Annex 3
<u>Template RT full proposal (step 2)</u>

CONFIDENTIAL

Targeted Research, step 2
RT FULL PROPOSAL

FOOD SAFETY

RT 24/06 PUR(I)SALT

Purity and impurities of salt and salt substitutes

Zuiverheid en onzuiverheden van zout en zoutvervangers

Purité et impuretés du sel et des substituts de sel

Total duration	12 months
Total budget	€ 110000
Requested grant	€ 110000
If appropriate:	
percentage of own contribution	0 %
origin / nature of own contribution	
Proposed start date	01 /06 /2024

1. IDENTIFICATION OF THE CONSORTIUM

1.1 IDENTIFICATION OF THE COORDINATOR (TE)

Name : [redacted]
First name : [redacted]
Title : Scientist
Institution and department : Sciensano, Trace Elements and Nanomaterials
Address for correspondence : Leuvensesteenweg 17, 3080 Tervuren, Belgium
(Mobile) phone : [redacted]
E-mail : [redacted]

1.2 IDENTIFICATION OF PROMOTER 2 (PEST)

Name : [redacted]
First name : [redacted]
Title : Scientist
Institution and department : Sciensano, Pesticides and Environmental Contaminants
Address for correspondence : Juliette Wytsmanstraat 14, 1050 Brussels, Belgium
(Mobile) phone : [redacted]
E-mail : [redacted]

1.3 IDENTIFICATION OF PROMOTER 3 (RHIA)

Name : [redacted]
First name : [redacted]
Title : Scientist
Institution and department : Sciensano, Risk and Health Impact Assessment
Address for correspondence : Victor Hortaplein 40, 1060 Sint-Gillis, Belgium
(Mobile) phone : [redacted]
E-mail : [redacted]

2. HISTORY OF CHANGES

Have significant changes– based on the recommendations formulated in the invitation for step 2 – been made to the full proposal compared to the pre-proposal?

During the evaluation of the pre-proposal several recommendations were formulated, and they are all considered in this proposal.

- In WP4 of the pre-proposal, it was indicated that a non-target screening of potential organic contaminants would be conducted in a selection of 100 samples using both GC-MS and LC-HRMS to screen for volatile and polar compounds, respectively. A comment was raised by the evaluators that conducting a screening without providing quantitative data may increase the likelihood of needing a follow-up project. To address this comment, it is now proposed in the full proposal to conduct **a semi-quantitative analysis** of volatile and polar organic compounds on the selection of 100 samples (see WP4, task 4.2).
- In WP2 of the pre-proposal it was indicated that a total of 200 salt samples would be collected including salts sold to final consumers and food industry, particularly bread manufacturing. During evaluation it was proposed to have more samples in sales channels for companies besides bakeries. In the present proposal, 20 additional samples will be collected **(total 220 salt samples)**, and the sampling will be extended to include salts used in the meat industry (see WP1, task 1.2).
- As requested, data on inorganic and organic impurities will be delivered in SSD2-format for EFSA.

3. GENERAL INFORMATION

3.1 Description of the context of the project proposal (maximum 1,5 pages)

Sodium chloride (NaCl), commonly known as salt, is an ingredient extensively used in food (Hutton, 2002). Salt is sourced from rock salt deposits (halite mineral), seawater or natural brine, and the main production methods are solar evaporation, rock salt mining and solution mining (European Salt Producers' Association [EUsalt])[i]. The last method yields high-purity salts and represents about 60% of the salt produced in Europe. Salt for human consumption is regulated by its purity and the presence of impurities. The standard issued by the Codex Alimentarius for food-grade salt establishes a minimum NaCl content of 97% on a dry matter basis, exclusive of additives (Joint FAO/WHO Codex Alimentarius, 1985). Regarding inorganic trace element impurities, maximum limits have been set for arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) (European Commission, 2023). In Belgium, the maximum levels for As, Pb and copper (Cu) in salt are set by the Royal Decree of 17 September 1968 on salt intended for human consumption[ii]. Although the royal decree has not yet been amended, the harmonised standards laid down in Regulation (EU) No 2023/915 for metals in salt are already applicable in Belgium. The following maximum levels apply for metals in salt: 0.5 mg/kg for As, 0.5 mg/kg for Cu, 0.50 mg/kg for Cd, 0.10 mg/kg for Hg and 1.0 mg/kg for Pb. The maximum level of Pb in the unrefined salts 'grey salt' and 'fleur de sel' manually extracted from salt marshes with a clay soil is 2.0 mg/kg.

[i] https://eusalt.com/
[ii] https://www.mycodex.be/doc/130443

Various edible salts, so-called 'specialty salts' are available on the market. These unrefined salts with variable chemical compositions are becoming popular since they are perceived as a healthier option than regular table salt and may induce taste differences in food (Carapeto et al., 2018). The main drawback is that specialty salts can contain impurities inherent to the raw material or from particular steps of salt processing. Furthermore, the consumption of salt with moderate iodine (I) content (10-15 mg/kg) (Superior Health Council, 2014) is advised by the Superior Health Council in Belgium to increase the I status of the population. Common sources for I are potassium iodide, sodium iodide and potassium iodate. It is also possible to find "natural" iodized salts on the market where the source of I is edible algae mixed in the salt. In these algae-containing salts, unfavourable contaminants like As may be introduced. Moreover, various food additives are permitted in table salt and salt replacers as anti-caking agents and flavour enhancers. For example, calcium carbonate (E 170), ferrocyanides (E 535-538), sodium carbonates (E 500), magnesium carbonates (E 504), magnesium chloride (E 511), magnesium oxide (E 530), iron tartrate (E 534), phosphoric acid, phosphates, and polyphosphates (E 338-452), glutamic acid and glutamates (E 620-625), and ribonucleotides (E 626-635) are among the permitted additives as indicated in Regulation EC No 1333/2008 of 16 December 2008 on food additives. Some of these food additives can also contain impurities.

Although sodium (Na) is an essential element for normal cell function, high levels of dietary Na have been associated with adverse cardiovascular health effects. The World Health Organization (WHO) has recommended that salt intake from all sources be limited to 5 g/day (2 g Na/day)[i], but in Belgium, the consumption of salt is substantially higher (9 g/day) (Vandevijvere et al., 2021). One of the measures to reduce Na intake is the use of salt substitutes. For example, in low-sodium salt, NaCl is (partially) replaced by potassium chloride (KCl) (Cepanec et al., 2017). KCl is regulated under EC No 1333/2008 if used as a food additive, but when used as a salt replacer, it does not fall within the scope of the regulation.

Information on the chemical composition of various salts and salt substitutes with attention to the purity and presence of impurities (inorganic and organic) can provide the Belgian regulatory authorities the evidence to amend the current national legislation or consumption advice, if necessary.

3.2 Executive summary of the project (about 20 lines)

The increasing variety of salts and the limited information on the chemical composition of some of them has raised questions on their purity and the nature and safety of impurities. The aim of the PUR(I)SALT project is to fill the data gaps regarding purity and impurities (organic and inorganic) of various types of salts and salt substitutes sold on the Belgian market. To reach this aim, first a literature review (**WP1**) will be conducted to map (i) available data on purity and impurities in salts, (ii) the presence of impurities according to the production methods and (iii) the current legislation (national and international) regarding maximum levels of toxic chemical impurities in salts for human consumption. Second, sampling of the types and brands of salt and salt substitutes representative for the Belgian market will be conducted (**WP2**). Chemical analysis of different salts and salt substitutes will be conducted to determine their composition and purity (**WP3**). The quantification of relevant inorganic impurities, the semi-quantitative analysis of potential high-concern organic impurities, and the quantification of selected organic impurities will be performed using ISO17025 accredited methods and/or relying on our analytical experience as national reference laboratories (**WP 4**). Although the

[i] https://www.who.int/news-room/fact-sheets/detail/salt-reduction

call does not explicitely include exposure and risk assessment of the impurities in the salts, such analysis is included in this proposal (**WP5**). This additional work will add value to the proposal and provide the authorities with more substantiated information on the impact of the current limits on food safety.

3.3 Research objectives (about 2x 20 lines)

[EN]

- What is the current scientific knowledge state about purity and impurities in salts and salt substitutes?
- Which salts (including table salts, specialty salts, iodized salts and nitrite curing salts) and salt substitutes are available on the Belgian market?
- What is the purity of these salts and salt replacers?
- Which impurities (trace element and organic contaminants) are present in salt and salt replacers?
- To which extent are we exposed to impurities coming from salts and salt replacers and does this contribute to a potential health risk?

[NL]

- Wat is de huidige wetenschappelijke kennis over zuiverheid en onzuiverheden in zouten en zoutvervangers?
- Welke zouten (waaronder keukenzout, speciaalzouten, gejodeerde zouten en nitrietzouten) en zoutvervangers zijn beschikbaar op de Belgische markt?
- Wat is de zuiverheid van deze zouten en zoutvervangers?
- Welke onzuiverheden (sporenelementen en organische verontreinigingen) zijn aanwezig in zout en zoutvervangers?
- In welke mate worden we blootgesteld aan onzuiverheden afkomstig van zouten en zoutvervangers en draagt dit bij aan een potentieel gezondheidsrisico?

3.4 Justification of the submission under this topic (about 15 lines)

Currently a wide variety of edible salts are available on the Belgian market, particularly the so-called specialty salts. There is a trend in consumers to switch from refined table salt to specialty salts as they are perceived as healthier options and because it is claimed that they can provide different tastes to foods (Di Salvo et al., 2023). Since many of these salts have minimal processing it is necessary to investigate their chemical composition with due attention to the presence of toxic elements. Besides inorganic contaminants, in recent years, efforts have been put to investigate the presence of microplastics in salts (Ercoşkun, 2023; Iñiguez et al., 2017) overlooking other persistent organic contaminants which can be potentially present in salts. In this study, inorganic and organic impurities will be investigated in various types of salts to fill this important data gap. In addition, the exposure estimation and risk assessment of inorganic impurities through the consumption of salt and salt substitutes will be conducted confering an added value to this project proposal.

Among the methods aimed at cutting down sodium consumption is through the use of salt replacers. There is no information regarding impurities and purity of salt replacers nor a legal framework for these food products. One example is KCl, when KCl is used as salt replacer it is excluded from the scope of the food additive regulation (EC1333/2008). The data that will be collected in this study can be used as a basis for decisions regarding the need for developing legislation for salt replacers.

3.5 Framework of the project proposal (about 2.5 pages)

Previous research in relation to the proposed subject

During the preparation of the proposal, four peer-reviewed articles (Di Salvo et al., 2023; Fayet-Moore et al., 2020; Karavoltsos et al., 2020; Serrano et al., 2011) and one scientific report (Infanger and Haldimann, 2016) were identified that provide information on the chemical composition, with focus on the analysis of impurities, of various types of salts. From the published papers, only the work by Serrano et al. (2011) and Karavoltsos et al. (2020) have investigated the presence of organic contaminants in salts. These studies are small in the number of samples analysed (max. 31 samples) nevertheless are a good starting point for the current project to identify the various types of salts available and the impurities that have been analysed. Moreover in these studies, state-of-the-art analytical techniques have been used for the analysis of impurities (i.e. ICP-MS, GC-MS) making the data comparable with the results that will be obtained in the present project. Below a short summary of the main findings:

1. Di Salvo et al. (2023): this study investigated inorganic impurities in 10 specialty salt samples purchased in local markets in southern Italy. The salts included Mozia, Atlantic grey salt, Persian blue, smoked salt, Guerande grey, Hawaii pink, Hawaii black, Himalayan pink, Maldon, Baule Volante. All the investigated salts contained levels of Pb that surpassed the maximum EU permitted limit. Nickel levels were elevated in Persia blue and smoked salts.
2. Fayet-Moore et al. (2020): this study analysed the mineral composition of 31 pink salts commercially available in Australia. Compared to regular white table salt, pink salts contained substantially higher levels of impurities including Al, Ni, Ba, Si, S. From the salts investigated, one sample exceeded the Food Standards Australia and New Zealand (FSANZ) maximum contaminant level for Pb (2.59 mg/kg vs. 2 mg/kg).
3. Infanger E. and M. Haldimann (2016): the Swiss Federal Food Safety and Veterinary Office conducted a research study to determine the elemental composition (including inorganic impurities) of 25 salts available on the Swiss market. The salts included in the study were standard iodized salts and specialty salts (i.e. Himalaya pink salts, Kala Namak salt, Blue Persian salt, Fleur de sel, etc). The study concluded that the NaCl content of specialty salts ranged between 83-99%. Higher levels of trace element contaminants (Al, As, Ni, Pb) were generally observed in Himalaya pink salts and Kala Namak salts. The levels of regulated inorganic impurities (As, Cd, Hg, Pb) were below EU maximum concentration limits for all salts.
4. Karavoltsos et al. (2020): this study investigated the presence of trace elements and PAH impurities in 24 unrefined salts available on the Greek market. This is one of the few scientific studies investigating the presence of organic contaminants in salts. Although this study investigated inorganic impurities, it did not consider Hg and As, two elements for which maximum limits are established.
5. Serrano et al. (2011). The study followed a screening methodology to identify organic compounds in sea salts obtained from solar saltworks located in the Spanish Mediterranean coast. Inorganic impurities were not analysed.

How is this project scientifically and technically innovative?

Many types of salts and salt substitutes are available on the Belgian market, but information regarding their chemical composition is limited, particularly for novel specialty salts, which are unrefined and prone to contain impurities. Along those lines, it is recommended in Belgium to use iodized salts. However, there is a lack of information on the iodine content in salts since labelling of I content is voluntary. The situation is similar for salt substitutes, like KCl, which are not regulated when used as salt. The present project proposal will differ from previous published work in that it will analyze a large number of samples including salt substitutes and nitrite curing salts in addition to standard table salts and specialty salts. Moreover, for the selection of salts, attention will be given to obtain samples from different natural origins (sea salt, rock salt, lake salt, well salt) as the presence of certain impurities and its levels may be associated with the particular origin and can be prevalent in salts that are not purified. The project also foresees sampling salts available for final consumers (retail) and salts intended for the bakery and meat industry (wholesale).

The project combines analysis of composition and impurities, both organic and inorganic, resulting in a thorough characterization of the salt products available on the Belgian market. No information is known in literature about the salt replacers, and most available publications focus on inorganic and microplastic impurities in salt without considering potential organic impurities (Ercoşkun, 2023). The combination of the analyses in this proposal on this scale of sampling will result in a complete database of information on the basis of which the competent authorities will gain insight into the exposure to the nutrients and impurities in question. Moreover, the exposure data will give information for the need for updating current legislation or consumption advices.

Achievements of the applicants/researchers in this field

All consortium partners have extensive expertise in the chemical analysis of inorganic and organic substances in food and food related products. They are involved in numerous research projects as partners (SPECENZYM 2017-2021, TEQFOOD 2022-2024) or coordinators (LECAHUNT 2018-2020, PARCLINTA 2019-2021, FLUOREX 2021-2023, TREFCOM 2021-2023, METALFOOD@ 2023-2026, PFASFORWARD 2023-2027) and frequently work together.

Trace elements and Nanomaterials (TE) – The service holds the Belgian National Reference Laboratory (NRL) since 2008 for trace elements and nanomaterials in food and feed and has a flexible scope accreditation (ISO 17025) for the analysis of trace elements and As species in food by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Ion chromatography (IC)-ICP-MS and Direct Mercury Analyser (DMA). Accredited methods are available for the determination of Pb, Cd, inorganic As (As_i), total As, Al, Cu, Ni and Hg in foodstuffs. As NRL, the service organized in 2022 sucessfully a proficiency test for As, As_i, Cd, Pb and Hg in unrefined salts (Rondelet and Cheyns, 2022).

Dr. Daniela Montalvo is a scientist with 8 years of research experience in topics related to environmental chemistry, including analysis of trace elements in soils, water, plant material, and fertilizers. Dr. Karlien Cheyns is a senior scientist, with more than 15 years of experience in trace element analysis in the food chain. She participates actively in many (inter)national working groups (e.g., CoE, EURL, EFSA, CEN, FASFC SciCom). TE will be responsible for the project coordination and management (WP0) and will coordinate WP1, 2, and 3.

Pesticides and Environmental Contaminants (PEST) is the Belgian NRL for process and environmental contaminants in collaboration with the University of Liege (CART) and Belgian NRL for pesticides. The laboratory has been ISO 17025 accredited by BELAC since 1997. The unit has a long experience in analytical chemistry (liquid and gas chromatography and mass spectrometry) and has state-of-the-art analytical instrumentation to analyze residues and contaminants in food. Non-targeted analyses are already performed in the framework for the national investigation unit (NOE-UNE) of the Federal Agency for Safety of the Food Chain (FASFC).

Dr. Laure Joly is a senior scientist with more than 15 years of experience in the analysis of pesticides and contaminants, and she is involved in numerous committees and working groups at the Belgian and EU levels.

Risk and Health Impact Assessment (RHIA) - The service focuses on the dietary exposure and risk assessment of contaminants using Belgian National Consumption Data. The service incorporates the dietary exposure assessments within the risk assessment for a full evaluation of the risk to human health *via* food and, where possible, *via* other routes of exposure. The team has broad expertise in national food consumption surveys and statistical methods to estimate the habitual intake of food additives and contaminants.

Dr. Nadia Waegeneers is a senior scientist, with more than 15 years of experience in trace elements, dietary exposure, and risk assessments. She was involved in the latest exposure assessments to Pb (SciCom Advices 36-2009 and 07-2011), Cd (SciCom Advice 35-2009; Vromman et al., 2010) and inorganic arsenic (SPECAS, 2010) for the Belgian population and will be coordinating the new exposure assessments for these trace elements in the Metalfood@ project (2023-2026). In her current function, she has access to the most recent food consumption data of the Belgian population.

3.6 Use of the expected research results (about 5 lines)

The results obtained in the project will be communicated to the Federal Public Service Health, Food Chain Safety and Environment to provide recommendations on whether and what changes to the legislation or consumption advice might be appropriate. Analytical data and all information gathered in the project will be summarized and reported.
Considering that knowledge transfer is a strategic objective of Sciensano, we will seek to disseminate the results of this project to all interested parties i.e. regional, national and international authorities, scientific community, regulators, and the general public. Research results will be published in peer-reviewed scientific journals and presented at international conferences/workshops on food safety. The results will also be vulgarized in articles for the general public.

3.7 Risks (about 15 lines)

Risks associated with the chemical analysis of samples are not expected to be a major problem since the partners have extensive experience for analyzing organic and inorganic impurities in various food matrices, including salt. Furthermore, back-up instruments are available.

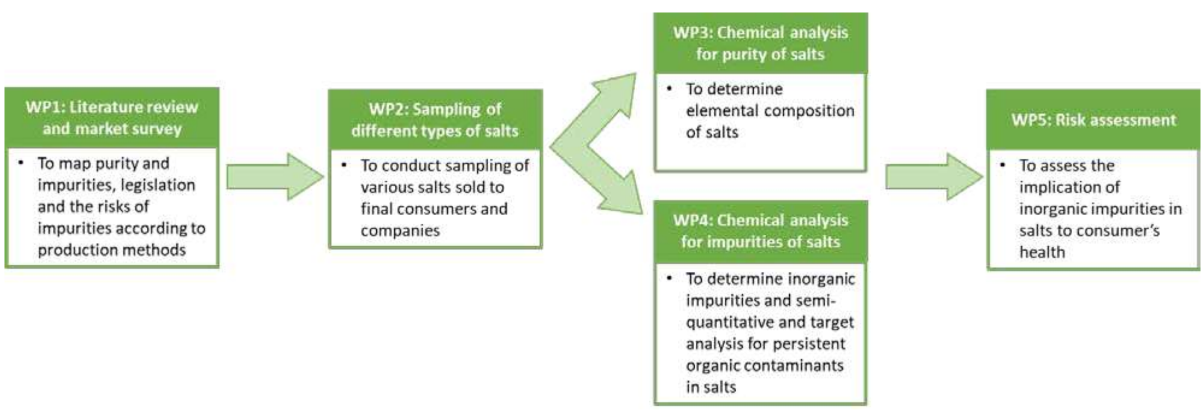
The analysis of chloride by ion chromatography (IC) has to be developed in the laboratory, though the instruments are available, method optimalisation is needed. In a worst case, the chloride content can be calculated based on the results of the analysis of the major elements following the procedure described in (Joint FAO/WHO Codex Alimentarius, 1985).

The untargeted analyses will be semi-quantitative, limiting the likelihood of needing a follow-up project. However, the untargeted analyses do not reach such low concentrations as the targeted analyses, and the toxic impurities at a very low level may not be detected at a sufficiently low level. Therefore, a targeted analysis performed on relevant suspected impurities will be included.

The purchase of salts from the wholesale market could represent a challenge in this project if certain products are sold explicitly to companies dedicated to the food industry. Main salt producers in Belgium (Zoutman N.V. and K+S Benelux) and representatives of salt producers in Belgium (EUsalt) will be contacted to inform about this proposal and to seek possible collaboration by means of obtaining samples of salts that are supplied to the food industry, specifically bakery and meat processing. Additionally the Federation of Large Bakeries of Belgium (FGBB) and the Federation of Belgian Meat Processing Industry (Fenavian) will be contacted as they can provide information regarding the type of salts that are used in their respective industries.

4. SPECIFIC INFORMATION

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



WP 0 – Coordination and project management

TE, RHIA, PEST Resources:1.25 Man-months (SW1, TE)

This work package is designed to ensure that the project will progress according to the work plan, particularly regarding the timing of the milestones and deliverables, as well as the planned resources. This will be ensured by performing regular internal meetings among the partners. Furthermore, project meetings (kick-off meeting and final meeting) will be organized with the Steering Committee and representatives from the funder to provide an update on the status of the project and discuss the obtained results. Special attention will be given to the advice of the Steering Committee regarding the sampling plan. The project coordinator will coordinate the progress report to the Steering Committee, and the final report, and facilitate the financial reporting to RCO. This WP will also include management of the collected data during and after the project.

This WP will also coordinate the dissemination of the results to a broad audience including end-users, consumers and policy makers. A wide range of communication media will be used such as press releases, scientific publications and international conferences.

<u>Milestones (M) & Deliverables (D)</u>

- D: Organization of data management after the project (including a contact person)
- D: Approved meetings minutes
- D: Progress report and final report
- D: Peer-reviewed scientific publication and vulgarizing publication

WP 1 – Literature Review and Market Survey

TE, RHIA, PEST
Resources:1.75 Man-months (SW1, TE)

T1.1. Literature review on edible salts

TE

In **WP1,** a literature review will be conducted on published scientific studies investigating the elemental composition of food-grade salts, including impurities (inorganic and organic). The literature study will be extended to map current national and international legislation regarding salt purity and the maximum permitted levels of contaminants. Additionally, the literature review will collect information on the common methods of salt production in order to link the presence of certain impurities to a particular production method. Relevant information will be summarized in a comprehensive database that will provide further guidance on which organic impurities should be prioritized.

T1.2. Market study and sampling plan

TE, RHIA

The information obtained in T1.1 will be used to conduct a market survey of the different types of salts available on the Belgian market. To identify salts sold to final consumers a market screening will be done using web scraping of the online offer of the major retailers in Belgium and by mapping low-scale retail and specialized web shops.

A list of salts available at the major retailers (Franz Colruyt NV, Carrefour SA, Koninklijke Ahold Delhaize NV, Aldi Group and Schwartz Beteiligungs GmbH (Lidl)) will be drawn from the FABEL dataset that was developed in the MULTI-EXP-ADD project. This dataset contains over 36000 unique food products that were available in 2020 from the retailers listed above. Additionally, the dataset will be updated with products available in 2022 using data collected by Sciensano.

Salt is widely used in the food industry. In the bread manufacture it is an essential ingredient for dough formation, fermentation rate and flavour. In the meat industry the main function of salt is preservation for products such as bacon, sausages, salami; while in cured products it acts in combination with nitrite to effect preservation (Hutton, 2002). Considering that the consumption of bread and processed meat products are most contributors for salt intake in the diet, the project will sample salts that are commonly used in those industries. Business operators and associations, such as the Federation of Large Bakers of Belgium and the Belgian Federation of Meat and Processing Industry will be contacted to obtain relevant information. Additionally, wholesale salt suppliers and key salt producers in Belgium (i.e. Zoutman N.V) will be also inquired.

Each salt or substitute will be listed in a matrix containing information which will be used for the selection of samples. Based on this matrix, a plan for the extensive sampling (**220 samples**) of different salts (common salt iodized or not, salt substitutes, specialty salts and nitrite curing salt) will be drafted. The sampling plan will include salts of different origin of the raw material (i.e. rock salt, sea salt, lake salt, well salt), various brands and will include salts that are sold to final consumers and salts sold to the food industry, particularly bread manufacturing and meat processing.

The sampling plan will be presented to the members of the Steering Committee for discussion and further approval.

<u>Milestones (M) and Deliverables (D)</u>

- M: Approved sampling plan by Steering Committee
- D: Database from literature with information on chemical composition of different types of salts, origin of raw material, presence and levels of inorganic and organic impurities, method of salt production
- D: Review on national and international legislation on maximum limits of contaminants in food grade salt and legislation on salt iodization
- D: Sampling plan for salt samples, including common table salt, specialty salt, iodized salt, salt replacers, nitrite curing salt products available on the Belgian market

WP 2 – Sampling of different types of salts

TE
Resources:0.5 Man-months (SW1, TE)

T2.1. Sampling of salts

TE

Following the approved sampling plan, salt samples will be purchased on the Belgian market in physical stores, wholesale shops and webshops. After purchase, samples will be labelled and stored in a cool and dry place until analysis. General available information such as date and place of purchase, lot number, detailed description of the salt as indicated on the package (i.e.

country of origin of the salt, origin of raw material) and the type of packaging material (plastic, cardboard, glass) will be collected in a database. Additionally, pictures of the samples will be taken. This database can be further extended with other relevant information and will serve as a tool for the evaluation and interpretation of the results.

Milestones (M) and Deliverables (D)

- M: Purchase of 220 samples
- D: Database containing all sample information

WP 3 – Chemical analysis for purity of salts

TE
Resources:1 Man-month (B, TE)

T3.1. Chemical analysis of major elements

TE

The chemical composition of food-grade salt as defined by the Codex Alimentarius Standard 150-1985 shall contain a minimum of 97% NaCl on a dry matter basis. Secondary elements and contaminants are also present in the final product depending on the salt origin and the method of production. Major secondary elements include Ca, Mg, K, and compounds Na_2SO_4, Na_2CO_3, NaBr, $CaCl_2$, KCl, $MgCl_2$ (Joint FAO/WHO Codex Alimentarius, 1985). In this task the analysis of major elements specifically Na, K, Ca, Mg, and S will be done by the Unit Trace Elements by ICP-OES in the totality of samples (approximately 220). The determination of Cl content in salts will analysed by ion chromatography (IC). The dry matter content will be determined simultaneously in order to report results on a dry matter basis.

T3.2. Chemical analysis of iodine

TE

Total iodine (I) will be determined by ICP-MS in iodized salts and salts that claim to contain this element. The method of analysis will be based on the standard "EN 15111-2007[i] Determination of iodine by ICP-MS". The method uses an alkaline extraction with Tetramethylammoniumhydroxide (TMAH). A basic medium is usually preferred to avoid the loss of volatile iodine species (HI or I_2) produced in acidic media. Tellurium (Te) is used as an internal standard. The limit of quantification for I range 3-40 µg/kg (depending on the matrix).

Milestones (M) and Deliverables (D)

M: Occurrence data for Na, K, Ca, Mg, S, Cl in all the samples and I in iodized salts
D: Report on major nutrient elements in salts (dry matter base) and calculation of salt purity expressed as % of NaCl.
D: Report on I content in iodized salts.

[i] https://www.nen.nl/en/nen-en-15111-2007-en-116294.

WP 4 – Chemical analysis for impurites of salts

TE, PEST
Resources:1 Man-month (B, TE), 5 Man-Months (B, PEST)

T4.1. Chemical analysis of inorganic impurities

TE

The analysis of inorganic impurities Al, As, Cd, Cu, Ni and Pb in the salts will be conducted by the Unit Trace Elements. The Laboratory holds a flexible scope ISO 17025 accreditation for the analysis of elements including As, Cd and Pb in foodstuff by ICP-MS. The limits of quantification (LOQ) range (depending on matrix) 0.5-2 µg/kg for As, 0.7-2.8 µg/kg for Cd, 7-28 µg/kg for Cu, and 4-16 µg/kg for Pb. The Unit holds also an ISO 17025 accreditation for the analysis of Hg in foodstuff by Direct Mercury Analysis (DMA). The limit of quantification ranges (depending on the matrix) 0.1-0.6 µg/kg Hg. In addition, the unit holds a flexible scope ISO 17025 accreditation for the analysis of As species by IC-ICP-MS in food of vegetable origin. The limit of quantification ranges (depending on the matrix) 1-8 µg/kg inorganic As. The validation of the analysis methods in foodstuff is done conform the performance criteria specified in Commission Regulation (EC) No 333/2007 (i.e. determination of LOQ, precision, trueness).

The analysis of Al, As, Cd, Cu, Ni and Pb will be determined by ICP-MS and Hg by DMA on the totality of samples (approximately 220) using validated methods. It will be considered that the total amount of As measured in the salts corresponds to inorganic As, unless there is indication in the package that organic As is expected to be present (e.g. algae mixed with salts). For these specific samples inorganic As will be determined by IC-ICP-MS. The dry matter content will be determined simultaneously in order to report on a dry matter basis.

T4.2. Semi-quantitative analysis of organic contaminants by LC-HRMS and GC-MS

PEST

The semi-quantitative analysis of organic impurities will be performed on a selection of **100 samples** guided by the literature review (WP1), the results obtained in WP3 and a discussion with the Steering Committee. Priority will be given to samples with potential contamination by organic impurities. For example, the production methods and the associated possibility of including organic impurities will be considered. As a first approximation, sea salt (the most predominant salt) and smoked specialty salts seem the most relevant, but at least 5 samples of each of the four origins (sea salt, lake salt, rock salt and well salt) will be analyzed.

As a wide range of organic contaminants may be present, a non-target screening of potential environmental contaminants from available spectra databases will be carried out using both GC-MS/MS and LC-HRMS to screen for volatile and more polar compounds, respectively. As suggested by Malm et al. (2021) a minimal sample preparation method will be performed since salt is not a complex matrix, thereby avoiding the loss of contaminants. The procedure will most likely consist of diluting salt in water followed by a QuEChERS extraction to enable direct analysis in LC and GC. LC analysis will be performed in positive and negative modes in combination with high-resolution mass spectrometry (HRMS), while GC analysis will be conducted with tandem mass spectrometry. Generic columns (C18 for LC, DB5 for GC) and chromatographic parameters (elution gradients using water/acetonitrile for LC, and linear temperature gradients up to until 220°C for GC) will be used. A wide scan range will be applied, typically from 100 to 1000 Da. The contaminants will be identified using the methodology developed by Schymanski et al. (2014). They defined five identification levels, giving the maximum confidence level when an exact mass, a referenced mass spectrum after fragmentation, the retention time and reference standard are available (i.e. confirmed structure - level 1). Depending on the available data, other levels were defined, varying from probable structure (i.e. level 2), tentative candidate (i.e. level 3), unequivocal molecular formula (level 4) to exact mass of interest (level 5). After identification, the availability and analysis of the analytical standard will enable a semi-quantitative analysis.

A quality control plan will be implemented for each batch of samples to monitor the daily method's performance and ensure the reliability of the reported results. A solution with a set of compounds with known low concentrations will be injected before and after each sample batch to ensure that no loss of sensitivity or instrumental deviation has occurred during the batch (deviation $\leq$ 30 % for the end batch standards). Measurements include analysis of procedural blanks to ensure that no contamination occurred during the extraction procedure nor in the chromatographic and mass spectrometry systems. Samples will be analyzed at two dilutions by injecting two different volumes to estimate the slope of the calibration curve.

T4.3. Target analysis of selected organic contaminants

PEST

The targeted analysis of organic impurities will be performed on the selection of **100 samples** selected in Task 4.2. The analysis of a specific class of impurities will allow to apply a dedicated method, leading to lower quantification limits compared to the semi-quantitative analysis. The selection of target class of contaminants will be guided by (i) a literature review identifying the most severe contaminants in seawater, and (ii) the results from the semi-quantitative analysis. Potential candidates are poly-and-perfluoroalkyl substances (PFASs), polycyclic aromatic hydrocarbons (PAHs), polar pesticides, apolar pesticides or non dioxin like-PCBs. The final selection of the targeted class will be discussed with the Steering Committee.

Once the class of contaminants is selected, the analytical methodology already available at Sciensano will be optimized for the analysis of salt. Sciensano can already rely on a broad scope of methodologies in various matrices. No issues are expected in this optimization step since well-known extraction and purification steps will be applied, followed by LC-MS/MS or GC-MS/MS according to the selected contaminants.

<u>Milestones (M) and Deliverables (D)</u>

- M: Occurrence data for inorganic and organic impurities
- D: Report on inorganic impurities present in salt samples
- D: Report on semi-quantitative results for organic impurities in selected salt samples
- D: Report on quantitative results for one class of organic compounds in selected salt samples
- D: SSD reporting on inorganic and organic impurities in salts

WP 5 – Risk assessment

TE, RHIA, PEST
Resources:0.5 Man-months (SW1, TE)

T5.1. Exposure estimation and risk assessment of impurities

RHIA

To estimate the potential exposure to trace elements (impurities) through the consumption of salts and salt substitutes, the analytical results obtained in WP 4 (Task 4.1) for Al, As, Cd, Cu, Hg, Ni and Pb will be combined with salt consumption data. The total amount of salt consumption for adults will be based on (1) the WHO recommended maximum intake of 5 g salt/day (WHO, 2012) and (2) the actual salt intake by the Belgian adult population. Salt intake assessed from the Belgian food consumption survey (6.0 g/d for adults (De Ridder and Teppers, 2016), which used duplicate 24h dietary recalls, appears to be less accurate than salt intake assessed from urinary Na excretion (De Keyzer et al., 2015). The most recent estimates of mean salt intake by the Belgian adult population, based on urinary Na excretion, is 8.3-9.4 g/day (Vandevijvere et al., 2021). These data will be used for estimating the potential exposure to trace elements through the consumption of salts for adults. This will represent a conservative estimate as part of the total salt intake originates from the natural presence of NaCl in food commodities (e.g. in milk and vegetables).

For children, the total amount of salt intake will be based on (1) the recommended daily Na intakes by the Superior Health Council (Hoge Gezondheidsraad, 2009), which will be recalculated to salt intake and (2) the salt intake assessed from the Belgian food consumption survey (De Ridder and Teppers, 2016), as no recent urinary Na excretion data are available for Belgian children.

The obtained exposure estimates will be compared to the general dietary exposure and health-based guidance values for the respective trace elements to evaluate the contribution of salt to trace element exposure.

T5.2. Policy recommendations

TE, RHIA, PEST

The results from the chemical analysis for purity and impurities in the different types of salts collected in Belgium will be considered a baseline study to formulate policy recommendations and, if necessary, propose amendments to current limits for contaminants in salts. The obtained knowledge will be translated into outputs aligned with the needs of the FPS Health authorities.

Currently in Belgium there is no legal framework to control iodine concentration in salt for bread production and household use. Iodine concentration of 10-15 mg/kg salt is recommended but not controlled. A more strict regulation on I concentration is encouraged as the tendency on public health policy is a reduction in the consumption of salt (Vandevijvere, 2012). In that respect this study will provide such information.

<u>Milestones (M) and Deliverables (D)</u>

- M: General exposure assessments for inorganic impurities (Al, As, Cd, Cu, Hg, Ni and Pb) through the consumption of salts and salt substitutes
- D: Risk assessments for the inorganic impurities
- D: Knowledge transfer outputs: provide FPS Health authorities one evidence brief or policy brief to facilitate informed decision-making

Foreseen timeframe of the PUR(I)SALT project

Code	Year 1				Total budget per WP*
	t1	t2	t3	t4	
WP 0: Coordination and project management					9550
WP 1: Literture review and market survey					13300
T1.1. Literature review on edible salts	x				
T1.2. Market study and sampling plan	x				
WP 2: Sampling of different types of salts					7950
T2.1. Sampling of salts	x	x			
WP 3: Chemical analysis for purity of salts					14200
T3.1. Analysis of major elements		x	x		
T3.2. Analysis of iodine		x	x		
WP 4: Chemical analysis for impurities of salts					56200
T4.1. Analysis of inorganic impurities		x	x		
T4.2. Semi quantitative analysis of organic contaminants by LC-HRMS and GC-MS		x	x		
T4.3. Target analysis of selected organic contaminants			x	x	
WP 5: Risk assessment					8800
T 5.1. Exposure estimation and risk assessment of impurities				x	
T 5.2. Policy recommendations				x	

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

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

Code	Task description	Contracting institution(s)	Required personnel (qualifications)	PM
WP 0	Coordination and project management			1.25
WP 1	Literature review and market survey			
T1.1	Literature review on edible salts	Sciensano - TE	SW1	1.25
T2.1.	Market study and sampling plan	Sciensano - TE, RHIA	SW1	0.5
WP 2	Sampling			
T2.1	Sampling of salts	Sciensano - TE	SW1	0.5
WP3	Chemical analysis for purity of salts			
T3.1	Analysis of major elements	Sciensano - TE	B	0.5
T3.2	Analysis of iodine	Sciensano - TE	B	0.5
WP4	Chemical analysis for impurities of salts			
T4.1	Analysis inorganic impurities	Sciensano - TE	B	1
T4.2	Semi quantitative analysis of organic contaminants by LC-HRMS and GC-MS	Sciensano - PEST	B	3
T4.3	Target analysis of selected organic contaminants	Sciensano - PEST	B	2
WP5	Risk assessment	Sciensano - RHIA		
T5.1	Exposure estimation and risk assessment of impurities	Sciensano - RHIA	SW1	
T5.2	Policy recommendations	Sciensano - TE	SW1	0.5

4.3 Collaboration, complementarity and/or parallel applications

The project will be conducted in collaboration between three services of Sciensano, TE, PEST and RHIA. The services TE and PEST have many years of experience in the analysis of contaminants and hold ISO 17025 accreditation for most analytical methods. The service RHIA has elaborate experience in assessing the risk and impact of exposure of contaminants on the public health. Overall the services have the knowledge and analytical equipment to successfully execute the project.

Important salt producers in Belgium (i.e. Zoutman N.V. and K+S Benelux) will be contacted to inform about this research proposal and seek possible collaboration by means of obtaining samples of salts that are supplied to the bakery and meat processing industry. Additionally, Federation of Large Bakeries of Belgium (FGBB), the Federation of Belgian Meat Processing Industry (Fenavian), and the EUSalt association will be also contacted to inquire if they can provide information on the types of salts that are most commonly used in the food industry (i.e. bakery and meat processing) and if salt samples could be provided for the analysis. The collaboration with such associations and federations will be useful in this proposal not only to obtain samples but also as channels to communicate the information and results of the project.

This project proposal was only submitted to the Contractual Research of the FPS Health, Food Chain Safety and Environment.

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

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]

5. BUDGETARY INFORMATION

5.1 Budgetary overview table

Type of cost	*TE* [redacted]	*PEST* [redacted]	*RHIA* [redacted]	Total per item
Staffing	€ 35,100	€ 25,520	€ 0	€ 60,620
Operational	€ 24,390	€ 13,928	€ 5,000	€ 43,318
General	€ 3,510	€ 2,552	€ 0	€ 6,062
Total per partner	€ 63,000	€ 42,000	€ 5,000	€ 110,000
Own contribution	€ 0	€ 0	€ 0	€ 0
% own contribution	0.00%	0.00%	0.00%	0.00%
FPS Grant	€ 63,000	€ 42,000	€ 5,000	€ 110,000
% FPS Grant	100.00%	100.00%	100.00%	100.00%

5.2 Detailed budget proposal

4.1.	**Staffing costs**						**€ 60,620**
		year	seniority in years	number of person-months	budget in euros		
4.1.1.	Sciensano - Trace Elements (TE)					35,100	
4.1.1.1.	SW10 [redacted]	2024	*1*	*2*	12,000		
		2025	*2*	*2*	12,400		
4.1.1.2.	B ([redacted]	2024	*4*	*2*	10,700		
4.1.2.	Sciensano - Pesticides and Environmental Contaminants (PEST)					25,520	
4.1.2.1.	B ([redacted]						
	Pay scale	2025	*5*	*5*	16,400		
4.1.2.2.	- double holiday pay				9,120		
4.2.	**Operational costs**						**€ 43,318**
4.2.1.	Sciensano - Trace Elements (TE)					24,390	
4.2.1.1.	Standard operational costs (flat-rate)				5265		
4.2.1.2.	Specific operational costs				19125		
4.2.1.2.1.	*purchasing salts*			*4125*			
4.2.1.2.2.	*element analysis*			*15000*			
4.2.2.	Sciensano - Pesticides and Environmental Contaminants (PEST)					13,928	
4.2.2.1.	Standard operational costs (flat-rate)				2552		
4.2.2.2.	Specific operational costs				11376		
4.2.2.2.1.	*Semi quantitative analysis of organic contaminants by LC-HRMS and GC-MS*			*7376*			
4.2.2.2.2.	*Target analysis of selected organic contaminants*			*4000*			
4.2.3.	Sciensano - Risk and Health Impact Assessment (RHIA)					5,000	
4.2.3.1.	Standard operational costs (flat-rate)				0		
4.2.3.2.	Specific operational costs				5000		
4.2.3.2.1.	*Costs related to exposure analysis*			*5000*			
4.3.	**General costs**						**€ 6,062**
4.3.1.	Sciensano - Trace Elements (TE)					3,510	
4.3.1.1.	Overheads (flat-rate)				€ 3,510		
4.3.2.	Sciensano - Pesticides and Environmental Contaminants (PEST)					2,552	
4.3.2.1.	Overheads (flat-rate)				€ 2,552		
TOTAL							**€ 110,000**

6. ADMINISTRATIVE INFORMATION

6.1 Proposal for a guidance committee

Title – First name – Name	Institution	E-mail
Dr. [redacted]	FAVV	[redacted]
Ir. [redacted]	FAVV-AFSCA-Section Sècuritè des Produits	[redacted]
Dr. [redacted]	FAVV-AFSCA-UNE-NOE	[redacted]
[redacted]	FPS HFCSE	[redacted]
Dr. [redacted]	FOD Volksgezondheid	[redacted]
Prof. Dr. [redacted]	KU Leuven	[redacted]
Prof. Dr. [redacted]	KU Leuven	[redacted]
Prof. Dr. [redacted]	ULiège	[redacted]
Prof. Dr. ir. [redacted]	UGent	[redacted]
Prof. Dr. [redacted]	University of Antwerp	[redacted]
Dr. [redacted]	VITO	[redacted]

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

Institution coordinator, promoter 2 and 3	Located at	Name representative institution coordinator	Position
Sciensano	Brussels	Dr. Christian Léonard	Director
		Name coordinator	Position
		Daniela Montalvo Grijalva	Scientist

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

Company registration number : [redacted]
IBAN : [redacted]
BIC : PCHQ BE BB
Name and address of the account holder : Sciensano, Juliette Wytsmanstraat 14, 1050 Elsene

7. BIBLIOGRAPHY

Carapeto, C., Brum, S., Rocha, M., 2018. Which table salt to choose? J Nutr Food Sci 8. https://doi.org/10.4172/2155-9600.1000701

Cepanec, K., Vugrinec, S., Cvetković, T., Ranilović, J., 2017. Potassium Chloride-Based Salt Substitutes: A Critical Review with a Focus on the Patent Literature. Comprehensive Reviews in Food Science and Food Safety 16, 881–894. https://doi.org/10.1111/1541-4337.12291

De Keyzer, W., Dofková, M., Lillegaard, I.T.L., De Maeyer, M., Andersen, L.F., Ruprich, J., Řehůřková, I., Geelen, A., van 't Veer, P., De Henauw, S., Crispim, S.P., de Boer, E., Ocké, M., Slimani, N., Huybrechts, I., 2015. Reporting accuracy of population dietary sodium intake using duplicate 24 h dietary recalls and a salt questionnaire. British Journal of Nutrition 113, 488–497. https://doi.org/10.1017/S0007114514003791

De Ridder, K., Teppers, E., 2016. Natrium (No. Rapport 4. WIV-ISP), In: Bel S, Tafforeau J (ed). Voedselconsumptiepeiling 2014-2015. Brussel, België.

Di Salvo, E., Tardugno, R., Nava, V., Naccari, C., Virga, A., Salvo, A., Corbo, F., Clodoveo, M.L., Cicero, N., 2023. Gourmet Table Salts: The Mineral Composition Showdown. Toxics 11. https://doi.org/10.3390/toxics11080705

Ercoşkun, H., 2023. Impurities of natural salts of the earth. Food Additives & Contaminants: Part B 16, 24–31. https://doi.org/10.1080/19393210.2022.2114016

European Commission, 2023. Commission Regulation (EU)2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, Official Journal of the European Union.

Fayet-Moore, F., Wibisono, C., Carr, P., Duve, E., Petocz, P., Lancaster, G., McMillan, J., Marshall, S., Blumfield, M., 2020. An Analysis of the Mineral Composition of Pink Salt Available in Australia. Foods 9. https://doi.org/10.3390/foods9101490

Hoge Gezondheidsraad, 2009. Voedingsaanbevelingen voor België (Herziening 2009) (No. nr. 8309). Brussel, België.

Hutton, T., 2002. Sodium Technological functions of salt in the manufacturing of food and drink products. British Food Journal 104, 126–152. https://doi.org/10.1108/00070700210423635

Infanger, E., Haldimann, M., 2016. Report on the composition of prevalent salt varieties (No. FSVO). Switzerland.

Iñiguez, M.E., Conesa, J.A., Fullana, A., 2017. Microplastics in Spanish Table Salt. Scientific Reports 7, 8620. https://doi.org/10.1038/s41598-017-09128-x

Joint FAO/WHO Codex Alimentarius, 1985. Standard for food grade salt. CODEX STAN 150-1985.

Karavoltsos, S., Sakellari, A., Bakeas, E., Bekiaris, G., Plavšić, M., Proestos, C., Zinelis, S., Koukoulakis, K., Diakos, I., Dassenakis, M., Kalogeropoulos, N., 2020. Trace elements, polycyclic aromatic hydrocarbons, mineral composition, and FT-IR characterization of unrefined sea and rock salts: environmental interactions. Environmental Science and Pollution Research 27, 10857–10868. https://doi.org/10.1007/s11356-020-07670-2

Malm, L., Palm, E., Souihi, A., Plassmann, M., Liigand, J., Kruve, A., 2021. Guide to Semi-Quantitative Non-Targeted Screening Using LC/ESI/HRMS. Molecules 26. https://doi.org/10.3390/molecules26123524

Rondelet, G., Cheyns, K., 2022. Determination of As, Asi, Cd, Pb, and Hg in Unrefined salts. Final Report on the 2022 Proficiency Test Organized by the NRL for TE in Food and Feed. (No. PT-2022-NRL-TE-FASFC.).

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying Small Molecules via High Resolution Mass Spectrometry:

Communicating Confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105
Serrano, R., Nácher-Mestre, J., Portolés, T., Amat, F., Hernández, F., 2011. Non-target screening of organic contaminants in marine salts by gas chromatography coupled to high-resolution time-of-flight mass spectrometry. Talanta 85, 877–884. https://doi.org/10.1016/j.talanta.2011.04.055
Superior Health Council, 2014. Strategies to increase iodine intake in Belgium (No. No. 8913).
Vandevijvere, S., 2012. Sodium reduction and the correction of iodine intake in Belgium: Policy options. Archives of Public Health 70, 10. https://doi.org/10.1186/0778-7367-70-10
Vandevijvere, S., Ruttens, A., Wilmet, A., Marien, C., Hautekiet, P., Van Loco, J., Moreno-Reyes, R., Van der Heyden, J., 2021. Urinary sodium and iodine concentrations among Belgian adults: results from the first national Health Examination Survey. European Journal of Clinical Nutrition 75, 689–696. https://doi.org/10.1038/s41430-020-00766-5
Vromman, V., Waegeneers, N., Cornelis, C., De Boosere, I., Van Holderbeke, M., Vinkx, C., Smolders, E., Huyghebaert, A., Pussemier, L., 2010. Dietary cadmium intake by the Belgian adult population. Food Additives & Contaminants: Part A 27, 1665–1673. https://doi.org/10.1080/19440049.2010.525752
WHO, 2012. Guideline: Sodium intake for adults and children. World Health Organization (WHO), Geneva.

On behalf of the consortium,

Friday, 22nd of September2023

[redacted]

Coordinator of the consortium

[redacted]