose nanofibres from biomass, which typically includes agricultural waste or forestry residues (see Figure 12). The fibers exhibit a high aspect ratio and possess numerous hydroxyl groups, making them highly hydrophilic and suitable for chemical modifications. NCHs are formed by creating a hydrogel from NFCs. This hydrogel is a three-dimensional network that can absorb and retain large amounts of water, making it ideal for use in dry or arid environments where water retention is crucial. Finally, NFs involve the creation of foams from NFCs, which are structured to have well-defined nano or micro pore sizes, enhancing their utility in encapsulating and slowly releasing active substances like fertilizers. An important aspect of the synthesis of NCs is the extraction of cellulose. Cellulose can be extracted from a broad range of plants. The source is very important because it affects the size and properties of the extracted cellulose. The extraction of nanocellulose from wood or non-wood materials requires a multistage process involving vigorous chemical and/or mechanical operations. To extract the cellulose from the raw materials is very important to select a correct pretreatment method which will be dependent on the cellulose source and to a lesser degree, on the desired morphology of the initial cellulose for further pretreatments. Choosing an appropriate pretreatment of cellulose fibers promotes accessibility, increases the inner surface, alters crystallinity, breaks hydrogen bonds and boosts the reactivity of the cellulose; thus, it decreases the energy demand and facilitated the process of nanocellulose production.

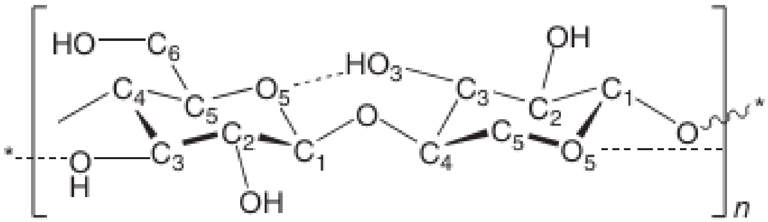

Figure 12. Single cellulose chain repeat unit, showing the directionality of the 1–4 linkage and internal hydrogen bonding.

The synthesis of NCs consists of several individual steps.

Step 1: Pulping process of lignocellulosic biomass (see Figure 14)

Step 2: Production of nanofibrillated cellulose from (ligno)cellulose pulps

Step 3: Production of cellulose nanofoams (NF)

Step 4: Chemical modification.

Each of these steps include several substeps.

Step 1. Pulping process of lignocellulosic biomass

Different possible pulping processes of lignocellulosic biomass are possible and will be explored, namely 1) semi-chemical/thermochemo-mechanical pulping process, 2) alkaline and 3) alkaline peroxide pretreatment processes. To this end, it is important to choose the correct pulping process in order to extract cellulose from the agricultural residue. Pulping is used to isolate cellulose fibers. The correct combination of pulping processes will be studied in the project. In the following a list of possible chemical reagents are mentioned.

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As it is expected, in AGRI4AGRO, non-wood byproducts (agrifood residues) are going to be used to extract cellulose. For this kind of lignocellulosic materials, it is usual to carry out alkaline pulping processes with chemical reagents such as sodium hydroxide that can be applied together with a catalyst. This catalyst can be anthraquinone which preserves the carbohydrates from degradation. However, the use of this catalyst is optional.

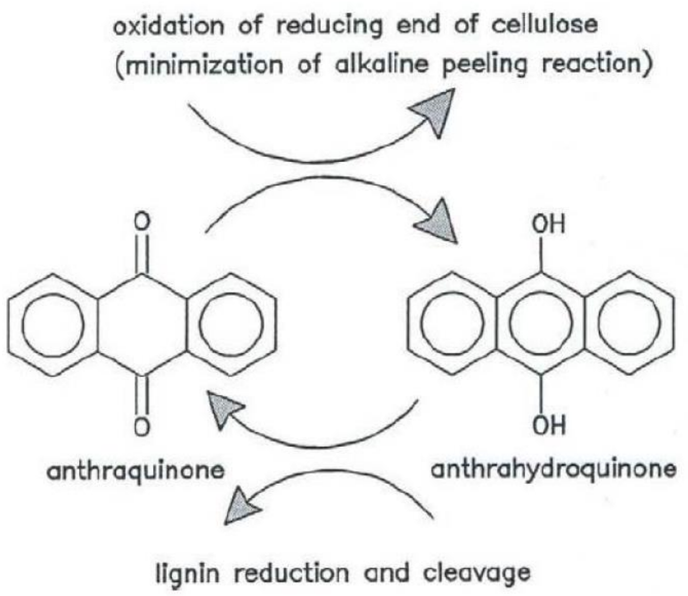

Figure 13. Cyclic action of anthraquinone

During soda pulping, lignocellulosic materials are typically treated with NaOH at temperatures below 160 C during which the lignin is solubilized by the deprotonation of the phenolic lignin subunits. These subunits are derived by the cleavage of lignin-carbohydrate linkages and the depolymerisation of lignin by the cleavage of α- and β-aryl ether bonds (Chakar and Ragauskas, 2004). In terms of pretreatment, NaOH has often been used as the predominant alkaline pretreatment method with NaOH's strong base, catalytic nature resulting in effective hemicellulose and lignin removal (Takada et al., 2020).

Another kind of alkaline pulping process considered in AGRI4AGRO is related to alkali pretreatment which uses hydrogen peroxide. Hydrogen peroxide has been shown to efficiently remove lignin, resulting in the effective bioconversion of agricultural residues. In the process chemical reagents such as NaOH (up to 20%), H2O2 (up to 5-10%) and Anthraquinone are used as well as water for the subsequent washing of cooked materials. Temperatures are up to 180°C with NaOH and below 95°C with H2O2 (see Figure 13).

Finally, alkaline-acid pretreatment processes will be explored as pretreatment to extract cellulose. This process consists of soaking fibers in sodium hydroxide, followed by an acid step with hydrochloric acid solution (HCl) and another sodium hydroxide step. This kind of alkaline-acid treatment also removes lignin and hemicelluloses partially. This process involves chemical reagents such as NaOH (up to 20%) and HCl (up to 2M) in combination with water for the subsequent washing of cooked materials. Temperatures are up to 100-150°C.

Semi-chemical pulping process and thermochemo-mechanical pulping process will be completed by applying a moderate mechanical refining treatment and harsh refining treatment, respectively. Only water and energy is used at this step of the process. After the refining process, a washing step with water and screening is needed. This process will result in the obtaining of non-bleached cellulose pulp. Depending on the cellulose purity needed, an additional step could be performed. This step includes delignification and bleaching of cellulose fibers. In this process, the pulp can be bleached to remove the residual lignin and other impurities. Various bleaching agents can be used, such as hydrogen peroxide, pressurized oxygen, peracetic acid or sodium chlorite. One or more sequential steps can be needed to this end. The exact selection of chemical reagents will be studied in the project.

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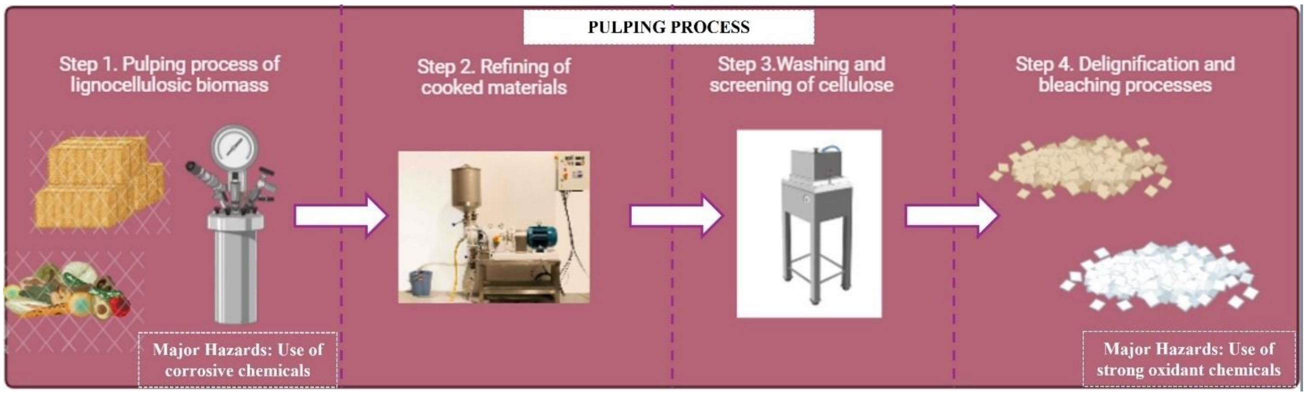

Figure 14. Pulping process for cellulose production.

Step 2. Production of nanofibrillated cellulose from (ligno)cellulose pulps

Once cellulose pulps are ready, it is possible to produce nanocellulose fibers (NFCs) as well as nanocellulose hydrogels (NCHs) from them, simultaneously. Both NFCs and NCHs are produced at the same time from cellulose pulps. There are several techniques to produce them. They can be produced directly through high-shear mechanical processes or even subjecting cellulose to a chemical or mechanical pretreatment before the main mechanical process. During the project the need of applying such kind of pretreatments (which are optional) will be studied. Then, if cellulose pulps are subjected to strong mechanical disintegration, the original structure of cellulose fibers is turn to nanofibrils (NFC) or their microfibril bundles (MFC) with diameters in the range of 10-100 nm, depending on the disintegration power. The three following main mechanical technologies are susceptible to be used in AGRO4AGRI: Sonication (S), High-pressure homogenization (HPH) and Ultra-fine grinding (UFG). As a result of the fibrillation process, nanofibrillated cellulose (NFCs) and Nanocellulose Hydrogels (NCHs) are obtained in a water base. No chemicals are used in the fibrillation processes. Only energy and a water suspension of cellulose pulp is used and subjected to the mechanical forces. Depending on the recalcitrance of the cellulose pulps to the fibrillation process, a mechanical or chemical pretreatment could be applied previously to the main mechanical process (S, HPH, UFG). The mechanical pretreatment will consist of the combination of mechanical fibrillation technologies. For example, a first step of UFG followed by HPH or S followed by HPH. The most known chemical pretreatment is TEMPO mediated oxidation. TEMPO-mediated oxidation allows the introduction of carboxylate and aldehyde groups into solid native cellulose under aqueous and mild conditions. TEMPO mediated oxidation pretreatment decreases the energy consumption of repeated cycles of a high-pressure homogenizer. The basic principle of this form of pre-treatment consists in the oxidation of cellulose fibers via the addition of NaClO to aqueous cellulose suspensions in the presence of catalytic amounts of 2,2,6,6 tetramethyl-1-piperidinyloxy (TEMPO) and NaBr at pH 10-11 at room temperature. The C6 primary hydroxyl groups of cellulose are thus selectively converted to carboxylate groups via the C6 aldehyde groups, and only the NaClO and NaOH are consumed. As a result, the nanofibrils within the fibers separate from each other better due to the repulsive forces among the ionized carboxylated, which overwhelm the hydrogen groups holding them together.

Step 3: Production of cellulose nanofoams (NF).

The terms "foam" and "aerogel" are commonly used interchangeably to describe nanocellulose-based porous materials. Previous reports have defined a (nanocellulose-based) aerogel as "a highly porous solid of ultra-low density and with nanometric pore sizes formed by replacement of liquid in a gel with gas". Foams are usually more broadly defined, commonly referred to as "solid porous materials with micrometric pore sizes". Agro4agri will produce both: nanocellulose based foams and nanocellulose based aerogels (see Figure 14). A nanocellulose(-based) foam is a multi-phase porous material with a porosity larger than 50% in which gas (*e.g.* air) is dispersed in a liquid, solid or hydrogel. The diameter of the bubbles (or the pore size) is usually larger than 50 nm. A nanocellulose(-based) aerogel is a mesoporous solid material (*i.e.*pore size in the range 2–50 nm) of high porosity (>90%) (Lavoine et al. 2017). Two method production are susceptible of being applied for the production of nanofoams. One method is by freeze- drying. Using liquid nitrogen as chemical reagent for freezing followed by a sublimation phase whereas another is by adding gas (air) in the nanocellulose hydrogel and convective drying. Besides water, this requires the use of liquid nitrogen to freeze and surfactants. Which surfactants is yet to be defined.

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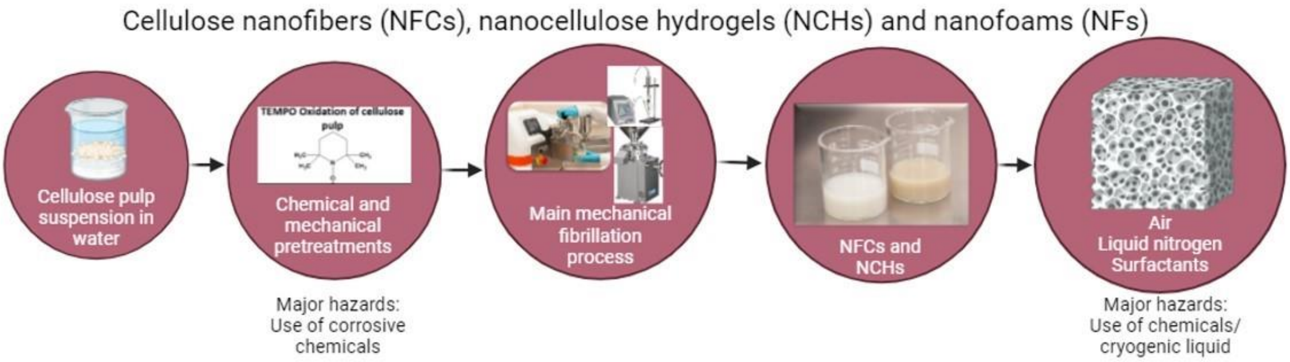

Figure 14 Cellulose nanofibers (NFCs), nanocellulose hydrogels (NCHs) and cellulose nanofoams (NFs) processes.

Step 4: Chemical modification

Given the presence of hydroxyl groups on the surface of NCs, the routes for their chemical modification are very varied (Mokhena et al., 2018), and can be categorized into 3 groups: a) Modification of the -OH groups of NCs with small molecules, b) "Grafting to" of known polymers on NCs and, c) "Grafting from" which involves the incorporation of a polymerization initiator for *in-situ* polymerization on the NC (see Figure 15). Both "Grafting from" and "Grafting to" are complex processes that do not provide apparent advantages compared to case (a) in terms of the objective of the Project. Hence, we will focus on modification processes with small molecules, which are much easier to control and reproduce.

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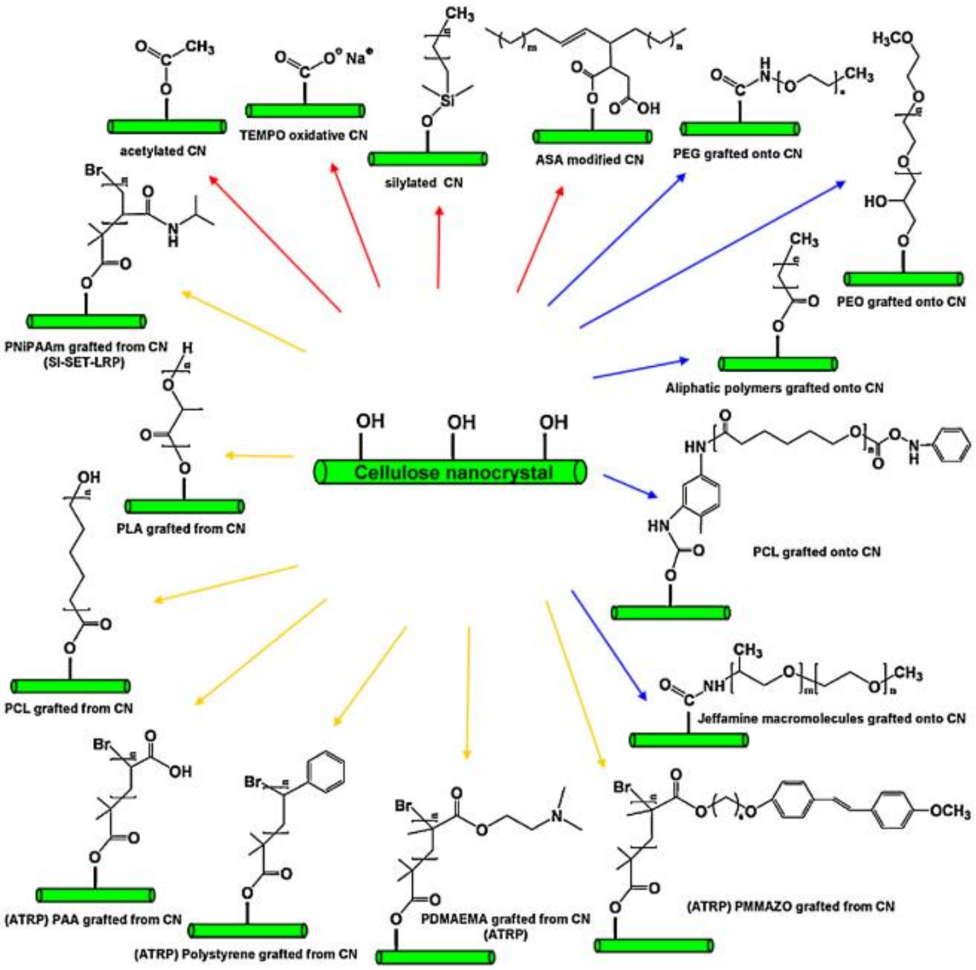

Figure 15. Examples of chemical modifications of MFCs (Sharma et al., 2019).

One potential strategy for the project will be the introduction of anionic or cationic moieties onto cellulose as this can significantly modify its surface properties, allowing its interaction with oppositely charged compounds. However, this depends on the active substance to be incorporated that still needs further analysis. Still, many of the chemical modification routes that can be followed may lead to a anionic or cationic charges, or could lead to a neutral group (e.g. amine, carboxylic acid) dependent on the reactant In the following some common routes include: esterification, silylation, sulfation, carboxymethylation, phosphorylation, oxidation, sulfonation, quaternization, amidation, cationic polymer grafting, deductive amination, amine functionalization, epoxide ring-opening reaction and mannich reaction (see Table 7).

ESTERIFICATION	Esterification of cellulose involves introducing ester groups onto the cellulose backbone, which can enhance its hydrophobicity, thermal stability, and compatibility with various organic matrices. This modification is widely used in applications such as coatings, films, and composite materials. There are several methods for esterifying cellulose, each with its own advantages and specific conditions.			
	a. Direct Esterification with Carboxylic Acids	b. Esterification with Acid Anhydrides	c. Esterification	d. Transesterification

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	This method involves reacting cellulose with carboxylic acids in the presence of a catalyst. Here are the main routes for esterification of cellulose: Reagents: Carboxylic acids (e.g., acetic acid, butyric acid) Catalysts: Acid catalysts like sulfuric acid, p-toluenesulfonic acid Solvents: Organic solvents like toluene or acetic anhydride can be used to facilitate the reaction. Conditions: Typically, high temperatures (100-150°C) are required to drive the esterification reaction. Cellulose-OH + R-COOH → Cellulose-O-CO-R + H_2O	Acid anhydrides are highly reactive and can efficiently esterify cellulose. Reagents: Acid anhydrides (e.g., acetic anhydride, succinic anhydride) Catalysts: Pyridine or other bases are often used to catalyze the reaction. Solvents: Reactions can be carried out in solvents like pyridine or in the presence of an organic solvent. Conditions: Typically carried out at room temperature to moderate heating (20-100°C). Cellulose-OH + $(R\text{-}CO)_2O$ → Cellulose-O-CO-R + R-COOH	with Acyl Chlorides Acyl chlorides react readily with hydroxyl groups on cellulose, often in the presence of a base to neutralize the resulting hydrochloric acid. Reagents: Acyl chlorides (e.g., acetyl chloride, benzoyl chloride) Catalysts: Bases like pyridine, triethylamine Solvents: Organic solvents such as dichloromethane or toluene Conditions: Reactions are usually conducted at room temperature to moderate heating (20-60°C). Cellulose-OH + R-COCl → Cellulose-O-CO-R + HCl	This method involves exchanging the ester groups from an ester with the hydroxyl groups on cellulose. Reagents: Esters (e.g., ethyl acetate, methyl benzoate) Catalysts: Acid or base catalysts such as sodium methoxide or sulfuric acid Solvents: Organic solvents like methanol, ethanol, or toluene Conditions: Typically conducted under reflux conditions to drive the reaction to completion. Cellulose-OH + R-COOR' → Cellulose-O-CO-R + R'-OH
SILYLATION	Silylation of cellulose involves the introduction of silyl groups (Si-containing groups) onto the cellulose backbone. This modification can enhance the hydrophobicity, thermal stability, and compatibility of cellulose with various organic matrices, making it suitable for a wide range of applications including composites, coatings, and functional materials.			

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	There are many different sylilation routes that can be followed.			
	a. Direct Silylation Direct silylation typically involves reacting cellulose with silylating agents in an appropriate solvent. Reagents: Common silylating agents include chlorosilanes (e.g., trimethylchlorosilane), alkoxysilanes (e.g., trimethylethoxysilane), and silyl isocyanates. Solvents: Organic solvents like toluene, dichloromethane, or pyridine are often used. Conditions: The reaction is usually carried out under anhydrous conditions to prevent hydrolysis of the silylating agent. Heating may be applied to accelerate the reaction. Cellulose-OH + R3SiCl → Cellulose-OSiR3 +HCl	b. Silylation Using Silane Coupling Agents Silane coupling agents can be used to introduce functional silyl groups that can form covalent bonds with the cellulose hydroxyl groups. Reagents: Silane coupling agents such as (3-aminopropyl)triethoxysilane (APTES), (3-glycidyloxypropyl)trimethoxysilane (GPTMS). Solvents: Aqueous or organic solvents can be used, depending on the solubility of the coupling agent. Conditions: Reactions can be carried out at room temperature or with gentle heating. The process typically involves hydrolysis of the alkoxy groups followed by condensation with the cellulose hydroxyl groups. Cellulose-OH + (EtO)3Si-(CH2)3NH2 →Cellulose-OSi-(CH2)3NH2 + 3EtOH	c. Silylation via Silyl Halides and Bases This method involves the use of silyl halides and a base to deprotonate the cellulose hydroxyl groups, facilitating the silylation reaction. Reagents: Silyl halides such as trimethylsilyl chloride (TMSCl), and a base such as pyridine or triethylamine. Solvents: Anhydrous organic solvents like toluene, dichloromethane. Conditions: Anhydrous conditions and inert atmosphere (e.g., nitrogen) are typically used. The reaction is often carried out at room temperature or with mild heating. Cellulose-OH + TMSCl + Et3N → Cellulose-	d. Use of aminosilanes The use of aminosylanes require water medium, appropriate catalyst and and moderate temperature. The versatility of the reaction relies on the different reactants that can be used, for example, 3-aminopropyl trimethoxysilane (APMS), 2-aminoethyl 3-aminopropyl trimethoxysilane (DAMS)and 3-2-(2-aminoethylamino) ethylamino propyl-trimethoxysilane (TAMS), all of them being very soluble in aqueous medium.

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	OSiMe3 + Et3N·HCl
SULFATION	Sulfation introduces sulfate ester groups onto the cellulose backbone. The process typically involves reacting cellulose with a sulfating agent such as sulfuric acid, chlorosulfonic acid, or sulfamic acid. Reagents: Sulfuric acid, chlorosulfonic acid, sulfamic acid Conditions: Often carried out at low temperatures to avoid cellulose degradation. Product: Cellulose sulfate with $-OSO_3^-$ groups
CARBOXYMETHYLATION	Carboxymethylation introduces carboxymethyl groups ($-CH_2COOH$) onto the cellulose molecule, converting it into carboxymethyl cellulose (CMC). Reagents: Sodium monochloroacetate, sodium hydroxide Conditions: Alkaline conditions, typically performed in aqueous or alcohol-water solutions. Product: Carboxymethyl cellulose with $-CH_2COOH$ groups
PHOSPHORYLATION	Phosphorylation introduces phosphate groups onto the cellulose backbone. Reagents: Phosphoric acid, phosphorus oxychloride, or other phosphate sources Conditions: Typically involves heating and may use catalysts. Product: Phosphorylated cellulose with $-PO_4^{2-}$ groups
OXIDATION	Selective oxidation of cellulose can introduce carboxyl groups directly on the C6 position of the glucose units. Reagents: TEMPO (2,2,6,6-Tetramethylpiperidine-1-oxyl radical), sodium hypochlorite, sodium bromide Conditions: Mild conditions, usually carried out in water. Product: Oxidized cellulose with -COOH groups (often called TEMPO-oxidized cellulose)
SULFONATION	Sulfonation introduces sulfonic acid groups ($-SO_3H$) onto cellulose. Reagents: Sulfonating agents like sulfur trioxide or chlorosulfonic acid Conditions: Typically involves heating and careful control of reaction conditions to avoid degradation. Product: Sulfonated cellulose with $-SO_3H$ groups
QUATERNIZATION	Quaternization involves introducing quaternary ammonium groups onto the cellulose backbone. Reagents: Quaternary ammonium compounds such as glycidyl trimethylammonium chloride (GTMAC) or 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC). Conditions: Typically carried out in alkaline conditions, often in an aqueous or alcohol-water solution. Product: Quaternary ammonium cellulose with $-NR_4^+$ groups

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AMIDATION	Amidation introduces cationic amide groups onto cellulose. Reagents: Amines such as chitosan or polyamines, often in the presence of activating agents like carbodiimides (e.g., EDC, DCC) or acid chlorides. Conditions: Generally performed in aqueous or organic solvents, sometimes with heating. Product: Amidated cellulose with cationic $-NH_2$ or $-NH_3^+$ groups
CATIONIC POLYMER GRAFTING	Grafting cationic polymers onto cellulose involves the polymerization of cationic monomers on the cellulose surface. Reagents: Cationic monomers such as diallyldimethylammonium chloride (DADMAC) or methacryloyloxyethyl trimethylammonium chloride (MTAC). Conditions: Typically involves radical polymerization using initiators like potassium persulfate or azobisisobutyronitrile (AIBN) in aqueous or organic solvents. Product: Cellulose grafted with cationic polymers, introducing various cationic groups depending on the monomer used.
REDUCTIVE AMINATION	Reductive amination introduces primary or secondary amine groups onto the cellulose backbone. Reagents: Aldehydes or ketones (e.g., formaldehyde, acetone) and amines (e.g., methylamine, ethylenediamine) in the presence of reducing agents like sodium cyanoborohydride or sodium borohydride. Conditions: Typically carried out in aqueous or organic solvents under mild conditions. Product: Aminated cellulose with primary or secondary amine ($-NH_2$, -NHR) or their protonated forms ($-NH_3^+$, $-NHR^+$)
AMINE FUNCTIONALIZATIO N	Amine functionalization introduces amine groups onto cellulose directly through reaction with amine-containing reagents. Reagents: Amine reagents like ethylenediamine, diethylenetriamine, or polyethyleneimine. Conditions: Carried out in aqueous or organic solvents, often under mild to moderate heating. Product: Aminated cellulose with $-NH_2$ or $-NH_3^+$ groups
EPOXIDE RING-OPENING REACTION	Epoxide ring-opening reactions introduce cationic groups by reacting cellulose with epoxide-containing cationic compounds. Reagents: Epoxide-containing cationic compounds such as 2,3-epoxypropyltrimethylammonium chloride. Conditions: Typically performed in alkaline conditions to facilitate the ring-opening reaction. Product: Cationic cellulose with $-NR_3^+$ groups
MANNICH REACTION	The Mannich reaction involves the condensation of formaldehyde, an amine, and cellulose, introducing cationic amine groups.

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	Reagents: Formaldehyde, secondary amines (e.g., dimethylamine), and cellulose. Conditions: Conducted in aqueous or organic solvents, often under mild heating. Product: Mannich base cellulose with cationic -NR_2 or -NR_2^+ groups

Table 7. Chemical modification routes that could be followed in AGRO4AGRI.

4.5.3 AGRO4AGRI R&D on NCs

As mentioned, many other routes for chemical modifications of cellulose can be envisioned. In the frame of the project, reactions that can be done under aqueous medium will be preferred so it can be avoid the pre-drying of the cellulose that if energy demanding, and also to avoid the use of organic solvents. To define the routes a more detailed analysis should be done, and a better understanding of the active substance to be incorporated is required, so the new chemical functionalities introduced into the cellulose chains favors the interactions with these active substances. These nanocellulose formats will be integrated into fertilizer delivery systems to utilize their properties for controlled release and effective nutrient delivery. Their ability to be chemically modified allows for tailored interactions with fertilizers to optimize release profiles based on environmental conditions.

The hydrophilic nature of NFCs facilitates the retention and controlled release of water and nutrients. NCHs leverage their gel-like structure to provide sustained release of water and fertilizers, enhancing soil moisture over extended periods. NFs utilize their porous structure to physically encapsulate fertilizers, allowing for gradual diffusion based on soil conditions. The nanocellulose materials undergo various tests to characterize their physical and chemical properties, including their capacity for water absorption, nutrient retention, and release efficiency. These materials are also tested for their biocompatibility and environmental safety, ensuring that they do not introduce any harmful effects into agricultural settings. The effectiveness of NFCs, NCHs, and NFs in agricultural applications is validated through field trials, where their impact on crop yield, nutrient efficiency, and soil health is evaluated. These trials are crucial to demonstrate the practical benefits of nanocellulose-based delivery systems in real farming conditions.

4.5.4 Levels of SSbD design

The development of cellulose nanofibers (NFCs), nanocellulosa hydrogels (NCHs) and cellulose nanofoams (NFs) in the AGRO4AGRO project spans all three levels of design: molecular design, process design, and product design.

Molecular Design:

- At this level, the molecular structure of nanocellulose is engineered to enhance its properties such as hydrophilicity, hydrophobicity, or the ability to bind with nutrients and water molecules effectively. The chemical modification of nanocellulose to optimize its interaction with fertilizers and to respond to external stimuli like water demonstrates a deep engagement with molecular design.

Process Design:

- The AGRO4AGRI project includes designing the processes for extracting nanocellulose from agro-industrial biomass residues and chemically modifying it. These processes aim to be sustainable, using residual biomass as a feedstock, thus minimizing waste and reducing the environmental footprint. The focus on sustainable process design also involves improving the efficiency and scalability of these processes to ensure they are economically viable and less resource-intensive.

Product Design:

- The project focuses on integrating the modified nanocellulose into agricultural products as a delivery system for fertilizers. This application falls under product design, where nanocellulose derivatives are used to meet specific functional demands, such as controlled release and water retention in agricultural settings. The end products are designed to be effective in real-world agricultural applications, with validations conducted through trials in relevant environments.

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4.5.5 REACH and/or CLP classification of NFC, NCHs and NFs

In general, NFC, NCHs and NFs are believed to be biodegradable, non-toxic, and sourced from renewable materials, making them environmentally friendly options for agricultural applications. The chemcials and materials are used in the synthesis of NFC, NCHs and NFs:

- NaOH (CAS no.:1310-73-2): According to the harmonised classification and labelling(CLP00) approved by the European Union, this substance causes severe skin burns and eye damage. Additionally, the classification provided by companies to ECHA in REACH registrations identifies that this substance causes serious eye damage and may be corrosive to metals.
- H_2O_2 (CAS no.:7722-84-1): According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance causes severe skin burns and eye damage, may cause fire or explosion (strong oxidiser), is harmful if swallowed and is harmful if inhaled. Additionally, the classification provided by companies to ECHA in REACH registrations identifies that this substance causes serious eye damage, is harmful to aquatic life with long lasting effects and may cause respiratory irritation.
- HCl (CAS no.:7647-01-0). According to the classification provided by companies to ECHA in REACH registrations this substance causes severe skin burns and eye damage, is toxic if inhaled, may damage fertility or the unborn child, causes serious eye damage, may cause damage to organs through prolonged or repeated exposure, may be corrosive to metals, may cause respiratory irritation and contains gas under pressure and may explode if heated.
- Oxygen (CAS no.:7782-44-7): According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance may cause or intensify fire (oxidiser).
- Sodium chlorite (CAS no.: 7758-19-2): According to the classification provided by companies to ECHA in REACH registrations this substance is fatal in contact with skin, is toxic if swallowed, causes severe skin burns and eye damage, is very toxic to aquatic life, may cause fire or explosion (strong oxidiser), may cause damage to organs through prolonged or repeated exposure and is harmful to aquatic life with long lasting effects.
- Liquid nitrogen (CAS no.: 7727-37-9): According to the classification provided by companies to ECHA in CLP notifications this substance contains gas under pressure and may explode if heated and contains refrigerated gas and may cause cryogenic burns or injury.

4.6 Fertilizer raw materials to be selected for all delivery systems

All the delivery systems developed in AGRO4AGRI on NCs, NCLs, MSN and biochar will be combined with fertiliser raw materials. Raw materials for controlled release fertilisers that will be explored include urea and ammonium nitrate as nitrogen sources (Table 8), monoammonium phosphate and phosphoric acid as phosphorous sources (Table 9) and potassium chloride and potassium sulfate as potassium sources (Table 10).

NITROGEN SOURCES	Urea	Ammonium nitrate
Chemical	Urea, CO(NH2)2, CAS 57-13-6, is the most popular solid nitrogen fertilizer worldwide. The urea nitrogen content is 46%. It must undergo two transformations in the soil before it becomes available to the plants, first hydrolysis to ammonia and then nitrification, by oxidation in the soil by	Ammonium nitrate, NH4(NO3), CAS 6484-52-2, is a white crystalline substance with a nitrogen content of 35% and a density of about 1.725 kg/m3. Ammonium nitrate is the most popular form of nitrogen fertilizer in most European countries, applied as straight fertilizer or in combinations. It is a principal ingredient

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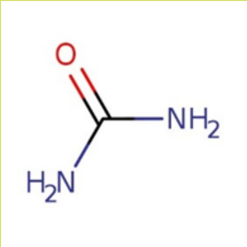
	microorganisms, in two steps to nitrite and finally to nitrate. These reactions proceed rapidly in warm, moist soil.	of most liquid fertilisers. The nitrogen in ammonium nitrate is more rapidly available to most crops that take up nitrogen mainly in the nitrate form.
Chemical structure		
REACH and/or CLP	The urea is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 10 000 000 tonnes per annum. According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified. Additionally, the classification provided by companies to ECHA in CLP not classified as hazardous within the meaning of Regulation (EC) No 1272/2008.	According to the classification provided by companies to ECHA in REACH registrations this substance may intensify fire (oxidiser) and causes serious eye irritation. Additionally, the classification provided by companies to ECHA in CLP notifications identifies that this substance may cause respiratory irritation and causes skin irritation. In accordance with Regulation (EC) No 1272/2008 making a breakdown of all 1117 C&L notifications submitted to ECHA: Hazard statements: H319 Causes serious eye irritation. Eye Irrit. H272: May intensify fire; oxidiser. 2. Ox. Sol. 3 and 2. Ox. Liq. 3. H335 May cause respiratory irritation. STOT SE 3.
Stability	Urea is stable in aqueous solution. Hydrolysis is not seen and is not predicted based on a theoretical assessment of the structure of the molecule. No data are available: a waiver is proposed for this endpoint. Urea is stable in aqueous solution. Hydrolysis is not seen and is not predicted based on a theoretical assessment of the structure of the molecule.	In aqueous solution, ammonium nitrate is completely dissociated into the ammonium ion (NH4+) and the nitrate anion (NO3 -). Hydrolysis of ammonium nitrate does not occur.
Biodegradation	Urea is considered to be readily biodegradable. The degradation of urea was investigated in psychrophilic bacteria in an aqueous test system. The maximum degradation rate per hour at 20 °C was 11.6 mg/L. Biodegradation in	Biodegradation: Ready biodegradation studies do not need to be conducted since the substance is inorganic (Annex VII REACH). In addition, in the anaerobic transformation of ammonium, one group of bacteria oxidizes ammonium to nitrite while another group oxidizes nitrite into nitrate. The average biodegradation rate

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	water screening test: The degradation of urea was investigated in psychrophilic bacteria in an aqueous test system. The maximum degradation rate per hour at 20 ºC was 11.6 mg/L. The average degradation rate per hour at 20 ºC was 10.9 mg/L. The maximum degradation rate per hour at 2 ºC was 4.0 mg/L. The average degradation rate per hour at 2 ºC was 3.2 mg/L. The biodegradation of urea was investigated in activated sludge from a laboratory sewage treatment facility fed with adapted domestic and synthetic sewage. Degradation levels of 3% (3 hours), 52% (7 hours), 60% (10 days), 85% (14 days) and 96% (16 days) was seen. Urea is ultimately biodegradable according to this study. The biodegradation of dimethyl urea was investigated in a DOC-die away study. The biodegradation (DOC removal) after 21 days is 90-100%. Dimethyl urea is therefore readily biodegradable according to OECD criteria. The degree of degradation of the reference substance was 100% after 21 day and the degradation degree in inhibition control was 101%. The validity criteria is therefore met according to OECD criteria. Biodegradation in water and sediment: simulation test: From other studies in water and soil it can be concluded that urea is readily biodegradable and therefore a study does not need to be conducted for this endpoint. Biodegradation in soil: The main mode of urea degradation is enzymatic mineralization. In soil and water, urea is expected to biodegrade fairly rapidly to ammonia and bicarbonate if the temperature is not too low. The main factors affecting the rates of nitrogen metabolism are the initial concentration of the ureolytic bacteria, the physical state of the nitrifying micro-organisms and the concentration of toxic organics.	in wastewater plant at 20 degrees Celsius is 52 g N/kg dissolved solid/day. Nitrate degradation is fastest in anaerobic conditions. In the anaerobic transformation of nitrate into N2, N2O and NH3, the biodegradation rate in wastewater plant at 20 degrees Celsius is 70 g N/kg dissolved solid/day. Biodegradation in water screening test: no data. Biodegradation in water and sediment: simulation test: no data. Biodegradation in soil: no data

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Bioaccumulation	A study is not required: due to the low log Kow value urea is not likely to undergo bioaccumulation. Additionally, urea is utilised by fish species as a nutrient and is excreted by some species as a product of protein catabolism. Bioaccumulation is not predicted. Bioaccumulation aquatic/sediment: A study is not required: due to the low log Kow value urea is not likely to undergo bioaccumulation. Additionally, urea is utilised by fish species as a nutrient and is excreted by some species as a product of protein catabolism. Bioaccumulation: terrestrial: Low potential for bioaccumulation.	Bioaccumulation: Simple inorganic salts with high aqueous solubility will exist in a dissociated form in an aqueous solution. Such a substance has a low potential for bioaccumulation. Bioaccumulation aquatic/sediment: no data Bioaccumulation: terrestrial: no data.

Table 8. Information and data on urea and ammonium nitrate as nitrogen sources.

PHOSPHORUS SOURCES	Monoammonium phosphate	Phosphoric acid
Chemical	Monoammonium phosphate, $(NH_4)H_2PO_4$, CAS 7722-76-1, is a water soluble, white crystalline salt with the chemical. Ammonium phosphates (diammonium phosphate, DAP, and monoammonium phosphate, MAP) are the leading form of phosphate fertilizer in the world because of their high nutrient content and good physical properties. The nutrient content in MAP is 11% N y 52%P (11-52-0, DAP is 18-46-0).	**Phosphoric acid,** H_3PO_4, CAS 7664-38-2, also known as orthophosphoric acid and monophosphoric acid, is primarily used to manufacture liquid fertilisers. The phosphorus content is 52% expressed as P_2O_5. It mainly occurs as an aqueous solution with an 85% concentration.

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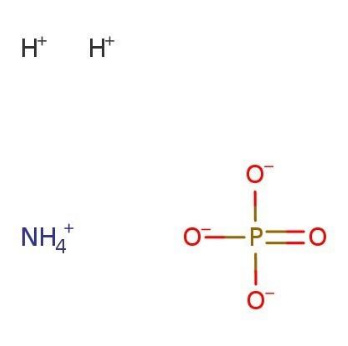
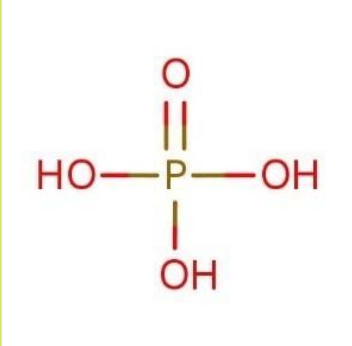
Chemical structure		
REACH and/or CLP	According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified. Additionally, the classification provided by companies to ECHA in CLP notifications identifies that this substance causes serious eye irritation and causes skin irritation. In accordance with Regulation (EC) No 1272/2008 making a breakdown of all 1150 C&L notifications submitted to ECHA: 10% of companies indicate Hazard statements: **H319** Causes serious eye irritation. Eye Irrit 2. **H315** Causes skin irritation. Skin irrit 2.	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance causes severe skin burns and eye damage. Additionally, the classification provided by companies to ECHA in REACH registrations identifies that this substance is harmful if swallowed, causes serious eye damage and may be corrosive to metals. In accordance with Regulation (EC) No 1272/2008 making a breakdown of all 4247 C&L notifications submitted to ECHA: **H314:** Causes severe skin burns and eye damage. Skin corr. 1B. **H290:** May be corrosive to metals Met. Corr.1 **H318:** Causes serious eye damage Eye DAm.1 **H302:** Harmful if swallowed. Acute tox.4. This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 1 000 000 to < 10 000 000 tonnes per annum.

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Stability	In aqueous solution, ammonium dihydrogenorthophosphate is completely dissociated into the ammonium ion (NH4+) and the phosphate anion (PO4 3-). Hydrolysis of the substance does not occur, and it is also not susceptible to photodegradation.	No data. Hydrolysis: study scientifically not necessary
Biodegradation	Readily biodegradation study does not need to be conducted since the substance is inorganic (Annex VII REACH). In addition, in the anearobic transformation of ammonium, one group of bacteria oxidizes ammonium to nitrite while another group oxidizes nitrite into nitrate. The average biodegradation rate in wastewater plant is 20 degrees Celsius is 52 g N/kg dissolved solid/day. Nitrate degradation is fastest in anaerobic conditions. In the anaerobic transformation of nitrate into N2, N2O and NH3, the biodegradation rate in wastewater plant at 20 degrees Celsius is 70 g N/kg dissolved solid/day. **Biodegradation in water screening test:** the study does not need to be conducted because the substance is inorganic **Biodegradation in water and**	**N**o data**. Biodegradation in water screening test:** The study does not need to be conducted because the substance is inorganic**. Biodegradation in water and sediment: simulation test:** study scientifically not necessary / other information available. **Biodegradation in soil:** study scientifically not necessary / other information available.

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	sediment: simulation test: study scientifically not necessary / other information available**. Biodegradation in soil:** study scientifically not necessary / other information available	
Bioaccumulation	Simple inorganic salts with high aqueous solubility will exist in a dissociated form in an aqueous solution. Such a substance has a low potential for bioaccumulation. **Bioaccumulation aquatic/seddiment:** study scientifically not necessary. **Bioaccumulation terrestrial:** no data.	**N**o data. **Bioaccumulation aquatic/seddiment:** study scientifically not necessary / other information available. **Bioaccumulation: terrestrial:** no data

Table 9. Information and data on monoammonium phosphate and phosphoric acid as phosphorous sources

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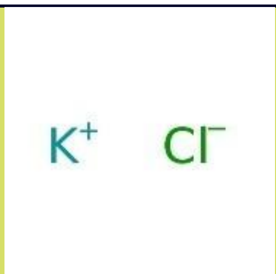
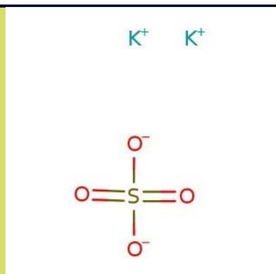
POTASSIUM SOURCES	Potassium chloride	Potassium sulfate
Chemical	KCl, CAS 7447-40-7, also known as muriate of potash (MOP), is the most applied K fertiliser. It is guaranteed to have at least 60% K_2O. Usually is marketed in a powdered form.	**Potassium sulfate**, K_2SO_4, CAS 7778-80-5, is the second largest tonnage potassium compound, and it is preferred for certain crops that do not tolerate the chloride ion well and in areas where chloride accumulation in the soil is a problem. It is guaranteed to have at least 50% K_2O. It is more expensive than potassium chloride per Kilogram of K_2O, the cost limits its use.
Chemical structure		
REACH and/or CLP	According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified. According to the majority of notifications provided by companies to ECHA in CLP notifications no hazards have been classified.	According to the classification provided by companies to ECHA in REACH registrations this substance causes serious eye irritation and causes skin irritation. Additionally, the classification provided by companies to ECHA in CLP notifications identifies that this substance causes serious eye damage. In accordance with Regulation (EC) No 1272/2008 making a Breakdown of all 542 C&L notifications submitted to ECHA: 70% not classified. 10% **H318:** causes serious eyes damage. Eye Dam.1

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Stability	no data.	In aqueous solution, potassium sulphate is completely dissociated into the potassium ion (K+) and the sulfate anion (SO4 2-). Hydrolysis of potassium sulfate does not occur. Hydrolysis: study scientifically not necessary / other information available.
ROW HEAD **Biodegradation:**	no data**. Biodegradation in water screening test:** no data**. Biodegradation in water and sediment: simulation test:** no data. **Biodegradation in soil:** no data.	**Biodegradation:** Due to the inorganic nature of the substance standard testing systems are not applicable. Sulfates can be retained in soil, both by incorporation into organic matter (e.g. as sulfate esters of humic acids) and adsorbed tosoil particles such as hydrous iron and aluminum sesquioxides**. Biodegradation in water screening test:** no data. **Biodegradation in water and sediment: simulation test:** no data. **Biodegradation in soil:** no data.
Bioaccumulation	no data. **Bioaccumulation aquatic/sediment:** no data **Bioaccumulation: terrestrial:** no data.	**Bioaccumulation:** Simple inorganic salts with high aqueous solubility will exist in a dissociated form in an aqueous solution. Such a substance has a low potential for bioaccumulation. **Bioaccumulation aquatic/sediment and terrestrial:** no data.

Table 10. Information and data on potassium chloride and potassium sulfate as potassium sources.

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4.7 Target-specific nematicides based on RNAi

4.7.1 Goal of the synthesis of target-specific nematicides based on RNAi

The goal of the synthesis of target-specific nematicides based on RNAi is to utilize RNA interference, a biological process where RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. RNA interference (RNAi) technology leverages a natural cellular mechanism where RNA molecules inhibit gene expression or translation by neutralizing specific messenger RNA (mRNA) molecules. This process is a vital regulatory pathway in many organisms, playing a key role in controlling gene expression and maintaining cellular function (see Figure 16).

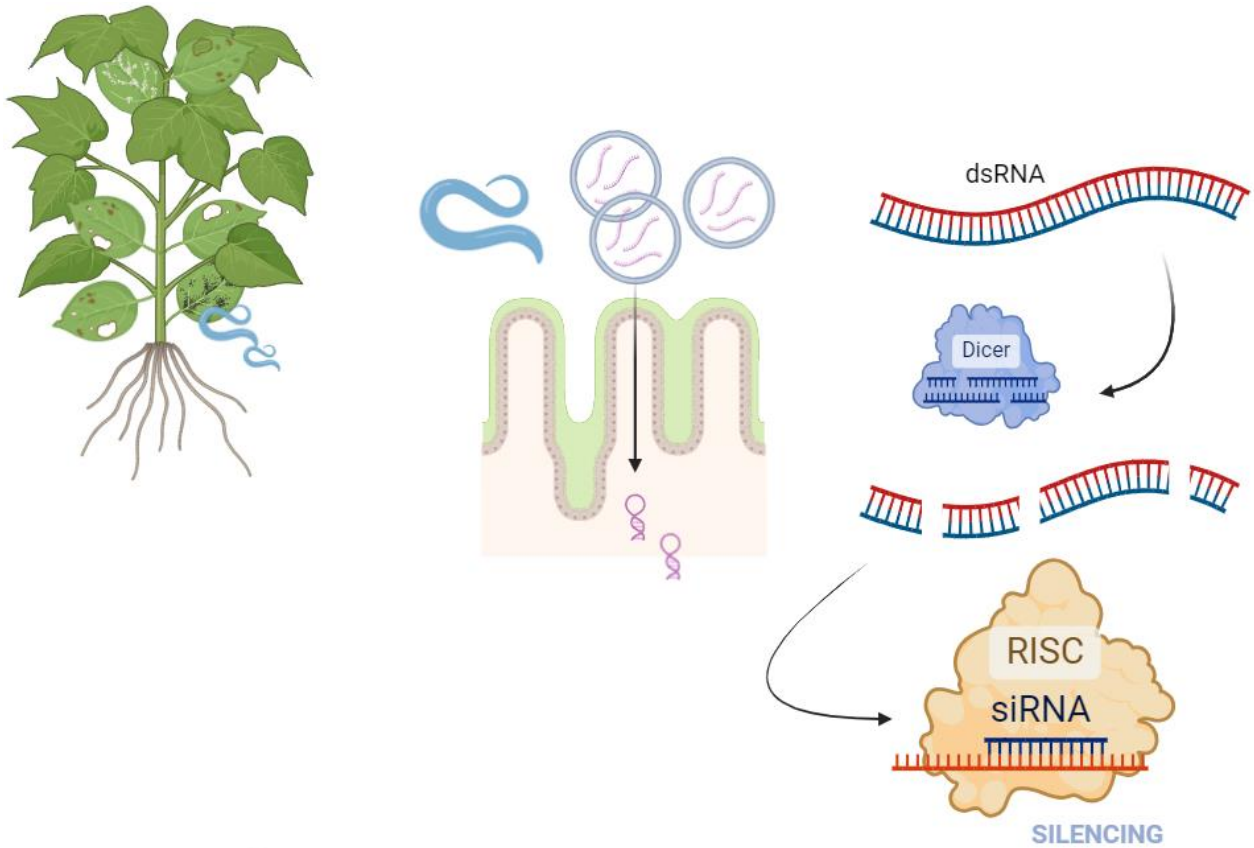

Figure 16. Synthesis of target-specific nematicides based on RNAi is to utilize RNA interference

The focus is specifically on the root-knot nematode, *Meloidogyne incognita*, a prevalent parasite that significantly impacts a wide range of food crops in warm regions. The focus is specifically on the root-knot nematode, Meloidogyne incognita, a prevalent and highly destructive parasite that significantly impacts a wide range of food crops in warm regions worldwide. This nematode species inflicts considerable damage by invading plant roots, causing the formation of characteristic galls or "knots," which disrupt the plant's ability to absorb water and nutrients effectively. The resulting damage leads to stunted growth, reduced yields, and in severe cases, the death of the plant.

4.7.2 Synthesis at the lab scale development of target-specific nematicides based on RNAi

The synthesis of of target-specific nematicides based on RNAi involves are range of steps.

Step 1. Gene Target Identification

The initial step involves screening the genome of the nematode to identify essential genes related to vital biological functions, such as chitin synthesis. The project targets these genes to disrupt the lifecycle of the

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nematodes, thereby controlling their population and mitigating crop damage. This bioinformatics-driven gene target identification phase is entirely computational and does not require any physical materials or substances.

Step 2. Design and Synthesis of dsRNA

Specific dsRNA sequences corresponding to the identified target genes are designed. These sequences are crafted to ensure specificity, minimizing the risk of affecting non-target organisms. This bioinformatics-driven gene target identification phase is entirely computational and does not require any physical materials or substances. The dsRNA constructs are then synthesized using biotechnological tools. Typically, this involves the use of bacterial systems like *E. coli* for the production of dsRNA. Once the target gene sequences have been successfully cloned into the plasmid vector, the production of dsRNA in *E. coli* involves several steps. Here is a detailed explanation of the process along with a list of materials and substances required. The specific steps of the process are:

1. Transformation: Introduce the recombinant plasmid containing the target gene sequence into competent *E. coli* cells (e.g., HT115(DE3)). Plate the transformed cells on LB agar containing the appropriate antibiotic to select for transformants.
2. Colony Selection and Culturing: Pick individual colonies and grow them overnight in LB broth with the corresponding antibiotic to maintain plasmid selection.
3. Induction of dsRNA Expression: Subculture the overnight culture into fresh LB broth with the antibiotic and grow until the culture reaches the mid-log phase (OD600 ~0.4-0.6). Add IPTG (isopropyl β-D-1-thiogalactopyranoside) to induce the expression of the dsRNA. The concentration of IPTG is typically 0.1-1 mM, and the induction period is usually 3-4 hours at 37°C with shaking.
4. Harvesting Cells: After induction, harvest the *E. coli* cells by centrifugation. Collect the cell pellet.
5. dsRNA Extraction: Extract the dsRNA from the harvested cells using an RNA extraction kit or a suitable protocol. The extraction process involves cell lysis, removal of proteins and other contaminants, and purification of dsRNA.
6. Verification of dsRNA Production: Verify the presence and size of the dsRNA by running an agarose gel electrophoresis and visualizing the bands under UV light.

Specifically, when using DNA ligase for cloning, the following reagents and components are typically needed:

- DNA Ligase: The enzyme that facilitates the formation of phosphodiester bonds between the 3'-hydroxyl and 5'-phosphate ends of DNA fragments. Common choices include T4 DNA Ligase.
- Ligase Buffer: A buffer solution that provides the necessary conditions for the ligation reaction, typically containing:
- Tris-HCl (pH 7.5-8.0)
- $MgCl_2$ (magnesium chloride)
- ATP (adenosine triphosphate), which is a necessary cofactor for the ligase enzyme.
- DNA Constructs:
- Vector DNA: The plasmid vector that has been digested with appropriate restriction enzymes to create compatible sticky or blunt ends.
- Insert DNA: The DNA fragment containing the target gene, also digested with the same restriction enzymes used to cut the vector.
- Water: RNase-free or sterile water to adjust the final volume of the reaction mixture.

4.7.3 AGRO4AGRI R&D on of target-specific nematicides based on RNAi

To protect the dsRNA from environmental degradation and ensure its delivery to the nematodes, the dsRNA is encapsulated using advanced materials that can withstand field conditions and release the dsRNA in the target organism's gut. Encapsulation technology is chosen to respond to specific triggers, such as the acidic environment in the nematode's digestive tract, ensuring that the dsRNA is released precisely where and when needed. The development of novel pesticides based on RNAi technology has gained great attention due to their significant advantages over traditional small molecule pesticides (e.g., lower toxicity and higher specificity). However, the direct use of naked dsRNA for agricultural use is restricted due to its low environmental stability,

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especially under conditions like rainfall, high humidity, or UV light. In addition, it has been reported that dsRNA is poorly assimilated in highly destructive pest species, which may limit its pesticide efficacy to a higher extent. In this regard, the development of dsRNA-loaded encapsulated systems has been proposed as a promising strategy to both: i) protect dsRNA from environmental degradation and ii) ensure its effective delivery in the target organism's gut (i.e., nematodes). Therefore, the materials - and, consequently, the encapsulation technology - need to be chosen for their ability to withstand challenging field conditions and can be engineered to respond to triggers such as the acidic environment found in the nematode's digestive tract. As widely reported in the literature, the encapsulation of dsRNA is mainly governed by the electrostatic interactions between the negatively charged nucleic acid phosphate group of the genetic material and the carrier. Therefore, the production and/or surface functionalization of the encapsulating material is often the first step for the development of dsRNA-loaded encapsulates. In the literature, different dsRNA-loaded encapsulation systems have been reported such as polyplex nanoparticles (e.g., star polycation, SPc), BioClays (i.e., Layered Double Hydroxide, LDH) or virus like-nanoparticles (VLPs). However, these delivery systems present some disadvantages that might retrain their use in agriculture due to the difficult synthesis and formulation of the nanocarriers (e.g., surface functionalization of non-charged polymers such as dextran or synthesis of SPc), thus limiting the scalability of the process, or the use of (cyto)toxic ingredients (e.g., polyamidoamine, PAMAM), among others. Based on the latter, recent changes in the materials used for dsRNA encapsulation have significantly driven the development of lipid-based delivery systems, such as liposomes (LPNs). LPNs have been extensively proven to be effective pharmaceutical carriers. Therefore, the inspiration gathered from the medical field, together with their high biocompatibility, biodegradability, low toxicity, and capacity to transport hydrophobic loads, make them a promising encapsulation technology for agricultural applications. Indeed, the use of LPNs as dsRNA carriers for oral delivery aimed for pest control purposes has been recently reported and the results proved the effectiveness of the encapsulates to successfully suppress the marker gene expression, thus resulting in a higher mortality rate, than naked dsRNA (Xie et al., 2024). In brief, LPNs aimed as dsRNA delivery systems generally consist of 4 main components, being: i) an ionizable lipid, ii) a phospholipid, iii) cholesterol and iv) a poly(ethylene glycol)-conjugated lipid. The ionizable lipid, which is positively charged at low pH-values, allows the binding with the negatively charged RNA through electrostatic interactions. This ionization is also responsible for the RNA release inside endosomal compartments due to the low pH-derived protonation (pH ~ 5). As for the synthesis of the LPNs, several methodologies are available, the Thin-Film Hydration (TFH; Bangham Method) and Microfluidic-Based Synthesis (MBS, e.g., Emulsiflex C3) the most reported.

To produce dsRNA-loaded LPNs, the ingredients described in the study by Xie et al., (2024) will be used where the carriers were prepared by employing the formulation of Pfizer BioNTech containing four different types of lipids. The lipids included (1) ALC-0315, (2) ALC-0159, (3) 1,2-distearoyl-sn-glycero-3-phos-phocholine (DSPC) and (4) cholesterol. The four lipid components were dissolved in absolute ethanol to obtain a lipid stock solution with a total concentration of 20 mM (ALC0315: ALC-0159: DSPC: Cholesterol = 46.3:1.6:9.4:42.7).

To produce the delivery systems, the emulsifier EmulsiFlex®-C3 (AVESTIN, Inc.2450 Ottawa, ON, Canada) will be used, and the dsRNA load and emulsification pressure will be optimized accordingly.

Following synthesis and encapsulation, the RNAi formulations undergo rigorous testing to verify their efficacy in silencing the target genes.

Before applying RNAi-based strategies in field conditions, it is essential to conduct in vitro efficacy tests to determine the direct impact of dsRNA on nematode viability. In vitro assays provide a controlled environment to precisely measure the effects of dsRNA treatment on nematode mortality, behaviour, and gene expression. These preliminary tests are crucial for optimizing dsRNA concentrations, identifying effective target genes, and ensuring the reproducibility and reliability of the RNAi approach.

This study outlines an in vitro protocol to assess the efficacy of dsRNA on Meloidogyne incognita. By exposing nematode juveniles to encapsulated dsRNA in petri dishes, we aim to evaluate the potential of this method to reduce nematode populations. The protocol includes the preparation of dsRNA solutions, nematode collection and exposure, incubation, and subsequent analysis of nematode mortality and gene expression changes. The results from these in vitro tests will provide foundational data to support further development and eventual field application of RNAi-based nematode control strategies. Laboratory tests assess the reduction in nematode populations, and the specificity of the RNAi effect is confirmed to ensure there are no off-target impacts.

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Effective formulations are tested in controlled field trials to evaluate their performance under real agricultural conditions. These trials help determine the appropriate dosages and application methods.

Upon successful validation, production processes are scaled up to meet commercial needs, ensuring that the production method is viable for large-scale manufacturing.

4.7.4 Levels of SSbD design

The development of target-specific nematicides based on RNA interference (RNAi) in the AGRO4AGRO project spans all three levels of design: molecular design, process design, and product design.

Molecular Design:

- **Objective**: At this level, the focus is on the design of specific RNA molecules (dsRNA) that target crucial genes within the nematode's genome. The objective is to create dsRNA sequences that are highly specific to the nematodes, ensuring that these sequences can effectively silence or disrupt the expression of essential genes, leading to the nematodes' death or significant disruption in their ability to harm plants.
- **Applications**: This involves understanding the genetics of the nematodes and identifying potential target genes that are critical for their survival and reproduction. The design also considers the stability and efficacy of dsRNA molecules in agricultural environments.

Process Design:

- **Objective**: This level involves developing the processes for synthesizing, encapsulating, and delivering the RNAi molecules in a way that they reach the target nematodes effectively while remaining stable and active in field conditions. Process design also includes the methods for mass production of these RNAi molecules, ensuring they can be produced at scale and cost-effectively.
- **Applications**: Key processes include the biotechnological production of dsRNA, encapsulation techniques to protect the RNAi molecules from environmental degradation, and the formulation of delivery systems that ensure the dsRNA reaches the nematode's gut where it can be most effective.

Product Design:

- **Objective**: The final product design integrates the molecularly designed RNAi agents and their production processes into a practical, easy-to-use agricultural product. This product must effectively deliver the RNAi to the target nematodes when applied in the field, under real agricultural conditions.
- **Applications**: The product is designed to be user-friendly and compatible with existing agricultural application methods (e.g., spraying equipment or drip irrigation system). It must also meet safety and regulatory standards, ensuring it is safe for the environment, non-target organisms, and human handlers.

4.8 Encapsulation technologies for controlled release of dsRNA

4.8.1 Goal of synthesis and production of encapsulation technologies for controlled release of dsRNA

The main goal is to protect the dsRNA from degradation due to environmental factors such as UV radiation and enzymatic breakdown, ensuring that it reaches the target nematodes effectively. The encapsulation technologies utilize biobased materials like nanocellulose derivatives, clays, chitosan, alginate or liposomes, which will be selected for their biocompatibility, biodegradability, and ability to effectively encapsulate and protect dsRNA.

4.8.2 AGRO4AGRI R&D on encapsulation technologies for controlled release of dsRNA

The dsRNA is encapsulated using materials that can form stable complexes with the RNA molecules, providing a barrier against external degrading factors. The core (dsRNA) and the shell (coating material) must not react because the core can be degraded or modified. Technologies such as spray drying, spray chilling, coacervation, and molecular complexation are explored to create these encapsulates, focusing on achieving optimal size, stability, and release characteristics.

A crucial aspect of the encapsulation strategy is designing mechanisms that release dsRNA in response to specific environmental stimuli—namely, the pH levels typical of a nematode's gastrointestinal tract. This targeted release ensures that the dsRNA is activated only within the nematode, maximizing its efficacy and minimizing waste.

Post-encapsulation, the dsRNA formulations are subjected to thorough physical and morphological characterization to confirm their integrity and the effectiveness of the encapsulation. The release dynamics are tested under simulated conditions that mimic the nematode's digestive environment, ensuring that the release mechanism functions as intended.

Laboratory tests assess how effectively the encapsulated dsRNA silences target genes in nematodes, measuring the consequent reduction in nematode populations. The scalability of the encapsulation process is also tested to ensure that it can be economically and efficiently upscaled to meet agricultural demands.

The final step involves field trials where the encapsulated dsRNA's performance is validated in real agricultural settings. These trials are crucial for assessing the practical application and overall effectiveness of the RNAi nematicides in controlling nematode populations under typical farming conditions.

4.8.3 Levels of SSbD design

The development of encapsulation technologies for the controlled release of dsRNA in the AGRO4AGRO project also encompasses all three levels of design: molecular design, process design, and product design.

Molecular Design:

- **Objective**: This level involves the design of the dsRNA molecules themselves and the encapsulation system at the molecular level. The goal is to ensure that the dsRNA is stable, targeted, and effective against the nematodes. Molecular design includes selecting materials for encapsulation that interact favorably with the dsRNA, protecting it from degradation by environmental factors such as UV light and enzymes while ensuring its release in the specific environment of the nematode's gut.
- **Applications**: Key tasks include the chemical modification of encapsulation materials to enhance their protective qualities and interaction with dsRNA, as well as adjusting the molecular structure of the encapsulation matrix to achieve the desired release profiles.

Process Design:

- **Objective**: At this level, the focus is on developing and optimizing the processes used to encapsulate dsRNA effectively. This includes designing the methods for encapsulating dsRNA into the selected materials, such as liposomes, biopolymers, or other nanostructures, and ensuring that these processes are scalable and economically viable for large-scale production.
- **Applications**: Process design involves setting up the encapsulation parameters, such as solvent conditions, temperatures, and mixing techniques, that result in stable and functional encapsulates. It also includes integrating these processes with existing manufacturing systems to produce the encapsulated dsRNA at commercial volumes.

Product Design:

- **Objective**: This level concerns the formulation of the final product that will be used in agricultural settings. The product design integrates the encapsulated dsRNA into a form that is easy to apply, effective in the field, and safe for the environment and non-target organisms.
- **Applications**: The final product might be designed for application via common agricultural spraying systems or through other means suitable for targeting nematode-infested areas. Product design must also consider regulatory compliance, shelf life, storage conditions, and user safety.

4.9 Plant biostimulants for enhanced fertiliser efficiency

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4.9.1 Goal of synthesis of Plant biostimulants for enhanced fertiliser efficiency

The primary goal is to increase nitrogen use efficiency (NUE) in plants, thereby reducing the amount of fertilizer required for optimal growth and minimizing environmental impact. The biostimulants are derived from natural sources, including protein hydrolysates, seaweed extracts, and humic substances. These materials are chosen for their proven effects on promoting root development and enhancing nutrient uptake.

4.9.2 AGRO4AGRI R&D on Plant biostimulants

New formulations are created to be applied through foliar spray, targeting efficient uptake and immediate impact on the plants' metabolic processes. The formulations are designed to interact beneficially with plant physiology, improving nutrient assimilation and stress resilience. AGRO4AGRI will test different application methods to determine the most effective way to deliver these biostimulants to the plants, ensuring that they are absorbed and utilized efficiently. The biostimulants undergo extensive laboratory and greenhouse testing to measure their impact on plant growth parameters such as root mass, canopy development, and overall nutrient use efficiency. Tests are designed to quantify improvements in NUE and yield enhancements across various crop types.

Following successful laboratory tests, the biostimulants are trialed in field conditions to evaluate their performance in real agricultural settings. These trials assess the compatibility of the biostimulants with different soil types, climates, and crop species, providing comprehensive data on their effectiveness and versatility. Processes for synthesizing and producing the biostimulants at scale are developed, focusing on maintaining quality and efficacy while increasing production volume.

4.9.3 Levels of SSbD design

The development of plant biostimulants aimed at enhancing fertilizer efficiency in the AGRO4AGRO project encompasses all three levels of design—molecular design, process design, and product design:

Molecular Design:

- Objective: At this level, the focus is on understanding and engineering the molecular composition and biological activity of the biostimulants. This involves selecting and combining natural or synthetic ingredients that can enhance plant growth, nutrient uptake, and stress resilience. The molecular interactions between the biostimulants and plant cells are key to optimizing how these substances improve plant metabolism and nutrient use efficiency.
- Applications: The design includes the formulation of compounds that can influence plant hormonal balances, enhance enzyme activities, or improve nutrient solubilization and assimilation.

Process Design:

- Objective: This level involves developing the processes for manufacturing the biostimulants in a consistent, safe, and economically viable manner. Process design includes the methods for extracting, synthesizing, and combining the active ingredients into a stable formulation that maintains efficacy during storage and use.
- Applications: Process design also involves scaling up production from laboratory to industrial scales, ensuring quality control throughout the manufacturing process, and addressing any environmental impacts associated with production.

Product Design:

- Objective: At the product design level, the biostimulants are formulated into products that are practical for farmers to use and tailored to specific crops or growing conditions. This involves ensuring that the biostimulants are compatible with existing agricultural application systems, such as foliar sprays or root applications, and that they are effective under field conditions.

Applications: The final product design also takes into account regulatory compliance, market needs, packaging, labeling, and distribution logistics to ensure that the biostimulants reach the market in an effective and usable form.

5. Identification of hotspots using Early4AdMa and needs to redesign

5.1 Use of REACH and/or CLP classified chemicals in the synthesis of AGRO4AGRI solutions

The preliminary hotspot analysis reveals that there is a lack of data and information on the hazards of the envisioned AGRO4AGRI solutions in - and of themselves, which underlines the importance of generating this information in WP7.

The hotspot analysis also reveals that several of the potential chemicals and materials involved in the development of NCs, HDES and SAPs, etc. have many different hazard classifications in the EU. I

Depending on the chemical composition and nanoparticle morphology, NCs are organized into various groups such as montmorillonite, bentonite, sepiolite kaolinite, hectorite, and halloysite. Different hazard notifications have been provided by companies to the European Chemicals Agency in accordance to the Regulation on Classification, Labelling and Packaging ranging from no hazards to H315 (Causes skin irritation), H318 (Causes serious eye damage), H335 (May cause respiratory irritation) and H350 (May cause cancer, state route of exposure if conclusively proven that no other route applies). For biochar Biocahr notified as a flammable solid (H228), whereas hazards associated with the synthesis of biochar include: The heat source of the kiln, the release of toxic and flammable gases when extracting biochar from the reactor and possible leakage of toxic and flammable gases which could lead to an explosion.

HDESs and SAPs were chosen for their ability to modify the release properties of fertilizer carriers and candidates for materials to be explored in AGRO4AGRI obtain deep eutectic solvents with different hydrophobicity properties include: Betaine, Menthol, Thymol and Hexanoic acid. No hazards have been reported on Betaine and Decanoic acid whereas ECHA Registration data indicates that Menthol is slightly toxic and skin and eyes irritation (H315 and H319) are reported. Thymol is reported Acute Toxicity 4 (H302), Skin corrosion 1B (H314) and ecotoxicity for aquatic chronic 2 (H411). Finally, Hexanoic acid has been reported to be slightly ecotoxic in fresh water fishes. There are several different chemicals and materials involved in the development of SAPs and some of these have CLP classifications such as cellulose (Respiratory hazards (H335) and acute toxicity if its contacted to skin or swallowed (H312 and H302 respectively), Starch (Eye Irritation 2 (H319, H320), Acute toxicity by inhalation (H332, H335), Toxicity to aquatic life (H411)), Chitosan (Skin and eye irritation (H315 and H319), Affects to organs (lungs if it is inhaled (dry powder) and Carrageenan (Eye Irritation 2 (H319) and suspect to be carcinogenic Category 2 (H351)). Due to the hazard properties of Carrageenan, this material will be not used moving forward in AGRO4AGRI. In general, NFC, NCHs and NFs are believed to be biodegradable, non-toxic, and sourced from renewable materials, making them environmentally friendly options for agricultural applications. The chemicals and materials used in the synthesis of NFC, NCHs and NFs include:

- NaOH (CAS no.:1310-73-2): According to the harmonised classification and labelling(CLP00) approved by the European Union, this substance causes severe skin burns and eye damage. Additionally, the classification provided by companies to ECHA in REACH registrations identifies that this substance causes serious eye damage and may be corrosive to metals.
- H2O2 (CAS no.:7722-84-1): According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance causes severe skin burns and eye damage, may cause fire or explosion (strong oxidiser), is harmful if swallowed and is harmful if inhaled. Additionally, the classification provided by companies to ECHA in REACH registrations identifies that this substance causes serious eye damage, is harmful to aquatic life with long lasting effects and may cause respiratory irritation.
- HCl (CAS no.:7647-01-0). According to the classification provided by companies to ECHA in REACH registrations this substance causes severe skin burns and eye damage, is toxic if inhaled, may damage fertility or the unborn child, causes serious eye damage, may cause damage to organs through prolonged or repeated exposure, may be corrosive to metals, may cause respiratory irritation and contains gas under pressure and may explode if heated.

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- Oxygen (CAS no.:7782-44-7): According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance may cause or intensify fire (oxidiser).
- Sodium chlorite (CAS no.: 7758-19-2): According to the classification provided by companies to ECHA in REACH registrations this substance is fatal in contact with skin, is toxic if swallowed, causes severe skin burns and eye damage, is very toxic to aquatic life, may cause fire or explosion (strong oxidiser), may cause damage to organs through prolonged or repeated exposure and is harmful to aquatic life with long lasting effects.
- Liquid nitrogen (CAS no.: 7727-37-9): According to the classification provided by companies to ECHA in CLP notifications this substance contains gas under pressure and may explode if heated and contains refrigerated gas and may cause cryogenic burns or injury.

All the delivery systems developed in AGRO4AGRI on NCs, NCLs, MSN and biochar will be combined with fertiliser raw materials. Raw materials for controlled release fertilisers that will be explored include urea and ammonium nitrate as nitrogen sources, monoammonium phosphate and phosphoric acid as phosphorous sources and potassium chloride and potassium sulfate as potassium sources. Collectively, these raw materials have been classified as may intensify fire (oxidiser) (Ammonium nitrate) and causes serious eye irritation (Ammonium nitrate, Phosphoric acid, Potassium sulfate), may cause respiratory irritation (Ammonium nitrate) and causes skin irritation (Ammonium nitrate, Phosphoric acid, Potassium sulfate), harmful if swallowed (Phosphoric acid) and may be corrosive to metals (Phosphoric acid)).

For Target-specific nematicides based on RNAi, Encapsulation technologies for controlled release of dsRNA and PB for enhance NUE no information on CLP was available highlighting an important knowlege gap.

5.2 Early4AdMa

In order to futher identify hotspots and needs to redesign regarding the synthesis of the new agrichemicals, we used the Early4AdMa framework developed by the OECD (2023). The Early4AdMa questionnaire was circulated among the developers of AGRO4AGRI technologies to gauge the applicability of the current framework to very early design phase technologies. At the current state of development, it was possible to apply the framework to three case-studies: MSN, NC and biochar. It is important to highlight that the framework is still in development and have so far only been applied to few case-studies. Furthermore, it is filled out by developers who does not necessarily have expert knowledge about the state-of-the-art literature within each of the specific categories. The full results of the questionnaires are available in supplementary information files (Early4AdMA_MSN.xls, Early4AdMa_NC.xls, Early4AdMa_Biochar.xls), herein a summarization of the key findings will be discussed.

5.2.1 Early4AdMa evaluation of mesoporous silica nanoparticles

The summary of the Early4AdMa assessment for mesoporous silica nanoparticles is shown in Figure 17. Awareness flags resulting from the human health perspective mainly revolves around the exposure to workers either through the production or application phase and the potential nanoparticles size of the porous structures. However, it is not deemed that the awareness flags raised in this regard cannot be mitigated through already established abatement technologies such as personal protection equipment. The largest knowledge gaps were identified within both safety assessment categories, human health and environment, mainly related to kinetics and fate of the MSN associated with the accumulation/persistence in organisms. Utilization of low-tier tests with regulatory relevant organisms such as crustaceans (*Daphnia magna)* could be proposed as a cost-effective approach to identify potential for bioconcentration or biomagnification (Pang et al., (2021)). The approach was also applied to case-studies throughout the EU Horizon 2020 project HARMLESS (GA No. 953183). The large fraction of awareness flags raised for the sustainability category (50%) is related to extraction of raw materials and utilization of potential critical raw materials in the synthesis which should be evaluated further in WP 7 and quantification of the impacts should be addressed and weighed against the benefits and impacts of comparable

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technologies. The uncertainties in the sustainability sub-group are related to recycling of raw materials and waste which at the current state of development cannot be quantified.

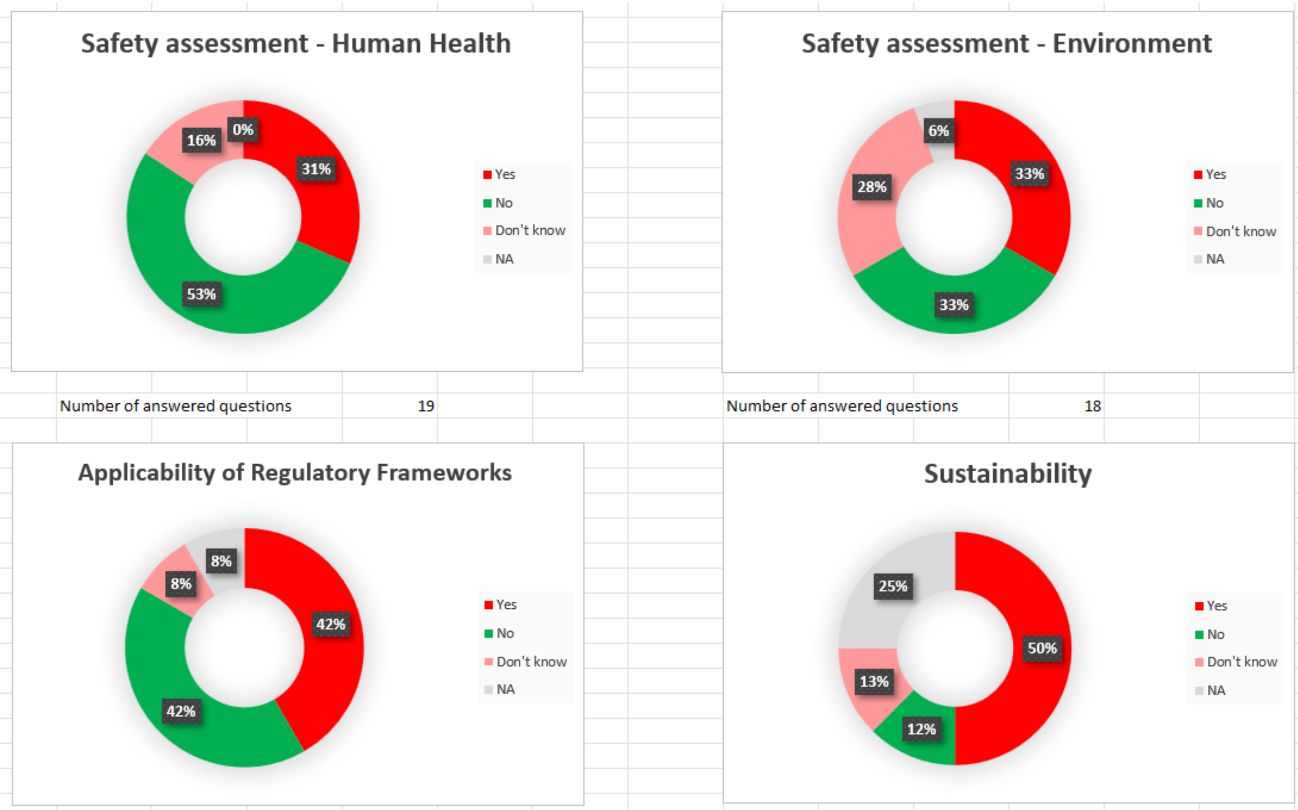

Figure 17. Summary of the four categories: human health, environment, regulatroy frameworks and sustainability, utilized in the Early4AdMa framework for mesoporous silica nanoparticles. "yes" highlights awareness flags, "no" no specific awareness flags, "don't know" no current information from the developer, "N/A" not applicable.

5.2.2 Early4AdMa evaluation of nanoclays

The results for the Early4AdMa assessment of nanoclays are shown in Figure 18. Similar to MSN main knowledge gaps for NC are identified for the categories related to safety assessment of human health and environment. Again, the understanding of kinetics and fate in environmental compartments and organisms related to uptake and mobility is lacking. However, the sustainability category markedly decreased in awareness flags (19%) raised due to no utilization of critical raw material.

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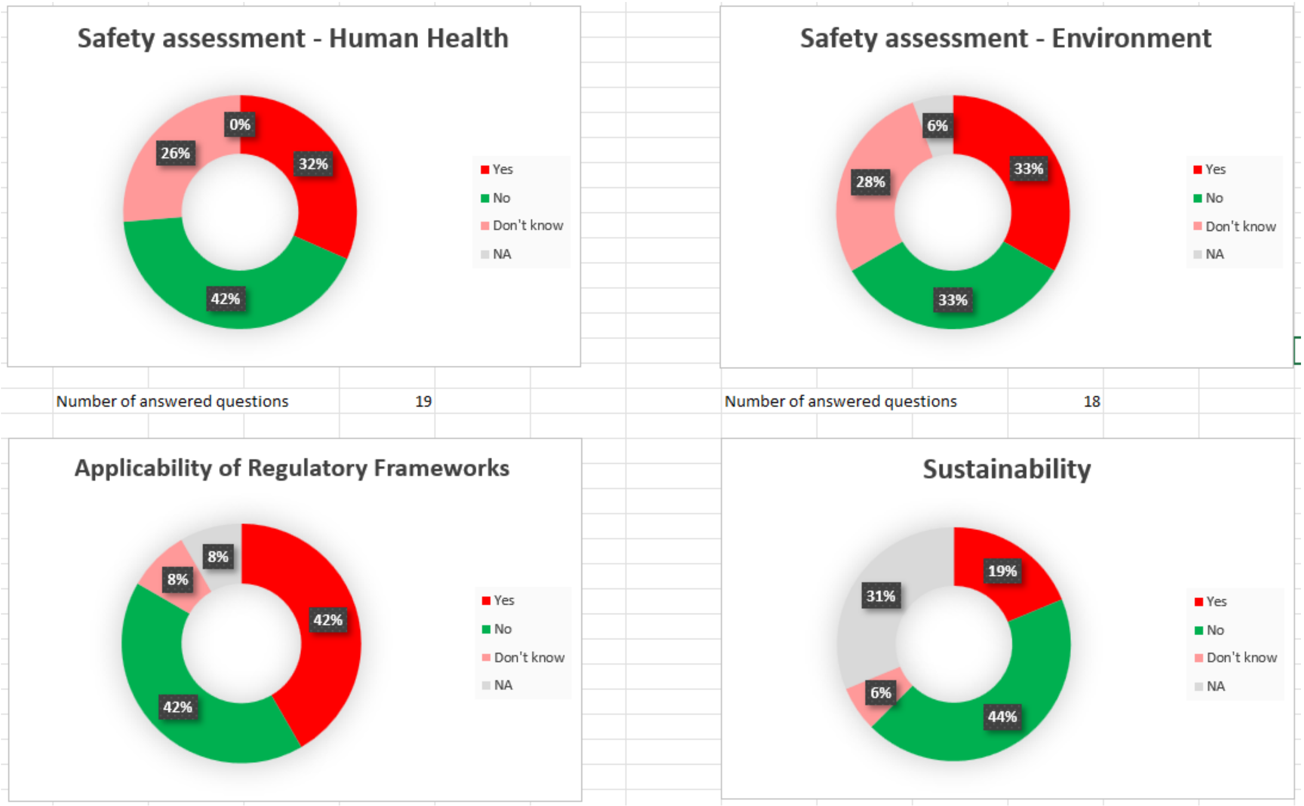

Figure 18. Summary of the four categories: human health, environment, regulatroy frameworks and sustainability, utilized in the Early4AdMa framework for nanoclays. "yes" highlights awareness flags, "no" no specific awareness flags, "don't know" no current information from the developer, "N/A" not applicable.

5.2.3 Early4AdMa evaluation of biochar

The results of the Early4AdMa screening of biochar is shown in Figure 19. It should be highlighted that compared to both MSN and NC there are markedly less uncertainty exemplified by only having 5 and 6% marked as "don't know" across the categories of human health and environment respectively. Also, for the sustainability category all questions were addressed. Across categories the awareness flags (amount of "no") decreased compared to MSN and NC. Only in the regulatory framework category was an increase in uncertainty (from 8% to 25% of questions answered "don't know"), with the uncertainty mainly revolving around the applicability of currently used regulatory test methods to assess the impact on human health and the environment. This discrepancy is interesting, as both categories of human health and environment uncertainty is decreasing, but there is uncertainty whether the currently employed method are applicable to address the safety for human health and the environment. However, based on the current questionnaire the biochar have the lowest uncertainty level and least awareness flags. It should be highlighted that the current findings are intial and should be used dynamically as the development and application of the designed technologies emerge.

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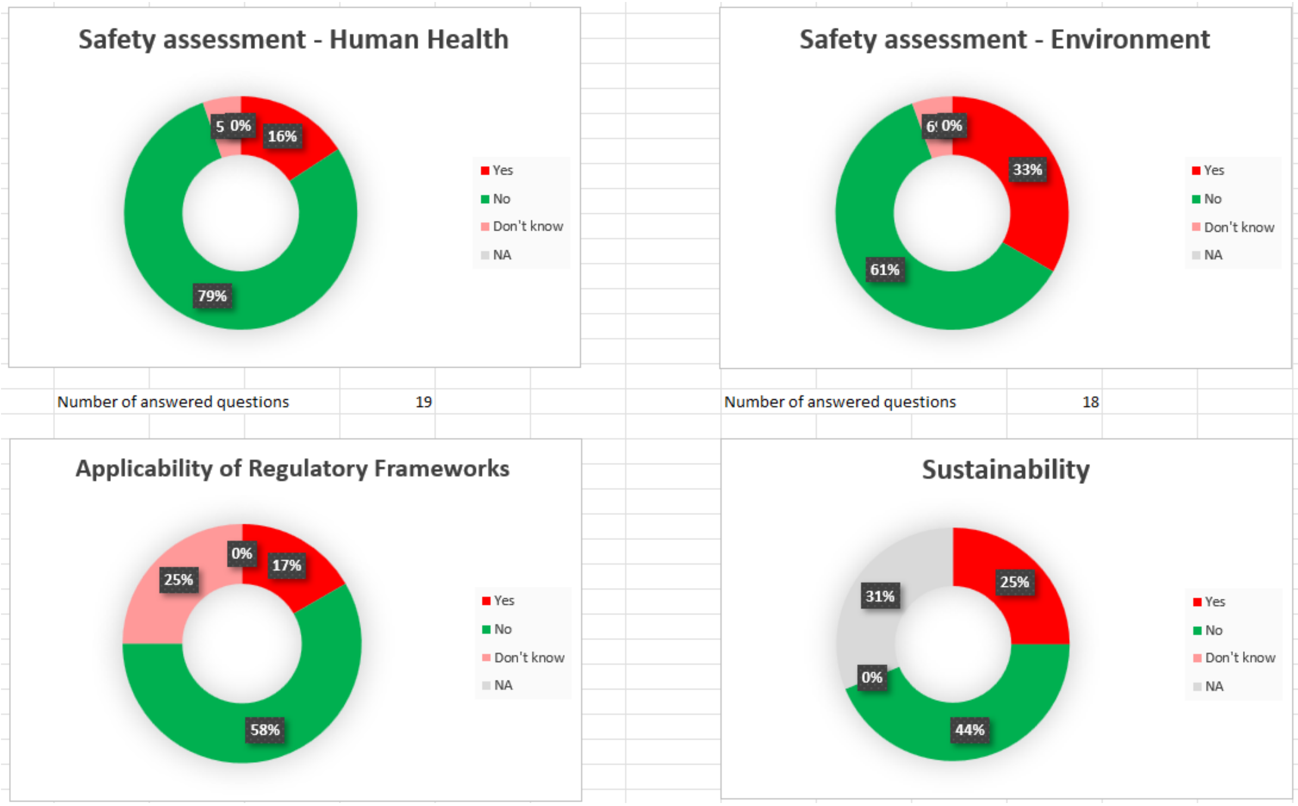

Figure 19: Summary of the four categories: human health, environment, regulatroy frameworks and sustainability, utilized in the Early4AdMa framework for biochar. "yes" highlights awareness flags, "no" no specific awareness flags, "don't know" no current information from the developer, "N/A" not applicable.

6. Conclusions

In this deliverable, we set out to apply the EC's SSbD framework to AGRO4AGRI solutions.

It is found that the SSbD framework can effectively guide the initial stages of innovation in AGRO4AGRI, emphasizing the integration of safety and sustainability from the outset. However, a key finding highlighted the critique of conventional sustainability assessments, which often neglect avoided impacts like human toxicological impacts related to indirect land use change (iLUC). This identified a need to explore these indirect impacts further, to be addressed in upcoming work packages (WP7).

The exploration of AGRO4AGRI solutions include molecular, process, and product design levels, ensuring comprehensive consideration of chemical interactions, production processes, and final product functionality. The study identified three primary SSbD principles: avoiding CMRs and reproductive toxicants, substances classified as PBT or Mobile, and unintended human and environmental exposure. In conclusion, the preliminary hotspot analysis reveals that there is a lack of data and information on the hazards of the envisioned AGRO4AGRI solutions in - and of themselves, which underlines the importance of generating this information in WP7. The hotspot analysis also reveals that several of the potential chemicals and materials involved in the development of NCs, biohcar, HDES and SAPs and synthesis of NFC, NCHs and NFs have many different hazard classifications in the EU. Identifying the least hazardous chemicals and materials in the development and synthesis of these AGRO4AGRI solutions will be a paramount importance in WP3, 4 and 5. The ppreliminary hotspot identification underscored the necessity for ongoing assessment and optimization throughout the product lifecycle.

Overall, the application of the SSbD and Early4AdMa frameworks provided a robust foundation for developing sustainable agrochemical solutions, with a strong focus on safety, functionality, and environmental impact. Future work will continue to refine these approaches, addressing identified knowledge gaps and indirect impacts to achieve comprehensive sustainability in agrochemical innovation.

The Early4AdMa framework was successfully applied for the initial agrochemical technologies (MSN, NC and biochar) highlighting marked uncertainty related to the categories of safety assessment of human health and environment. Uptake and transport across biological membranes was the most frequently highlighted issue and should be prioritized in further assessment of the technologies. Cost-effective tiered approaches should be used along the development chain focused on balancing functionality, safety and sustainability.

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