Novel foods as red meat replacers – an insight using Risk Benefit Assessment methods (the NovRBA project)

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Novel foods as red meat replacers – an insight using Risk Benefit Assessment methods (the NovRBA project)

Androniki Naska¹, Ermolaos Ververis¹, Aikaterini Niforou¹, Sara Monteiro Pires², Morten Poulsen², Lea S. Jakobsen², Nina Becker³, Mark Lohmann³, Vincent Tesson¹, Michel Federighi⁴ and Géraldine Boué⁴

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Abstract

The project entitled “Novel foods as red meat replacers – an insight using Risk Benefit Assessment methods (NovRBA)” aimed to develop and test harmonised Risk Benefit Assessment (RBA) methods to estimate the overall health impact of replacing red meat with an edible insect species (a novel food). Based on an appraisal of insect products that are more likely to be consumed in Europe, project participants decided to compare the consumption of a beef patty consisting of 100% minced beef meat (reference scenario) with a patty in which beef meat would be fully replaced by an edible insect dough (alternative scenario). The target population was adults. The RBA steps included the problem definition, the identification, prioritization and selection of components together with associated health outcomes. The assessment included the selection of dose-response relationships based on their internal validity (hazard characterisation) and an exposure assessment of nutrient intake and exposures to microbiological hazards and compounds of toxicological concern associated with the reference and alternative scenarios. All health outcomes were quantified according to the disability-adjusted life years (DALYs) composite metric. The project standardised the pivotal step of selecting model components through developing a tiered approach to prioritise components establishing the “long”, the “short” and the “final” list. The final list comprised thirteen nutrients, two heat-resistant spore-forming bacteria and inorganic arsenic. The RBA model was developed using @Risk® add-in software using Monte Carlo simulations considering variability and/or uncertainty. Overall the expected change in DALYs when moving from the reference scenario to the alternative one was estimated to be around 8,753 DALYs (per 100,000 population) saved in Greece, 6,572 DALYs in Denmark and 21,972 DALYs in France. This is mainly due to the overall beneficial nutritional and microbiological impacts. The proposed actions to communicate the project’s findings can be summarised in providing understandable scientific evidence to policy makers; creating opportunities for consumers to engage in-depth with information about insect consumption; making use of multipliers who enjoy a high level of trust and establishing informational exchange with trusted sources.

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Key words: Risk benefit assessment; Acheta domesticus; Edible insects; Beef meat; DALYs; Communication.

Question number: EFSA-Q-2022-00244

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Ermolaos Ververis is employed with EFSA but was part of the project’s Consortium solely in his capacity as a PhD student of the School of Medicine of the National and Kapodistrian University of Athens.

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Summary

In March 2018, the EFSA-supported partnering grant under the title "Novel foods as red meat replacers – an insight using Risk Benefit Assessment methods (NovRBA)" was launched with the aim to estimate the overall health impact of replacing red meat with a novel food, using Risk Benefit Assessment (RBA) methods. The project's primary objective was to perform a RBA through advancing and harmonising the existing RBA methodological framework with regard to the selection of the components to be considered in the assessment. Moreover, the project aspired to propose strategies to communicate the RBA results, using the replacement of red meat by an edible insect species as a case study.

After reviewing guideline and report documents relevant to the safety aspects of insects and products thereof published by competent authorities of EU Member States, 24 insect species that are more likely to be consumed in Europe were identified. The selection of the insect species to be considered in this case-study was based on two criteria related to the product's potential in the EU market and the availability of scientific publications with complete and reliable data on composition and related manufacturing processes. Taking into consideration the outcome of this appraisal as well as evidence on consumers' preferences and attitudes towards meat and insect eating, the project Consortium decided to focus on beef meat and Acheta domesticus (A. domesticus). In particular, project participants agreed to compare the consumption of a beef patty consisting of 100% minced beef meat with a patty in which beef meat would be fully (100%) replaced by a preparation based on an edible insect dough (A. domesticus powder to water ratio of 35:65). The target population was proposed to be adults (18 years old and over) as young adults are expected to be more willing to taste the product and systematic reviews on associations between diet and health primarily refer to adult populations.

To perform the RBA the NovRBA project followed the methodological approach developed under the RiskBenefit4EU project. The first step included the problem definition that aimed to formulate the risk-benefit question and define the two scenarios (reference and alternative) to be tested. The subsequent critical steps included the identification, prioritization and selection of components and their respective associated health outcomes. For each pair of “component-health outcome”, individual risk and/or benefit assessments were performed. This included the exposure assessment that aimed to estimate the intake of nutrients and the exposures to microbiological hazards and compounds of toxicological concern associated with the reference and alternative scenarios. It considered food intakes and the concentration of components in both foods. In parallel, dose-response relationships and associated inputs (e.g. measures of risk) were selected on the basis of critical appraising their internal validity and were analysed to estimate the probability or number of cases to be increased or decreased when comparing the alternative scenario with the reference one. Finally, all health outcomes were quantified according to the disability-adjusted life years (DALYs) composite metric.

In order to standardise the pivotal step of selecting model components and attribute similar importance to each field (nutrition, microbiology and toxicology), the NovRBA project developed and tested a 3-step tiered approach to prioritise the nutrients, microbiological agents and compounds of toxicological concern of minced beef and A. domesticus powder. The approach builds on assessments regarding exposure to microbiological hazards and relies on a similar framework being applied in all domains (nutrition, microbiology and toxicology). It follows the same steps, but with adaptation of criteria definitions to serve principles of these different domains. In practice, it included the establishment of three lists: the "long", the "short" and the "final" list. The long list of components was assembled based on an exhaustive literature review, data available from national food composition databases, and data from national food safety authorities. The short list was compiled on the basis of a set of standardized criteria taking into account data on the occurrence and the severity of the associated outcome combined with an additional set of criteria specific to nutrition, microbiology and toxicology. Food components included in the short list were further reviewed for inclusion in the final list primarily on the basis of available dose-response data for each "component-health outcome" pair. Finally, the final list comprised of thirteen nutrients, two spore-forming bacteria that are resistant to heat treatment and...
inorganic arsenic. Monte Carlo simulations were used to capture the variability by randomly selecting levels in concentration distribution and multiplying with reported levels of food intake (or their associated substitute estimate with insect powder). For each of the identified health outcomes, values of DALYs per case estimated on the basis of disease incidence were collected from the literature. When available, country-specific values were obtained. The RBA model was developed using Excel and the @Risk® add-in software for probabilistic modeling (Microsoft Excel version 7.6, Palisade Corporation, USA). The probabilistic model used Monte Carlo simulations with some parameters being described as distributions to consider variability and/or uncertainty.

The overall health impact was estimated in DALYs separately for each country, taking into account differences in each country’s size of the adult population, their national food intake values and their current incidence of diseases. The expected change in DALYs when moving from the reference scenario to the alternative one was estimated to be around 8,753 DALYs (per 100,000 population) saved in Greece, 6,572 DALYs (per 100,000 population) in Denmark and 21,972 DALYs (per 100,000 population) saved in France. This is mainly due to the overall beneficial nutritional and microbiological impacts.

In the context of NovRBA project, a review of the literature on risk perceptions and acceptance of beef and insects as foods was also undertaken in order to identify challenges related to the communication of the project results. The key findings on the consumption of edible insects were based on disgust, familiarity, and food neophobia state, processed vs. unprocessed forms of insects, contextual information, cultural differences and social norms. Socio-demographic disparities in attitudes and perceptions were also observed. An effort to communicate the results of the RBA case study should note that any communication must avoid triggering animal reminder disgust or increasing risk perception by evoking associations with live animals (either through visual or text information). The proposed communication actions can be summarised in the following: (a) to provide understandable scientific evidence about insect consumption to policy makers; (b) to create opportunities for consumers to engage in-depth with information about insect consumption; (c) to make use of multipliers who enjoy a high level of trust; (d) to establish informational exchange with trusted sources and (e) to inform industry about animal reminder disgust.

Overall, the NovRBA project has advanced previous assessments in the field of risk benefit assessment in the food sector through progressing specific steps of the process towards tailored and harmonized criteria for the selection of model components that have the potential to be adapted to other foods and risk-benefit questions, food consumption scenarios, countries and populations. It also allows for the inclusion of different components, while ensuring that assessments are done in a documented, standardized approach. Such harmonization will be crucial for the comparison of results across questions and countries.
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1. Introduction

Dietary choices are driven by multiple factors including personal preferences and ethical values, societal norms, changes in food availability, developments in products’ innovation, policy measures, as well as nutrition education. Meat consumption, for instance, is fundamental in the diet of several cultures worldwide with meat providing an array of nutrients necessary for growth and health maintenance. Concurrently, several epidemiological studies and their meta-analyses provide evidence that high consumption of red meat and especially of its processed products increases the risk of chronic diseases, including cardiovascular disease (CVD), colorectal cancer and type II diabetes (Barnard et al. 2014, Feskens et al. 2013, Larsson and Wolk 2006, Micha et al. 2010, Bouvard et al. 2015). Moreover, meat (including red meat) accounts for more than 10% of total European foodborne outbreaks (EFSA, 2015) and recent changes regarding its handling and consumption (e.g. longer storage before consumption, the increasing preference of consuming raw meat) will probably lead to new challenges in the products’ microbiological safety (ANSES, 2017a). Lastly, studies on the environmental impact of meat production raise concerns that could also shape consumers’ preferences (Steinfeld et al., 2006; Macdiarmide et al., 2016).

The health-related, environmental and ethical considerations of the public regarding red meat consumption have led to launching new products that could reduce and even replace red meat in the daily diet of the populations. Among the potential replacers are insects and products thereof (Shockley and Dossey, 2014), which fall under the provisions of the Regulation (EU) 2015/2283 on novel foods. Recently, the European Food Safety Authority (EFSA), the sole EU entity responsible for carrying out the safety assessment of novel foods (Ververis et al., 2020), has published four scientific outputs on the safety of insect-derived food products (EFSA NDA Panel, 2021a;2021b,2021c;2021d). The authority concluded that the assessed insect-derived novel foodstuffs, among which frozen and dried yellow mealworms, grasshoppers and house crickets, are safe to be consumed under the proposed uses and use levels.

Insects and insect-containing foodstuffs may contain various nutrients, as well as compounds of possible concern to human health (Belluco et al., 2013; Van der Spiegel et al., 2013). Consumption of insects may contribute to the supply of certain nutrients (e.g. polyunsaturated fatty acids (PUFAs), vitamins and minerals) (Rumpold and Schlüter, 2013). Compounds of possible concern can be anti-nutrients, i.e. naturally occurring substances which can impair nutrient absorption, and contaminants (EFSA NDA Panel, 2021a;2021b,2021c;2021d). It has been reported that certain insect species are able to bioaccumulate contaminants (e.g. heavy metals) in their tissues (Devkota and Schmidt, 2000; Vijver et al., 2003). Moreover, the occurrence of endogenously-produced hazardous compounds, such as cyanogenic glucosides (Zagrobelny et al, 2009) or benzoquinones (Gao et al., 2018) is also possible. Furthermore, insects can be a vector of food-borne human disease agents and a substrate for the growth of pathogenic micro-organisms (van Huis et al., 2013; EFSA, 2015; Kooch et al., 2019).

The potential replacement of red meat with an insect species bears multiple challenges since the simultaneous reduction of red meat consumption and the introduction of insects, a novel food source, in the diet can be linked with both adverse and beneficial health effects. An appropriate approach to investigate the impact of such a dietary shift is Risk-Benefit Assessment (RBA). RBA in the field of food and nutrition is an emerging decision-support tool that aims to evaluate risks and benefits in a harmonised approach in order to estimate the overall health impact of exposure (or lack of exposure) to a particular food, food component, and diet, as well as the substitution of one food item by another (Nauta et al., 2018; Membré et al., 2021). Towards this goal, RBA integrates knowledge on nutrition, toxicology, microbiology, chemistry and epidemiology for comprehensive health impact assessments (Assunção et al., 2019; Boué et al., 2015; Pires et al., 2019). The holistic approach that a RBA can offer could be a useful supplement to the existing safety evaluation standards and its’ integration into assessment methods will advance future risk assessments (Verhagen et al., 2021).

1.1. Background and Terms of Reference as provided by the requestor

In March 2018, the EFSA-supported project entitled “Novel foods as red meat replacers – an insight using Risk Benefit Assessment methods (NovRBA)” was launched with the aim to estimate the overall health impact of...
replacing red meat with a novel food, using the RBA methodology. The project’s primary objective was to perform a RBA through advancing and harmonising the existing RBA methodological framework with regard to the selection of the components to be considered in the assessment. Moreover, the project aspired to propose strategies to communicate the RBA results, using the replacement of red meat by an edible insect species as a case study.

To achieve these goals, the NovRBA project was segregated into the following sub-objectives:

- To identify an edible insect species which could function as a red meat replacer
- To identify both potentially hazardous, as well as the beneficial components of red meat and an edible insect species, based on information on their nutrient, microbiological, and toxicological profiles, through reviewing original research work, food composition databases, systematic reviews and meta-analyses
- To characterise hazards and benefits by identifying health outcomes related to a possible replacement of red meat by edible insects, based on the collected evidence about beneficial and hazardous components of both foodstuffs
- To perform the risk-benefit assessment of the dietary substitution, by designing and implementing RBA models which will integrate the exposure assessment of the consumers and will enable the estimation of the overall health impact of red meat replacement by the identified insect species
- To develop content and tools to communicate the findings of the RBA analysis to the general public.

The work undertaken in the context of the NovRBA project has been organised in the following Work Packages (WP):

WP1: To identify edible insects to replace red meat
WP2: To define the compositional profiles of red meat and its replacer
WP3: Individual hazards and benefit characterisations
WP4: Exposure estimates and Risk-benefit assessment
WP5: Communication of project’s activities and findings
WP6: Project management

This grant was awarded by EFSA to the: Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece

Beneficiaries: The Consortium is composed of the National and Kapodistrian University of Athens (coordinator - contact person Prof. Androniki Naska), the French National Research Institute for Agriculture, Food and the Environment/INRAE, France, (contact person Assoc. Prof. Géraldine Boué), the National Food Institute/DTU (contact persons Prof. Sara Monteiro Pires and Prof. Morten Poulsen), and the German Federal Institute for Risk Assessment (contact person: Dr. Mark Lohman).

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Grant number: GP/EFSA/ENCO/2018/03 – GA01
2. Data and Methodologies

2.1. Identification of the food items

2.1.1. Selection of insect species

Approximately 2000 insect species are consumed by various cultures worldwide (Jongema, 2017). For the identification of the insect species to be used in the substitution scenario, given the plethora of potential candidates, guideline and report documents relevant to safety aspect of insects and products thereof as food published by EU Member States (MS), were used as initial points of reference. Such documents have been published by the competent authorities of Belgium (FASFC, 2014), the Netherlands (NVWA,2014), France (ANSES, 2015), Finland (EVIRA,2017), Austria (BMGF,2017) and Spain (AECOSAN, 2018). Indicatively, according to the Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition on the microbiological and allergenic risks associated with the consumption of insects published in 2018 (AECOSAN, 2018), 24 insect species that are more likely to be consumed in Europe were identified. Additionally, in its publication “Risk profile related to production and consumption of insects as food and feed”, EFSA presented examples of insect species known to be farmed on commercial basis both within and outside the European Union (EFSA, 2015).

The selection of the insect species to be considered in this case-study was based on two main criteria comprised of the specific sub-criteria listed below. Any other relevant information retrieved that could not fall under these categories was classified as “other”.

Criterion 1: The product’s potential in the EU market

Sub-criteria

1. Prior consumption in EU countries

Based on different interpretations by the EU Member States (MS) of Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients, food products being or containing whole insects have been placed in the markets of certain EU MS and consumed by EU citizens prior to the initiation of the project.

2. Commercial potential in the EU

The availability of foodstuffs containing insects as ingredients in the markets of some EU MS could indicate countries with experience in farming and processing insect species and producing foodstuffs containing insects as ingredients. However, according to the Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods which came into force in 2018 and repealed Regulation (EC) No 258/97, insects and products thereof need to be authorized, after a positive assessment by EFSA on their safety, before being placed on the market. Therefore, possibly higher EU market potential have those insect species that have been already produced and consumed in certain EU countries.

3. Food technological potential

Edible insects are a relatively new scientific area that offers opportunities for food innovation and research. Attempts to investigate the rheological, textural, and structural properties of insect preparations, as well as the use of insect preparations to fortify and improve the nutritional and technological properties of other foodstuffs have been reported in the literature. Insect species already studied under this scope, may offer a better prospective in the Research & Development (R&D) food sector.

4. Sensorial aspects

The availability of studies on the sensorial attributes of edible insects is an important indicator of the commercial potential of an insect species.
2.2. Definition of the RBA scenario and formulation of the question

Discussions during the NovRBA project kick-off meeting (March 2019) took into consideration the findings of a representative consumer survey on the safety of edible insects, which was undertaken by the BfR Dept. of Risk Communication. According to the survey findings, approximately 14% of all respondents reported consuming insects in the past, mainly abroad. The majority of them were males aged 18 to 39 years with a high level of education. In addition, 34% of respondents identified the high protein content as a reason for consuming insects and recognised dislike as the biggest obstacle to the introduction of insects as foods. A report of the survey’s findings are available at https://www.bfr.bund.de/cm/364/insects-as-foods-and-feeds.pdf. Project participants reached the following decisions:

- To focus on beef meat, so as to include a type of meat broadly consumed in European countries by all age groups. Furthermore, excessive meat production and consumption, particularly of beef, is taking a toll on the environment (Eshel et al., 2014; Poore and Nemecek, 2018; Saget et al., 2021). Compared to other farmed animals, the production of 1 kg of beef has the highest global warming potential, and requirements regarding the use of land and energy (de Vries and de Boer, 2010). Considering the information above, the project’s Consortium decided to use beef in the substitution scenario. Beef comprises various cuts and forms; the minced beef was selected.

- To consider an insect preparation as a food ingredient (not in visible form), since consumers indicated that they would preferably consume insects if they could not distinguish them in the final product. The issue of product matrix was subsequently considered, since it relates to the risk profile of the foodstuff.

- To take up a case in which the product matrix remains unchanged and the only change in the product is the replacement of beef meat with the insect species.

- To study beef patties, such as those included in burgers, taking into consideration the finding of the BfR survey that young adults (18–39 years) were more frequently responding positively to the prospect of consuming edible insects.

Hence, the project participants agreed to compare the consumption of a beef patty consisting of 100% minced beef meat with a patty in which beef meat would be fully (100%) replaced by a preparation based on an...
edible insect dough. The insect dough would have an A. domestica powder to water ratio of 35:65, according to experiments undertaken in the laboratory of INRAE.

To answer this question, we defined two scenarios:

- **Baseline/Reference scenario**: actual consumption of minced beef patty in each country (g/day) (100% beef)

- **Alternative scenario**: consumption of patty in which beef meat would be fully (100%) replaced by a preparation based on an edible insect powder

In particular, in the reference scenario patties would weigh 150g including from 100g of minced beef meat and 50g of other ingredients up to 150g of minced beef meat only; and in the substitution scenario the minced beef part was replaced by 100g of rehydrated insect powder, consisting of 35g of insect powder and 65g of water.

The target population was proposed to be adults (18 years old and over) and young adults in particular as they are expected to be more willing to taste the product. In addition, since evidence from dose-response meta-analyses on associations between diet and health was primarily referring to adult populations, the project participants decided to focus on individuals aged 18 years and over.

**2.3. Stepwise approach of the Risk-Benefit assessment**

The RBA builds on the classic steps of the risk assessment framework, i.e. 1) hazard identification, 2) hazard characterization (dose-response assessment), 3) exposure assessment, and 4) risk characterization (WHO, 2010). The RBA includes multiple individual assessments of risks and benefits in the areas of nutrition, microbiology, and toxicology. These assessments are aggregated in a broader approach that aims to ultimately estimate the overall health impact through the comparison of different pre-defined consumption scenarios.

The NovRBA project followed the methodological approach presented by Assunçao et al. (2019) developed under the RiskBenefit4EU project (Figure 1). Briefly, the first step includes the problem definition that aims to formulate the risk-benefit question. This formulation includes defining the target population (general or a sub-population), the level of aggregation (food component, food or diet) and the type of assessment (qualitative or quantitative) (Boobis et al., 2013; Nauta et al., 2018). Then, the scenarios are defined, i.e. the description of hypothetical or real situations for which the health impact will be assessed. These two steps set the general frame and scope of the RBA. Consequently, a critical step is the identification, prioritization and selection of components and their respective associated health outcomes (effects). The inclusion of each component or health outcome will influence the final health impact estimated.

For each pair of “components-health outcomes”, individual risk and/or benefit assessments are performed. This includes the exposure assessment that aims to estimate the intake of nutrients and the exposures to microbiological and chemical hazards associated with the reference and alternative scenarios. It considers food intakes and the concentration of components in both foods. In parallel, dose-response relationships and associated inputs (e.g. measures of risk) are collected, selected and analysed in order to estimate the probability or number of cases to be increased or decreased when comparing alternative scenarios with the reference one. Finally, all health outcomes are quantified according to a comparable population-based composite metric, the disability-adjusted life years (DALYs) which can allow a quantitative comparison of scenarios assessed.
Figure 1. The risk-benefit assessment stepwise approach (Source: Assunçao et al., 2019)
2.4. Nutrient, microbiological and toxicological profiles of *Acheta domesticus* and beef meat

2.4.1. Nutrient, microbiological and toxicological profile of *Acheta domesticus*

A systematic search was undertaken in PubMed, Science direct, Scopus, Web of Science (all collections), websites of competent authorities and organisations (e.g. EFSA, Food and Agriculture Organization-FAO) through October 2021, for studies providing original data on the nutrient, microbiological and toxicological and profile of *A. domesticus*. The key search terms used in each database are listed in Table 1.

<table>
<thead>
<tr>
<th>Database</th>
<th>Search keywords</th>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>(Acheta domesticus) OR (Acheta domestica) OR (House cricket)</td>
</tr>
<tr>
<td>Science direct</td>
<td>(&quot;Acheta domesticus&quot;) OR (&quot;house cricket&quot;) OR &quot;Acheta domestica&quot;</td>
</tr>
<tr>
<td>Scopus</td>
<td>(Acheta AND domesticus) OR (Acheta AND domestica) OR (house AND cricket) in &quot;Article title, Abstract, Keywords&quot;</td>
</tr>
<tr>
<td>Web of Science- all collections</td>
<td>(Acheta AND domesticus) OR (House AND cricket) OR (Acheta AND domestica); in “topic”</td>
</tr>
<tr>
<td>Websites of competent authorities (search engine of each website)</td>
<td>Acheta domesticus, Acheta domestica, house cricket</td>
</tr>
</tbody>
</table>

After removal of duplicates, the remaining articles were screened by two project participants with the assistance of a third one to reach consensus when needed. Publications were selected for full-text screening if they contained quantitative compositional data on whole *A. domesticus* (adult stage, nymphs): nutrient profile (e.g. protein, fat, vitamins, minerals, ash, carbohydrates), microbiological and toxicological profile (hazards, risks, adverse health effects- linked to specific substances or to the consumption of the specific food). No restrictions were applied regarding the language and the publication year. Articles referring only to other insect species, to an irrelevant life stage of the insect or presenting results after the fortification of the insects’ diet (gut loading) were excluded.

In accordance to the RBA substitution scenario (rf. Section 2.2), the data extracted were primarily referring to oven dried *A. domesticus* (whole or in the form of powder). The powder produced through oven-drying is preferred since it currently is the most used type of insect powder in the industry (Bassett et al 2021).

2.4.2. Nutrient, microbiological and toxicological profile of beef meat

Data available through EFSA databases, food composition national databases as well as information provided by national food safety agencies were retrieved and reviewed. The nutrient composition of minced beef meat (rf. RBA scenarios described in Section 2.2), was retrieved from the Danish and French Food Composition Tables (Frida 2019, ANSES 2020). Because of the lack of data on the composition of beef available to Greek consumers, the Danish and French values were used.
2.5. Selection of model components and related health outcomes

Due to the fact that the RBA case-study investigates the substitution of foods, we firstly examined the possibility to include the whole foods (i.e. beef and A. domesticus) per se in the RBA model. Our initial goal was to find evidence for dose-response relationships between the intake of these specific food items and health outcomes, as this is a crucial element in the model building process. Nevertheless, epidemiological studies linking specific health effects to the consumption of beef in a dose-dependent manner were limited.

![Figure 2: Principles of the NovRBA process for selecting model components](image)

With respect to A. domesticus, such studies are simply non-existent. Hence, the project Consortium decided to proceed to a food component-based approach.

Regarding the selection of model parameters, the same strategy with the same criteria of selection of pairs of “components - health outcomes” should have been applied to achieve the best level of harmonization between all disciplines. This approach was however not feasible since all disciplines have their own specificities.

In order to standardise the selection process and attribute similar importance to each field, the NovRBA project developed and tested a 3-step tiered approach to prioritise the components (nutrients, microbiological agents and compounds of toxicological concern) of the two food items under assessment. This approach builds on assessments regarding exposure to microbiological hazards and has been adapted to serve criteria applicable to the other disciplines (Figure 2). It relies on a similar framework being applied in all domains (nutrition, microbiology and toxicology) following the same steps, but with adaptation of criteria definitions to serve principles of these different domains. In practice, this included the establishment of three lists: the “long”, the “short” and the “final” list (Figure 3).

The long list of components was assembled based on an exhaustive literature review, data available from national food composition databases, and data from national food safety authorities. Individual working groups (WG) were established in each domain (nutrition, microbiology and toxicology). The WG participants further applied a set of standardized criteria taking into account data on the occurrence and the severity of the associated outcome, as well as an additional set of criteria specific to nutrition, microbiology and toxicology in order to compile the short list. Food components included in the short list were further reviewed for inclusion
in the final list of components to be considered for the RBA model (Figure 4). The standardised criteria were discussed and pilot-tested in various project meetings. It is important to note that the final list, which is usually the one communicated in RBA studies, is the list of components and health outcomes that can be included into the model, whereas the short list is the one including all the component-health outcome pairs which have been identified as important to be evaluated with respect to the level of evidence. The elements missing from the final list are therefore essential and should also be communicated with the results obtained.
Figure 3: Description of the steps applied for the selection of components and related health outcomes in the areas of nutrition, microbiology and toxicology
Figure 4: Schematic presentation of the standardised process followed to select the food constituents (nutrient, microbiological and chemical hazards) to be considered in the RBA model.
2.5.1. Selection of nutrients to be considered in the RBA model

For the identification of nutrients to be considered in the model, a 3-step tiered approach was followed. All food components (nutrients, microbiological agents and chemical hazards) identified through the methodology described below are displayed in Tables 2-4 (Section 3.3).

- Tier 1: Assembling the long list

The long list includes all nutrients which were present in one foodstuff, but not in the other. Food components which were present in both foods were included in the long list only if the difference between the two concentration levels was, in absolute terms, higher than 20%. The cut-off of 20% difference was selected on the basis of an acceptable 10% deviation due to analytical errors (method of analysis, sampling etc.) and a minimum significant 10% difference when various foodstuffs are compared.

- Tier 2: Compiling the short list

For the selection of food components that would be prioritised for inclusion in the short list, the project Consortium developed, pilot-tested and finally applied a standardised grading system, based on two criteria. Criterion 1 relied on occurrence data and criterion 2 on public health considerations. Each nutrient received a grade estimated by the product of its score in each criterion. Hence:

\[
\text{INDEX of prioritization for inclusion in the short-list} = \text{Score in Criterion 1} \times \text{Score in Criterion 2}
\]

where:

**Criterion 1**

**Occurrence** = Concentration in raw material \( \times \) Effect of cooking on concentration

- Concentration in raw material (minced beef & insect powder).

A nutrient (e.g. protein) received a score of:

3: if all samples analysed contain the component (irrespective of concentration level)

2: the presence of the component was reported in some samples OR there is uncertainty regarding the presence of this component. Uncertainty may for instance exist due to sampling, farming practices, or source of data. Thus, data from national repositories were considered more robust than analysis reported in one single article.

1: the component is rare, i.e. reported to be found in <10% of the samples

- Effect of cooking on concentration (if data refer to raw insects, the effect of the powder’s production process is also taken into account)

A food component (e.g. protein or a vitamin) received a score of:

3: if cooking had no or only a mild impact on concentration levels (reduction <10%)

2: if cooking had a medium impact on concentration levels (10% < reduction <80%)

1: if cooking had a high impact on concentration levels (80% < reduction)

The percentages above are based on expert judgement and aim to capture extreme changes in occurrence levels.

**Criterion 2:**

Public Health Nutrition considerations = Food policy measures \( \times \) Contribution to intake \( \times \) Effects on bioavailability
Food Policy measures

Judgements relied on the importance of the nutrient in the formulation of national and/or regional food-based dietary guidelines. In addition, national food fortification measures (e.g. foods fortified with this nutrient as a measure to promote public health) were also taken into account. A nutrient received the score of:

3: if it was considered in the formulation of food-based dietary guidelines OR was included in food fortification measures in all three countries (Denmark, France and Greece)

2: if it was considered in the formulation of food-based dietary guidelines OR was included in food fortification measures in some but not all participating countries

1: if it was not considered in the formulation of food-based dietary guidelines OR was not included in food fortification measures in any country

Contribution to intake

This component aims to consider the contribution of the foodstuff (i.e. minced beef meat or *A. domesticus* powder) to the intake of this nutrient in the population under study (layer 1). When there was no information on the average contribution of the food to the nutrient’s intake, differences in the composition of beef and *A. domesticus* powder regarding this nutrient were taken into account (layer 2).

**Layer 1**: A nutrient received the score of:

3: if the food item is a principal source of this nutrient at population level

2: if the food item is a possible source of this nutrient at population level, but not the principal one

1: if the food item was not a source of this nutrient for the population under study

**Layer 2 (when the application of layer 1 was not feasible)**: A nutrient received the score of:

3: if difference in composition between minced beef meat and *A. domesticus* powder was ≥75%

2: if 35%≤ difference in composition between minced beef meat and *A. domesticus* powder < 75%

1: if 20%< difference in composition between minced beef meat and *A. domesticus* powder <35%

Effects on bioavailability, taking into consideration whether the food matrix (beef patty or cricket powder patty, RBA scenarios described in Section 2.2) is expected to reduce the nutrient bioavailability

A nutrient received the score of:

3: when the food matrix would not or only mildly reduce the bioavailability of the nutrient assessed (reduction< 10%)

2: when the food matrix would moderately reduce the bioavailability, or there was uncertainty on whether it would reduce the bioavailability (10% <reduction < 80%)

1: when the food matrix is expected to reduce significantly the nutrient bioavailability significantly (80%< reduction)

Tier 3: From the short to the final list

The availability of composition data regarding the assessed nutrient for both foods under study (beef meat and *A. domesticus* powder) was the first to define whether a nutrient would be considered in the final list of model components. For nutrients which were prioritised using this criterion, the project Consortium proceeded to a search of the literature for dose-response meta-analyses evaluating associations between nutrient intakes and health outcomes (feasibility criterion).
The subsequent condition for a nutrient to be included in the long list relied on the availability of dose-response data regarding associations with health outcomes.

2.5.2. **Selection of compounds of toxicological concern to be considered in the RBA model**

- **Tier 1: Assembling the long list**

  All data on chemical hazards potentially present in *A. domesticus* powder and minced beef were taken into consideration, including the recently published EFSA Opinion on the safety of frozen and dried formulations from *A. domesticus* as a novel food (EFSA NDA Panel, 2021c).

- **Tier 2: Compiling the short list**

  The two criteria relied on occurrence data and on the impact of the associated health outcomes. Similar to nutrients, each chemical received a grade estimated by the product of its score in each criterion. Hence:

  \[
  \text{INDEX of prioritization for inclusion in the short-list} = \frac{\text{Score in Criterion 1} \times \text{Score in Criterion 2}}{\text{Score in Criterion 1} + \text{Score in Criterion 2}}
  \]

  where in the case of chemical hazards:

  **Criterion 1**

  **Presence and exposure relative to reference doses (occurrence)**

  - **Presence relative to reference doses**

    A chemical hazard received a score of:

    3: if the concentration of the agent in the food is above the limit of detection/limit of quantitation (LOD/LOQ) AND for non-genotoxic carcinogens, the total exposure to the contaminant was exceeding the health based guidance value (HBGV), whereas for genotoxic carcinogens the MoE was below 10,000.
2: if the concentration of the agent in the food is above LOD/LOQ AND for non-genotoxic carcinogens, the total exposure to the contaminant was not exceeding the HBGV, whereas for genotoxic carcinogens the MoE was above 10,000.

1: Concentration of the agent in the food is below LOD/LOQ.

- **Contribution to total exposure**

A chemical hazard received the score of:

3: if the food is a major source of exposure (>50 %)
2: if the food is a significant source of exposure (10-50 %)
1: if the food is a minor or not a source of exposure (<10 %)

The score in criterion 1 was estimated as the multiplication of the two sub-scores on the presence and exceedance of reference doses of the contaminant and its contribution to total exposure.

**Criterion 2**

**Impact of associated health outcomes (severity)**

This criterion considers the severity of the health outcomes associated with the exposure to the chemical hazard. The severity is evaluated qualitatively and considers the input parameters for the DALYs. The score is based on how many of the following statements hold true:

a. The incidence of the health outcome is high in the population under study
b. The health outcome is fatal
c. The health outcome is associated with lifelong disability
d. The health outcome is associated with a high disability (rounded Disability weight (DW) > 0.4)

If the grade following points a to d is low, the agent will not be considered.

An agent receives a grade of:

3: if 3 or more of the statements above hold true
2: if two of the statements above hold true
1: in any other case

Finally, for the estimation of the overall grade and the decision on whether a chemical hazard will be forwarded to the short list, the score in criterion 2 was doubled so that criteria 1 and 2 contribute equally.

- **Tier 3: From the short to the final list**

Parallel to nutrients, the final list for chemical hazards was based on the availability of dose-response data/models.

### 2.5.3. Selection of microbiological hazards to be considered in the RBA model

- **Tier 1: Assembling the long list**

All data on microbiological agents in *A. domesticus* powder and minced beef were taken into consideration.

- **Tier 2: Compiling the short list**
In an approach similar to the one applied in the case of nutrients and chemical hazards, the two criteria considered relied on occurrence data and on the impact of the associated health outcome. Each microbial hazard received a grade estimated by the product of its score in each criterion. Hence:

**INDEX of prioritization for inclusion in the short-list =**

\[
\text{Score in Criterion 1} \times \text{Score in Criterion 2}
\]

where:

**Criterion 1**

**Presence in raw food and process effect (occurrence)**

- **Presence in raw food**
  
  A microbiological hazard received a score of:
  
  3: if the presence is very frequent (>$50\%$)
  2: if the presence is frequent ($10\%$ to $50\%$)
  1: if the presence is rare ($1\%$ to $10\%$)

- **Process effect**
  
  A microbiological hazard received the score of:
  
  3: if the process frequently introduces the hazard or cannot eliminate it when present
  2: if the process rarely introduces the hazard or can only partly eliminate it when present
  1: if the process does not introduce the hazard or eliminates it when present

The score in criterion 1 was estimated as the multiplication of the two sub-scores on the presence of the microbiological hazard in raw food and the process effect.

**Criterion 2**

**Impact of associated health outcomes (severity)**

This criterion considers the severity of the health outcomes associated with the exposure to the microbiological hazards. Specifically, the number of cases relating to microbiological compounds per year, the proportion of foodborne diseases attributable to beef for each compound and the proportion of beef patties consumed by the population are considered to estimate the number of cases per year due to beef patty intake. Finally, the severity is evaluated quantitatively and for each microbiological hazard considers the input parameters for the DALYs per disease-specific case.

A microbiological hazard receives a grade of:

3: if it relates to 100-1000 DALYs per 1000 cases
2: if it relates to 10-99 DALYs per 1000 cases
1: if it relates to less than 10 DALYs per 1000 cases

Finally, for the estimation of the overall grade and the decision on whether a microbiological agent will be forwarded to the short list, the score in criterion 2 was multiplied by 2 so that criteria 1 and 2 contribute equally.

- **Tier 3: From the short to the final list**

Similar to nutrients and chemical hazards, the final list for microbiological components was based on the availability of dose-response data.
2.5.4. Identification of associated health outcomes

In order to identify health outcomes associated with macro- and micro-nutrients that have been prioritised for inclusion in the final list, project participants screened EFSA’s Scientific Opinions on Dietary Reference Values and further undertook a PubMed Search focusing on systematic reviews and dose-response meta-analyses. Snowballing of key papers retrieved through the initial search provided additional references. From the roster of health outcomes identified, the project’s Consortium decided to focus on hard endpoints (i.e. incidence of disease) excluding data on intermediate factors (e.g., blood pressure, markers of glucose metabolism or inflammation). In relation to fatty acid intakes, project participants decided to consider the association between the substitution of saturated fatty acids (SFAs) with PUFAs and potential health effects.

When more than one dose-response meta-analyses were identified, the more recent one or that at a lower risk of bias was considered. To assess the risk of bias in the dose-response meta-analyses, we applied the ROBIS tool (A Risk of Bias Assessment Tool for Systematic Reviews), which has been designed by the University of Bristol to assess the risk of bias in systematic reviews (https://www.bristol.ac.uk/population-health-sciences/projects/robis/robis-tool/). ROBIS is implemented in three phases by assessing relevance (optional), identifying concerns related to the review process, and finally judging the risk of bias. The tool’s phase 2 particularly includes four domains through which bias may be introduced in the meta-analysis and refer to study eligibility criteria, identification and selection of studies, data collection and study appraisal (use of appropriate criteria to assess the risk of bias in the individual studies considered in the review); and, synthesis and findings (Whiting et al 2016). In the cases when more than one dose-response meta-analyses were available the one with the lower risk of bias was considered in the RBA model.

To identify evidence on causal associations between exposure to the compounds of toxicological concern and adverse health outcomes in humans, we conducted a scoping review of the literature, giving priority to epidemiological studies. Data collected in the context of studies on disease burden (which have previously assessed the strength of evidence) were reviewed. Health outcomes related to microbial hazards were collected in the literature.

Lastly, the burden of diseases associated with the components of interest was quantified through the application of DALYs, a composite metric that combines years of life lost due to premature mortality (YLL) and years of life lost due to time lived in states of less than full health (or years of healthy life lost due to disability, YLD). The YLL represents the product of the number of deaths due to the disease and the average remaining life expectancy at the time of death while the YLD is defined as the product of the incidence, the disability weight of the disease, which reflects the reduction in health-related quality of life on a scale of 0 (no impact on full-health) to 1 (death), and its duration. Hence, 1 DALY represents the loss of the equivalent of one year of full health (Murray, 1994). Estimates for DALYs and incidence rates for the selected diseases were extracted from the Global Burden of Disease (GBD) database. The WHO European Health for All database was explored to collect reliable data for the size of each country’s adult population (individuals aged 18 years old and over). However, the WHO database provided information on the size of the population of individuals aged 15 years and over.

2.6. Model development and simulations

The dual interplay between risks and benefits that is inherent in a RBA is described in Figure 5, which builds upon the traditional 4-step approach in risk characterisation
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Figure 5: The dual interplay of Risk and Benefit Characterisation in the RBA model. Adapted from (EFSA Scientific Committee, 2010; Thomsen, 2019)

The model was developed using Excel and add-in software for probabilistic modelling @Risk® software for Microsoft Excel version 7.6 (Palisade Corporation, USA). We applied a probabilistic model using Monte Carlo simulations, where some parameters were described as distributions to consider variability and/or uncertainty.

The first model used 1,000,000 iterations to capture the variability with one iteration corresponding to one day of patty consumption, and 1,000,000 iterations were run for the uncertainty. The convergence of the model was checked by running the model independently three times.

2.6.1. Exposure assessment

To estimate exposure to the selected components of both foods in each country, we combined national food consumption of beef patty with the concentration and prevalence of each component in the country. This was expressed in g or µg per day for nutrients, in CFU per day for microorganisms and in µg per kg body weight per day for chemicals. Also, for each country the frequency of patty consumption in the beef category was used to estimate the health impact associated to this sub-category only. In all cases, the populations included in the dietary surveys were adults above 18 years old.

2.6.1.1. Beef patty consumption data

Data on the consumption of minced beef in the form of patty were derived from national dietary surveys of each country, expressed in g per day, summing the weight of different meals in case of multiple intakes in the same day and excluding data when the person weight was not mentioned. Considering the fact that the data for each country are large, we used directly the data points without adjusting the distribution, in order to estimate the impact of the proposed substitution in each population which was selected to be representative.
Danish beef patty intake data were retrieved from the Danish National Survey of Diet and Physical Activity (DANSDA), 2011–2013 (Pedersen et al., 2015). DANSDA is a nation-wide, cross-sectional survey of diet and physical activity in a representative sample of the Danish population. DANSDA includes 3,946 participants between 4 and 75 years of age randomly sampled from the civil population registration system who answered pre-coded semi-closed food diaries for seven consecutive days, consisting of categories with common foods and dishes in the Danish diet (Knudsen et al., 2011). Food intake is reported at different levels (i.e. prepared dish and ingredients) and categorized according to standard recipes (Pedersen et al., 2015). Data on beef patties were collected as raw amount consumed in a meal (in g).

For France, the Third French Individual and National Food Consumption Survey (INCA3 survey) was used, a cross-sectional survey carried out between February 2014 and September 2015 among a representative sample of individuals living in mainland France (excluding Corsica) (ANSES, 2017b). A total of 3157 18- to 79-year-old adults, participated in the study, selected according to a three-stage cluster sampling design (geographical units, households and individuals). The dietary intake of the individuals was collected using the 24h-recall method, over three non-consecutive days (two weekdays and one weekend day) spread over around three weeks. Values collected were quantities reported in g per meal.

For Greece, data on the consumption of beef meat in the form of patties were retrieved from the Hellenic National Nutrition and Health Survey (Magriplis et al 2019), a population-based study conducted between September 2013 and May 2015, including a representative sample of adults permanently residing in Greece (N=3,780 participants), excluding institutionalized individuals, members of the armed forces, pregnant and lactating women and individuals with mental disabilities. Participants enrolled provided interviewer-administered 24-hour dietary recalls (two per participant) using the Automated Multiple Pass Method together with specific and validated food atlases as portion anchors.

2.6.1.2. Insect powder consumption estimations

The alternative scenario considering insect powder-based patty was estimated from patty food intake making an iso-weight substitution with rehydrated insect powder. A ratio of 35:65 powder to water was found to provide a suitable texture for replacement (INRAE personal data). The present study evaluates only the beef substitution by insect powder, expecting the other part of ingredients to play a key role in improving product taste and texture.

In the evaluation, we have assumed that the powders used will derive from house crickets (A. domesticus) which will have undergone a boiling step for 5 minutes at 100°C during their manufacturing process and subsequently oven-dried. The powder produced by oven-drying currently represents one of the most common processes used in industry (Bassett et al., 2021).

2.6.1.3. Concentration and prevalence of each component

Nutrients

For each of the thirteen nutrients included in the final list, concentration values in raw minced beef were collected from Danish (Frida, 2019) and French (ANSES, 2020) food composition databases. Data exist for raw minced beef with various fat contents. As the distribution of consumption of minced meat with different fat content in the population is not known, values for all types of raw minced meat were collected. The minimum and maximum for each nutrient among the collected values have been considered and a uniform distribution was implemented between the two extremes.

Levels in A. domesticus powder produced using oven-drying were collected in the international scientific literature and implemented using the same strategy of using a uniform distribution between minimum and maximum values.

Microbiological hazards
The concentration and prevalence was estimated only for the alternative scenario of insect patty consumption that uses a bottom up approach while the estimate of number of cases attributed to beef patty consumption used a top bottom approach based on disease burden estimate and source attribution.

Among the five hazards identified, the three vegetative bacteria (Salmonella spp., Cronobacter sakazakii and Listeria monocytogenes) were excluded as Kooh and al. (2019) reported that a boiling step for 5 minutes at 100°C enables to achieve more than 12 log reduction (commonly used in commercial sterility of canned food).

The two remaining are spore-forming bacteria that are resistant to heat treatment: Bacillus cereus and Clostridium perfringens. There is limited data available to approximate concentration and prevalence. Because they are partially resistant to heat treatment and in presence of limited information on occurrence levels in A. domesticus powders, values from raw and processed A. domesticus were used to approximate prevalence using a beta distribution. The concentration used a uniform distribution between the minimum value found for processed insect powder and the maximum value found in oven-dried insect. These strong assumptions were decided on a precautionary approach, applying the worst case strategy.

**Compounds of toxicological concern**

The level of inorganic arsenic in beef was integrated in the model using a uniform distribution between the lower and upper bounds found in beef meat reported by EFSA (2021e).

**2.6.1.4. Exposure calculations**

Monte Carlo simulations were used to capture the variability by selecting randomly levels in concentration distribution and multiplying with reported levels of food intake (or their associated substitute estimate with insect powder).

**2.6.2. Risk and benefit characterisation**

Risks and benefits were characterized using dose responses, combined with exposure assessment to estimate number of cases attributed to each pair of “component-health outcome”. The applied methodology depended on the type of available dose-response associations, with specificities related to nutrition, microbiology and toxicology.

**Nutrients and compounds of toxicological concern**

Epidemiological dose-responses were collected for each pair of “component – health outcome”. The selection was done considering recent meta-analyses available and, when more than one were available, the selection was based on the application of the ROBIS tool to identify the analysis of low risk of bias (rf. Section 2.4). The Relative Risk (RR) associated with the reference scenario (RRref, current situation) and the alternative (RRalt) associated with the substitution with insect powder were estimated using the log linear slope and the following equations.

\[
\beta = \ln \text{RR}_{\text{literature}} / \text{Dose} \\
\text{RR}(i) = \exp(\beta \cdot \text{exposure}(i))
\]

where RR\text{literature} is the RR reported in the meta-analysis for the specific dose (g/day), RR(i) and exposure(i) are the RR and the exposure estimated for each scenario considered (reference or alternative).

The annual number of increased/decreased cases was estimated using current incidence per country together with the Potential Impact Fraction (PIF) that corresponds to the change in disease burden associated with a change in exposure when moving from the reference to the alternative scenario. Lastly, in the estimation of the number of cases the frequency of patty consumption extracted from the national dietary studies introduced in Section 2.5.1, was also considered.
PIF = (RRalt – RRref) / RRref
Ncase(j) = PIF(j) . Incidence(j) . Frequency_beef

Microbiological hazards

A top to bottom approach was applied for beef, considering current disease incidence and source attribution estimates as performed in de Oliveira Mota et al. (2020). This calculation considers annual number of cases of *Clostridium perfringens*, *Toxoplasma gondii* and *Salmonella* spp attributable to beef. For the sake of harmonization, estimates for the European region from the WHO GBD values (Havelaar et al., 2015) were used for the three countries for *Toxoplasma gondii* (congenital and acquired forms) and *Salmonella* spp. Estimates for France were used for *Clostridium perfringens*, in the absence of other source. The proportion of foodborne disease attributable to beef for *Toxoplasma gondii* and *Salmonella* spp. were also extracted from the WHO GBD Study estimates (Hoffman et al 2017) for France for *Clostridium perfringens* (Fosse, 2008). All estimates were defined as a beta distribution. This was restricted to patty consumption by implementing the ratio of patty consumed among the beef category, specific to each country (collected from the national dietary surveys).

Number of cases (i) = Incidence(i) . Attribution_proportion (beef) . Ratio_patty/beef

The bottom-up approach applied for insect scenario considered levels estimated by the exposure assessment with a threshold dose-response for *Bacillus cereus* and an exponential dose-response for *C. perfringens*. The threshold dose-response is expressed as a limit of concentration (EFSA 2016) or a limit of exposure (Duc 2005). Both limits were used to estimate the number of cases of *Bacillus cereus* by considering that every exceedance results in a case while a probability of illness was calculated for *C. perfringens* and multiplied by the population size to acquire the number of cases.

2.6.3. Health impacts’ quantification in DALYs and comparison of scenarios

For each of the identified health outcomes, values of DALYs per case estimated on the basis of disease incidence were collected from the literature. When available, country-specific values were obtained. In nutrition and toxicology, incidence and DALY rates (i.e. new cases per 100,000 population and DALY per 100,000 population, respectively) were retrieved for each health outcome and country. In particular, values for cancer overall, different cancer sites (bladder, breast, colorectal, oesophageal, gastric, lung, ovarian, pancreatic, prostate, renal cell and non-melanoma skin cancer), CVD (overall, coronary heart disease, stroke), diabetes mellitus type II and Crohn’s disease have been extracted from the GBD database. Diverticular disease, colorectal adenoma, metabolic syndrome and gestational diabetes mellitus were not included in the RBA model since there was no incidence and DALY data available. The average DALY per disease-specific case as well as its 95% Confidence Intervals-CIs were estimated and introduced in the RBA model. In microbiology, the same value was used for the three countries in absence of more detailed data.

DALYs that could be gained or lost by substituting minced beef patty (reference scenario) to a patty with *A. domesticus* powder (alternative scenario) were expressed in terms of change in number of DALY, or ΔDALY. ΔDALY were calculated by subtracting the total DALY in one scenario (summed across components) with the total in the other (in microbiology and toxicology) or by directly considering number of cases saved or increased through the substitution (in nutrition).

Consequently, a negative “–ΔDALY” corresponds to an overall beneficial health impact whereas a positive “+ΔDALY” represents an increase in burden of disease.
2.7. Perceptions regarding the consumption of beef and insects

The Dept. of Risk Communication at BfR performed a review of the literature on risk perceptions and acceptance of beef and insects as foods, with a focus on environmental, emotional and health-related motivations to consume or avoid these foods, taking into consideration the sociodemographic characteristics of potential consumers. The search of the literature on insects yielded 150 unique references with 33 publications identified as most relevant, while 332 unique references were identified for red meat with 12 publications to be finally included. In general, issues related to the presence of microbiological hazards and emotional aspects were found to be primarily related to insects’ consumption; whereas nutritional issues i.e., health risks and food safety associated to meat intake were considered important among consumers.

The key findings on the consumption of edible insects were based on disgust, familiarity, and food neophobia state, processed vs. unprocessed forms of insects, contextual information, cultural differences and social norms. Socio-demographic disparities in attitudes and perceptions were also observed. Disgust towards eating insects also considering the fear of contamination and disease (animal reminder disgust) appears to be a barrier to insects’ consumption. First-time instead of repeated consumption, together with food neophobia, a general weariness of consuming novel foods, may also influence their consumption with individuals to be less likely to consume insects as a novel food and less willing to pay for or consume insect-containing products. Furthermore, several studies indicate that consumption of insect-based foods is more acceptable than the consumption of the item per se, particularly among individuals with low willingness to consume insects and those who have not tried insects before. Information given about insect-based food products seems to have inconsistent effects on attitudes and behaviour keeping in mind the cultural differences in the respective ratings of different sources of information. The view of insects as whole animal and visual reminders (e.g., images evoking thoughts of the live animals) may also limit their acceptability. Regarding socio-demographic characteristics, it is difficult to investigate the role of age on behaviours, partially due to the fact that most studies were conducted in disproportionately young populations. Women may be less likely to consume insects than men, linking to an increased animal reminder sensitivity. Associations of red meat with both positive and negative health outcomes found in various studies indicate the awareness among the European population about both risks and benefits of red meat intake. Fears regarding safety are one of the key barriers to red meat consumption.

This literature review has been accepted for publication to *Frontiers in Nutrition* Journal, Section on Nutrition and Food Science Technology Research (Topic Title: Risk-Benefit Assessment of Foods: Advances in Public Health). This review formed the basis of the strategy to communicate the outcome of the NovRBA case-study to the general public.

2.8. Project’s activities and results dissemination

2.8.1. Development of project logo

The NovRBA project developed its own logo, which aims to reflect opposed forces acting together when risks and benefits are simultaneously considered.
2.8.2. Dissemination activities in scientific events

Various stages of the project were/will be presented in several international Conferences and Scientific Symposia. Titles and venues of presentations are listed below:

- 3rd International Conference on Food Contaminants- Challenges in Risk Assessment, Aveiro, Portugal, September 2019
  

- 13th Federation of European Nutrition Societies (FENS) European Nutrition Conference 2019, Dublin, Ireland, October 2019

  *Edible insects instead of red meat? Assessing the health impact of the substitution via Risk-Benefit Assessment Methods. The NovRBA project* (poster presentation)

- Conference of the Greek Lipid Forum (virtual satellite event to the European Food Lipid Conference, October 2021)

  *Nutrient profiling of alternative vs conventional protein sources: into the lipids of bovine meat and house cricket, an edible insect species* (oral presentation)

- ONE – Health, Environment, Society – EFSA Conference 2022, Brussels June 2022

  *Towards a standardized and harmonised selection of nutritional, microbiological and toxicological components in Risk-Benefit Assessment of foods: a methodology developed under the NovRBA project* (abstract accepted for poster presentation)

- ONE – Health, Environment, Society – EFSA Conference 2022, Brussels June 2022

  *Integrating consumers’ perception in communication strategies for Risk-Benefit Assessment outcomes in food and nutrition. Case study: red meat vs novel protein sources, under the NovRBA project* (abstract accepted for poster presentation)

Moreover, the project was introduced to the scientific community and the public through the social media (LinkedIn, Twitter, ResearchGate, websites of institutes) and was presented in the newsletter of the RBA Network. The first post on LinkedIn related to introducing the project to the scientific community counted 11,070 views, while the second on the presentation of the project at the 3rd International Conference on Food Contaminants- Challenges in Risk Assessment, Aveiro, Portugal, September 2019, was viewed by 5,592 subscribers.
2.8.3. Dissemination in the scientific literature

- Manuscript submitted to *Frontiers in Nutrition* Journal, Section on Nutrition and Food Science Technology Research (Topic Title: Risk-Benefit Assessment of Foods: Advances in Public Health). Title: "Communicating food risk-benefit assessments: Edible insects as red meat replacers". The manuscript has been accepted for publication on 26/11/2021.

- Manuscript on the compositional profile of *A. domesticus*. A manuscript has been prepared and will be submitted to the Food Chemistry or Trends in Food Science and Technology journals.

2.9. Project management

The project's kick-off meeting took place in Parma in March 2019 and the project's interim meeting was carried out virtually in April 2020. To ensure the work progress according to the project's time plan, participants met regularly through web-meetings. NKUA, the project Coordinator, was responsible for the meetings' preparation and for drafting the minutes summarising the discussion, decisions and actions to be taken. Overall, 25 project meetings took place during the project's lifetime. In addition, several working sessions have been organised on a regular basis in order to address specific technical issues in relation to the formulation of the standardised methodology to select model components, the implementation of the RBA model and the interpretation of the model's outcome.
3. Milestones achieved and results obtained

3.1. Selection of the insect species to be studied

The five insect species with the highest scores overall as well as their scores by sub-criterion considered (rf Section 2.1) are presented in Figures 6 and 7 respectively. The scores refer to the initial 51 selected publications and documents reviewed.

![Score (out of 51)](image)

**Figure 6:** Insect species with the highest scores overall, according to criteria on their EU market potential and data availability

![Scores achieved by five insect species by sub-criterion considered in the selection process.](image)

**Figure 7:** Scores achieved by five insect species by sub-criterion considered in the selection process.

*Acheta domesticus* and *Tenebrio molitor* met all the selection criteria and achieved the highest scores. In particular, both species are among those commonly farmed in some EU countries (Mlcek et al., 2014), and their breeding is being continued up to date (Belluco et al., 2017; Caparros Megido et al., 2017; Vandeweyer et al., 2017). Foodstuff containing *A. domesticus* or *T. molitor* are already consumed in some EU countries and both species have been reported to have the potential to be used for food production in the whole EU (Van der Spiegel et al., 2013). *A. domesticus* and *T. molitor* are among the insect species products of which have receive a positive assessment by EFSA (EFSA NDA Panel 2021a, 2021c, 2021d).
Sensorial attributes of both species have been reported by Elhassan et al. (2019). Several studies have been conducted to characterise technological properties of the two species as food ingredients. Yi et al. (2013), Ndiritu et al. (2017) and Ndiritu et al. (2019) have investigated the physicochemical characteristics and functional properties of A. domesticus protein concentrates. Recovery and functionality of flour and protein concentrates of T. molitor larvae have also been studied (Yi et al., 2013; Zhao et al. 2016; Bussler et al., 2016; Zielińska et al., 2018; Roncolini et al., 2019).

Both insect species have been studied regarding their potential to replace meat in meat preparations. Kim et al., (2017) reported that A. domesticus flour can successfully replace lean meat/fat portion by up to 10% in meat emulsions without negative impact on texture or cooking properties, fortifying at the same time the product with protein and micronutrients (magnesium, potassium, phosphorus). Another study showed that it is possible to replace lean pork meat by up to 10% T. molitor larvae in frankfurters, maintaining at the same time the sensorial and structural characteristics of the product (Choi et al., 2017). Successful, from the technological point of view, attempts to use T. molitor and A. domesticus (Osimani et al., 2018; González et al., 2019) as protein fortification agents in bakery products have also been reported.

Payne et al. (2016) conducted a systematic review of nutrient composition data available for twelve commercially available edible insects, including A. domesticus and T. molitor. Complete nutrient composition for both species has also been previously reported (Finke, 2002; Kouřimská and Adámková, 2016). Protein quality of both species has been studied (Bosch et al., 2014; Zielińska et al., 2015; Nowak et al., 2016), and chitin content has been estimated (Finke, 2007). Lipid profiles for both species have been studied (Tzompa-Sosa et al., 2014; Paul et al., 2017). Microbiological aspects during production, processing and storage for both insect species have been addressed several authors (Klunder et al., 2012; Garofalo et al., 2017; Grabowski and Klein, 2017; Caparros Megido et al., 2017; Stoops et al., 2017; Fasolato et al., 2018; Vandeweyer et al., 2017). Poma et al. (2017) have investigated the occurrence of chemical agents of hazardous potential to humans in products containing A. domesticus (cricket balls) or T. molitor (whole), intended for human consumption.

Both A. domesticus and T. molitor can serve the purpose of the NovRBA project. However, to make the final selection the following details were taken into consideration. Payne et al. (2016) reported that A. domesticus is a species successfully reared on a large scale and sold for domestic consumption in non-EU countries. Furthermore, in their report Payne et al indicate that T. molitor has not been traditionally consumed by humans and its farming on a large scale for food and feed uses is a relatively recent. Furthermore, A. domesticus is indicated as the insect species with probably the longest history of insect mass-rearing in the United States. The already existing experience in large-scale breeding, that has been refined for several decades, renders A. domesticus one of the cheapest insects to farm, and the shifts that should be applied on the currently existing industrial practices to increase economic competitiveness shall be minimal (Paoletti, 2005; Hanboonsong et al., 2013; Morales-Ramos et al., 2013). Additionally, Fasolato et al. (2018) discussed that A. domesticus has an advantage regarding rearing, compared to T. molitor, the substrate can be readily removed before harvesting. Feeding substrate is one of the main sources for undesirable substances and microbiological hazards in insect farming.

A. domesticus has been reported to have superior taste, and larger versatility (House, 2018). Versatility is an important characteristic for a food ingredient, since it gives the potential for more uses and technological applications. Furthermore, the taste profile and protein content of crickets can be manipulated by the breeders, by implementing alterations on the feeding substrate (House, 2018).

Regarding the nutritional profile, it has been reported that vitamin B12 has been found in abundance in A. domesticus, and the levels reported were more than 10 folds higher than what was reported for T. molitor larvae (5.4 μg per 100 vs 0.47 μg per 100 g) (Kouřimská and Adámková, 2016). Such a nutritional characteristic is important, since a meat replacer should be able to provide some of the nutrients of the red meat. Paul et al., (2017) analysed and compared the lipid profiles of A. domesticus and T. molitor. They reported that A. domesticus contains higher amounts of essential fatty acids, with linoleic acid being in major quantities.
whereas *A. domesticus* n-6/n-3 ratio is 37.04. Studies suggest that a high dietary n-6/n-3 ratio can potentially be linked to a variety of physiological disorders (Miličević et al., 2014).

Based on the above assessment, the NovRBA project’s Consortium decided to select *A. domesticus* as the insect species to be considered as a potential red meat replacer in the project’s case study.

### 3.2. Nutrient, microbiological and toxicological profile of *A. domesticus*

Approximately 230 articles were screened for eligibility and 74 were selected to be used for data extraction. Among those, 64 articles included original quantitative data on nutrient composition, 18 on microbiological aspects and 3 on compounds of toxicological concern, all published between 1968 and 2021 (end of October).

Attempts to describe the nutrient composition and microbiological characteristics of *A. domesticus* forms are relatively recent, whereas the number of studies with data on the presence of toxicological compounds is limited. The majority of *A. domesticus* samples was produced in Europe (n=39), followed by samples produced in North and Central America (n=12 and n=1, respectively), Asia (n=13) and Africa (n=5).

In the raw and boiled *A. domesticus* (undried), the main component is water followed by crude protein and crude fat, whereas, in all the dried forms of *A. domesticus*, the main component is crude protein, with moisture levels varying from 0.6 to 12.4%. In dried *A. domesticus* products, fat appears as a major component at concentrations up to about 30%. The main components of the crude fat fraction are triacylglycerols, polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). The vitamin levels, for both undried and dried *A. domesticus* forms, have not been extensively investigated, since issues related to their feed (e.g. season), the effect of processing (affecting for instance thermally labile vitamins) and the method of analysis may impair estimations. *A. domesticus*, specifically in its dried form, contains several minerals, but the values reported vary possibly due to the effect of the feed on the mineral composition of the food product, as well as to the existence or not of the fasting step before killing. In both dried and undried forms of *A. domesticus*, total aerobic counts, Salmonella spp., *Listeria monocytogenes*, Bacillus cereus group, coagulase-positive staphylococci, Enterobacteriaceae, lactic acid bacteria, yeasts and moulds have been identified. Few studies provided information in relation to the toxicological profiling of *A. domesticus*, including the recently published Opinion by EFSA for the “Safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a Novel food pursuant to Regulation (EU) 2015/2283” (EFSA NDA Panel, 2021c). EFSA presented data on heavy metals and mycotoxins in dried *A. domesticus* adults reporting that concentrations of contaminants in *A. domesticus* forms are highly dependent on the occurrence levels of these compounds in the feed provided to the insects.

(Pre-meeting note: A manuscript has been prepared and will be submitted to the Food Chemistry or Trends in Food Science and Technology journals. The paragraphs above summarise main findings and discussion points)

### 3.3. Selection of the RBA model components

Tables 2, 3 and 4 present the nutrients, microbiological hazards and compound of toxicological concern which were included in the long list (Table 2), those which were selected for the short list (Table 3) and the ones which were prioritised to be included in the final list (Table 4).

The outcome of the literature search on dose-response associations between intakes and health outcomes is summarised in health trees included as Appendix A. An important challenge in incorporating estimates of disease risk in the model was related to curvilinear associations (U or J-shaped associations). In its current version, the RBA model cannot consider cases of non-linear associations and when identified they were treated as the type of evidence. For instance, a non-linear relationship was found between calcium intake and risk of metabolic syndrome (Cheng L et al, 2019). The study was however not considered as it solely relied on evidence from cross-sectional studies.
- the impact of the substitution to the nutrient intake and ultimately to disease risk. According to a recent meta-analysis by Vinceti et al (2021), the association between dietary selenium intake and risk of type 2 diabetes is non-linear, with risk increasing when selenium intake exceeds 80μg/day while there is no change in the risk of type 2 diabetes when the intake is below 55-60μg/day. Based on the substitution scenario tested in this case-study, we calculated the range of changes in selenium intake after the substitution of beef meat with *A. domesticus*. Taking into consideration the selenium intake from the background diet of the populations under study, we estimated that the selenium intake will not overpass 60μg/day and therefore an increase in the risk of type 2 diabetes is not expected to be observed. Thus, the association between selenium intake and risk of type 2 diabetes was not included in the RBA model.

The association between the substitution of SFAs with PUFAs and potential health outcomes was also investigated given the well-documented protection against coronary heart disease (CHD) (Mozaffarian et al 2010). In this meta-analysis of 11 prospective studies, the CHD risk was reduced by 13% (RR = 0.87, 95% CI 0.77- 0.97), when 5% of calories provided by SFA intake were substituted by PUFA intake. We evaluated the introduction of this element in our model assuming a total isocaloric substitution, i.e. that all other ingredients in the food product under study as well as all the other energy providing components of the person’s diet remain fully unchanged, a rather strong but necessary assumption. Under this assumption, we estimated that in our alternative scenario 4% less calories are provided by SFA, while PUFA intake increases only up to 2.5% energy. Under these circumstances, the expected changes in the SFA vs PUFA intake are far from the observed 5%, and under current knowledge this element cannot be introduced in our model.
### Table 2: The LONG list of potential nutrients, microbiological agents and compound of toxicological concern in beef meat and *A. domesticus* (identification based on literature search)

<table>
<thead>
<tr>
<th>MICROBIOLOGICAL HAZARDS</th>
<th>CHEMICAL HAZARDS</th>
<th>NUTRIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. domesticus</strong></td>
<td><strong>Beef</strong></td>
<td><strong>A. domesticus</strong></td>
</tr>
<tr>
<td><strong>Bacteria:</strong></td>
<td><strong>Beef</strong></td>
<td><strong>A. domesticus</strong></td>
</tr>
<tr>
<td><em>B. Cereus</em></td>
<td>Total PCB</td>
<td>Total PCB</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>(organochlorine</td>
<td>(organochlorine</td>
</tr>
<tr>
<td><em>C. Botulinum</em></td>
<td>compounds)</td>
<td>compounds)</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>Phosphorous</td>
<td>Phosphorous</td>
</tr>
<tr>
<td><em>Cronobacter</em></td>
<td>flame retardants (PFR,</td>
<td>flame retardants (PFR,</td>
</tr>
<tr>
<td><em>sakazakii</em></td>
<td>organochlorine</td>
<td>organochlorine</td>
</tr>
<tr>
<td><em>EHEC</em></td>
<td>compounds)</td>
<td>compounds)</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>Oxychlorodane (OCD,</td>
<td>Oxychlorodane (OCD,</td>
</tr>
<tr>
<td><em>monocyto genes</em></td>
<td>flame retardant)</td>
<td>flame retardant)</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Polybrinated diphenyl</td>
<td>Polybrinated diphenyl</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ethers (PBDE, flame</td>
<td>ethers (PBDE, flame</td>
</tr>
<tr>
<td><em>Vibrio sp.</em></td>
<td>retardant)</td>
<td>retardant)</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td><strong>Metals</strong></td>
<td><strong>Polyunsaturated</strong></td>
</tr>
<tr>
<td><em>HIV</em></td>
<td>(Methyl)mercury</td>
<td>Fatty acids</td>
</tr>
<tr>
<td><em>Norovirus</em></td>
<td>Cadmium</td>
<td>Total SFA</td>
</tr>
<tr>
<td><em>Rotavirus</em></td>
<td>Lead</td>
<td>C10:0</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>Inorganic arsenic</td>
<td>C12:0</td>
</tr>
<tr>
<td><em>enterocolitica</em></td>
<td>Aluminum</td>
<td>C14:0</td>
</tr>
<tr>
<td><strong>Parasites:</strong></td>
<td><strong>Chromium</strong></td>
<td>C15:0</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Chromium</td>
<td>C16:0</td>
</tr>
<tr>
<td><em>sp.</em> (veals)</td>
<td></td>
<td>C17:0</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td></td>
<td>C18:0</td>
</tr>
<tr>
<td><strong>POPs:</strong></td>
<td><strong>Minerals</strong></td>
<td><strong>Iodine</strong></td>
</tr>
<tr>
<td><em>Dioxin + di-PCBs</em></td>
<td>Aluminium</td>
<td><strong>Selenium</strong></td>
</tr>
<tr>
<td><em>Organochlorine</em></td>
<td>Calcium</td>
<td><strong>Chromium</strong></td>
</tr>
<tr>
<td><em>compounds</em></td>
<td>Copper</td>
<td><strong>Nickel</strong></td>
</tr>
<tr>
<td><em>Organochlorine</em></td>
<td>Iron</td>
<td><strong>Chromium</strong></td>
</tr>
<tr>
<td><em>compounds</em></td>
<td>Magnesium</td>
<td><strong>Phosphorus</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Manganese</td>
<td><strong>Potassium</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Sodium</td>
<td><strong>Sodium</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Sulphur</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Zinc</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Iodine</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Selenium</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Chromium</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><em>Oxychlorodane</em></td>
<td>Chloride</td>
<td><strong>Sulphur</strong></td>
</tr>
<tr>
<td><strong>Pesticides:</strong></td>
<td><strong>Fatty acids</strong></td>
<td><strong>Cholesterol</strong></td>
</tr>
<tr>
<td><em>(presence of 10 pesticides)</em></td>
<td></td>
<td>n-6/n-3</td>
</tr>
<tr>
<td><strong>Neoformed compounds or process contaminants</strong></td>
<td><strong>Total SFA</strong></td>
<td><strong>PUFA/SFA</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Amino acids</strong></td>
<td><strong>Histidine</strong></td>
</tr>
<tr>
<td><strong>Neoformed compounds or process contaminants</strong></td>
<td><strong>Total MUFA</strong></td>
<td><strong>Lysine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Total MUFA</strong></td>
<td><strong>Phenylalanine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Total PUFAs</strong></td>
<td><strong>Tyrosine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Total n-3 fatty acids</strong></td>
<td><strong>Prolin</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Total n-6 fatty acids</strong></td>
<td><strong>Serine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Total fatty acids</strong></td>
<td><strong>Threonine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Cholesterol</strong></td>
<td><strong>Valine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>n-6/n-3</strong></td>
<td><strong>Cystine/cysteine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>PUFA/SFA</strong></td>
<td><strong>Methionine</strong></td>
</tr>
<tr>
<td><strong>HAP</strong></td>
<td><strong>Tryptophan</strong></td>
<td><strong>tryptophan</strong></td>
</tr>
</tbody>
</table>

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**Table 3:** The SHORT list of significant nutrients, microbiological agents and compound of toxicological concern in beef meat and *A. domesticus* (through ranking considering occurrence and severity)

<table>
<thead>
<tr>
<th>MICROBIOLOGICAL HAZARDS</th>
<th>CHEMICAL HAZARDS</th>
<th>NUTRIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. domesticus</em></td>
<td>Beef</td>
<td><em>A. domesticus</em></td>
</tr>
<tr>
<td><strong>Bacteria:</strong></td>
<td><strong>Bacteria:</strong></td>
<td><strong>contaminants:</strong></td>
</tr>
<tr>
<td><em>Bacillus Cereus.</em></td>
<td><em>Clostridium Botulinum</em></td>
<td><em>Inorganic arsenic</em></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td><em>Clostridium perfringens</em></td>
<td></td>
</tr>
<tr>
<td><em>Cronobacter sakazakii</em></td>
<td><em>Salmonella spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td><em>Salmonella spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Salmomella spp.</em></td>
<td><em>Selenium</em></td>
<td></td>
</tr>
<tr>
<td><strong>Parasites:</strong></td>
<td><strong>Minerals:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td><strong>Fatty acids:</strong></td>
<td>Fatty acids</td>
<td></td>
</tr>
<tr>
<td><em>Total n-3 fatty acids</em></td>
<td><em>Total n-3 fatty acids</em></td>
<td></td>
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<tr>
<td><em>Total n-6 fatty acids</em></td>
<td><em>Total n-6 fatty acids</em></td>
<td></td>
</tr>
<tr>
<td><em>Total polyunsaturated fatty acids</em></td>
<td><em>Total polyunsaturated fatty acids</em></td>
<td></td>
</tr>
<tr>
<td><em>Total saturated fatty acids</em></td>
<td><em>Total saturated fatty acids</em></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamins:</strong></td>
<td><strong>Vitamins:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Vitamin B12 (Cyanocobalamin)</em></td>
<td><em>Vitamin B12 (Cyanocobalamin)</em></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4: The FINAL list of nutrients, microbiological agents and compound of toxicological concern in beef meat and *A. domesticus* (through ranking considering evidence and feasibility)

<table>
<thead>
<tr>
<th>MICROBIOLOGICAL HAZARDS</th>
<th>CHEMICAL HAZARDS</th>
<th>NUTRIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. domesticus</em></td>
<td><em>Beef</em></td>
<td><em>A. domesticus</em></td>
</tr>
<tr>
<td><strong>Bacteria:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus Cereus.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cronobacter sakazakii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Parasites:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteria:</strong></td>
<td><strong>Microbes:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td></td>
<td><em>Inorganic contaminants:</em></td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Parasites:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inorganic contaminants:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Inorganic arsenic</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Macronutrients:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fiber</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Minerals:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Calcium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Iron</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Magnesium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sodium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Zinc</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Selenium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamins:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vitamin B12</em> (Cyanocobalamin)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5 lists the health outcomes identified to be associated with each selected nutritional, microbiological and chemical components of the RBA model and provides the source of the evidence on the dose-response associations considered.

**Table 5:** Health outcomes associated with each of the selected nutrients, microbiological agents and compounds of toxicological concern of the RBA model

<table>
<thead>
<tr>
<th>Component</th>
<th>Health outcome</th>
<th>Type of dose-response implemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostate cancer</td>
<td></td>
</tr>
<tr>
<td>Fiber</td>
<td>CHD</td>
<td>Meta-analyses of epidemiological studies</td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crohn’s disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CVD</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>Oesophageal cancer</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>Diabetes mellitus type II</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>Diabetes mellitus type 2</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Oesophageal cancer</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>Oesophageal cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inorganic Arsenic in beef</td>
<td>Meta-analyses of epidemiological studies</td>
</tr>
<tr>
<td>Bacillus cereus in insect</td>
<td>Bladder cancer</td>
<td>Comparison with a threshold dose-response</td>
</tr>
<tr>
<td>Clostridium perfringens in insect</td>
<td>Lung cancer</td>
<td>Exponential dose-response</td>
</tr>
<tr>
<td>Cronobacter sakazakii in insect</td>
<td>Skin cancer</td>
<td>Calculation of heat treatment inactivation</td>
</tr>
<tr>
<td>Listeria monocytogenes in insect</td>
<td></td>
<td>Source attribution</td>
</tr>
<tr>
<td>Salmonella spp. in insect</td>
<td></td>
<td>Source attribution</td>
</tr>
<tr>
<td>Clostridium perfringens in beef</td>
<td></td>
<td>Source attribution</td>
</tr>
<tr>
<td>Salmonella spp. in beef</td>
<td></td>
<td>Source attribution</td>
</tr>
<tr>
<td>Toxoplasma gondii in beef</td>
<td></td>
<td>Source attribution</td>
</tr>
</tbody>
</table>

### 3.4. Exposure assessment results

The beef patty intake was estimated for each country based on national food consumption surveys. The cumulative distribution in Figure 8 provides the profile of intake in each country depicting the within- and between-country variability. Indicatively, the median patty portion of Greece and France are close and around 85g/day while it is around 150g/day in Denmark. These inputs were used to consider all different reported intakes and thus estimate a health impact reflecting real situations in each country. Low and high intakes were also included by collecting random values among these distributions. These values were used, in
combination with conversion factors provided in method section, to build the potential distribution of insect powder intake (Figure 8b). This results in a median exposure of insect powder around 14 g/day, 17 g/day and 30g/day for Greece, France and Denmark respectively.

![Cumulative distribution of a) current beef patty intake and estimated substitution with b) insect powder (in g per day) with Greece in red, France in green and Denmark in blue.](image)

**Figure 8:** Cumulative distribution of a) current beef patty intake and estimated substitution with b) insect powder (in g per day) with Greece in red, France in green and Denmark in blue.

Then, the combination of distributions of food intake data with distributions of concentration of nutrients provided estimates of each population's exposure to each nutrient (Table 6). For each country, a significant increase was observed in calcium, copper, crude fiber, magnesium, total n-6 fatty acids and total PUFA intakes while a decrease was observed for iron, sodium, total SFA, vitamin B12 (Cyanocobalamin) and zinc intakes.
Table 6: Mean values of nutrient exposure for reference (Ref) and alternative (Alt) scenario

<table>
<thead>
<tr>
<th></th>
<th>GREECE</th>
<th>DENMARK</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ref scenario</td>
<td>Alt scenario</td>
<td>Ref scenario</td>
</tr>
<tr>
<td>Calcium mg/day</td>
<td>7.38</td>
<td>28.22</td>
<td>14.85</td>
</tr>
<tr>
<td>Copper mg/day</td>
<td>0.05</td>
<td>0.57</td>
<td>0.09</td>
</tr>
<tr>
<td>Crude fiber g/day</td>
<td>0.00</td>
<td>1.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Iron mg/day</td>
<td>1.71</td>
<td>1.02</td>
<td>3.45</td>
</tr>
<tr>
<td>Magnesium mg/day</td>
<td>13.66</td>
<td>17.61</td>
<td>27.46</td>
</tr>
<tr>
<td>Selenium mg/day</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Sodium mg/day</td>
<td>40.96</td>
<td>36.01</td>
<td>82.30</td>
</tr>
<tr>
<td>Total n-3 fatty acids g/day</td>
<td>0.06</td>
<td>0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>Total n-6 fatty acids g/day</td>
<td>0.16</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>Total polyunsaturated fatty acids g/day</td>
<td>0.30</td>
<td>0.64</td>
<td>0.61</td>
</tr>
<tr>
<td>Total saturated fatty acids g/day</td>
<td>3.08</td>
<td>1.01</td>
<td>6.19</td>
</tr>
<tr>
<td>Vitamin B12 (Cyanocobalamin) mg/day</td>
<td>0.001</td>
<td>0.000</td>
<td>0.003</td>
</tr>
<tr>
<td>Zinc mg/day</td>
<td>3.04</td>
<td>2.74</td>
<td>6.11</td>
</tr>
</tbody>
</table>

In microbiology, considering the very limited number or data available, strong assumptions were made in terms of prevalence and levels of contamination in insect. This resulted in a high number of individuals with an exposure potentially exceeding the two thresholds tested for *B. cereus*: between 12% and 71% of individuals. Strong assumptions were also made for *C. perfringens*, leading to a median level of exposure between 1.6 and 2.2 log cfu/day.

Regarding toxicological assessment of inorganic arsenic, the median daily exposure level was estimated at 0.005, 0.007 and 0.012 µg/kgBW for Greece, France and Denmark respectively.
3.5. Comparison of scenarios in DALYs

The overall health impact was estimated in DALYs and reported for each country (Table 7). Each country had different results as the model considered their national food intake values and their current incidence of diseases to estimate number of cases increased or decreased by the change in exposure. Also, these three countries have a large difference in population size implemented with around 5, 9 and 53 million inhabitants in Denmark, Greece and France, respectively. The expected change in DALYs when moving from the reference scenario to the alternative scenario was estimated to be around 8,753 DALYs (per 100,000 population) saved in Greece, 6,572 DALYs (per 100,000 population) in Denmark and 21,972 DALYs (per 100,000 population) in France. This is mainly due to the beneficial overall nutritional and microbiological impacts.

Table 7: Change in DALYs (per 100,000 population) when moving from the reference to the alternative scenario, expressed in annual DALY change per country, 95th confidence interval and percentage of contribution of each component per domain.

<table>
<thead>
<tr>
<th></th>
<th>Greece</th>
<th>Denmark</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of ΔDALY</td>
<td>-8,753 (-14,484 ; -4,595)</td>
<td>-6,572 (-13,444 ; -2,737)</td>
<td>-21,972 (-34,400 ; -12,613)</td>
</tr>
<tr>
<td>Nutrition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>-5,206</td>
<td>-2,360</td>
<td>-17,400</td>
</tr>
<tr>
<td>Fiber</td>
<td>0,08%</td>
<td>0,19%</td>
<td>0,16%</td>
</tr>
<tr>
<td>Iron</td>
<td>0,06%</td>
<td>0,39%</td>
<td>0,36%</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0,50%</td>
<td>0,24%</td>
<td>0,70%</td>
</tr>
<tr>
<td>Sodium</td>
<td>84%</td>
<td>80%</td>
<td>82%</td>
</tr>
<tr>
<td>Vit B12</td>
<td>0,71%</td>
<td>1,69%</td>
<td>1,28%</td>
</tr>
<tr>
<td>Zinc</td>
<td>0,03%</td>
<td>0,17%</td>
<td>0,16%</td>
</tr>
<tr>
<td>Toxicology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic Arsenic</td>
<td>-0,10</td>
<td>-0,11</td>
<td>-0,69</td>
</tr>
<tr>
<td>Microbiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef - Clostridium perfringens</td>
<td>0,4%</td>
<td>0,3%</td>
<td>0,3%</td>
</tr>
<tr>
<td>Beef - Salmonella spp.</td>
<td>2%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Beef - Toxoplasma gondii</td>
<td>88%</td>
<td>92%</td>
<td>92%</td>
</tr>
<tr>
<td>AD – Bacillus cereus</td>
<td>10%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>AD – Clostridium perfringens</td>
<td>0,0000002%</td>
<td>0,00000003%</td>
<td>0,0000002%</td>
</tr>
<tr>
<td>AD – Cronobacter sakazakii</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>AD – Listeria monocytogenes</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>AD – Salmonella spp.</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
3.6. Challenges and limitations

The NovRBA project aimed to develop and test a harmonised tailored approach to implement a RBA of a substitution scenario. Through this process, the project Consortium identified a large number of knowledge gaps, which either lead to assumptions necessary to advance the assessment or to the identification of missing data with the subsequent limitations in the interpretation of results. These challenges are listed in the sections that follow.

3.6.1. Challenges related to the availability of food composition and occurrence data

Several impediments were encountered in relation to the compilation of compositional profiles for both food items under study. Reliable comparable data on the nutrient composition of *A. domesticus*, a novel food, were lacking. Our systematic literature search revealed several sources of compositional data, which were however of limited comparability and, in certain cases, of even limited use. Data were heterogeneous in relation to samples analysed and methods performed, while several important factors that can affect the composition (e.g. feed, rearing conditions) were left unreported. It is for this reason that results regarding the profiling of *A. domesticus* have to be considered and interpreted with caution. Furthermore, some of the reported analytical methods are not appropriate for insects (e.g. methods for protein or vitamin B12 quantification) and these can generate misleading information that could lead to an inappropriate selection of model components. Because of the substantial heterogeneity, average values for *A. domesticus* could not be used and uniform distributions using individual data points could not be integrated in the model. Hence, initiatives to ensure the application of a proper methodology, including standardised sampling, analysis and harmonised reporting, should also extend to novel food ingredients.

Regarding occurrence data and considering the fact that studies addressing *A. domesticus* are relatively recent, our sources of such data were particularly limited. In the case of chemical contaminants in particular, our only source was the recently published EFSA Opinion (EFSA NDA Panel, 2021c).

In relation to beef, a staple food in Europe, the occurrence data regarding minced beef meat were limited at national level and, when available, they were mainly referring to raw beef and were not always comparable. No nutrient composition data were available for beef consumed in Greece. In this case, our assessment had to rely on a combination of nutrient values available from the other two countries.

3.6.2. Challenges related to the identification of health outcomes

On the basis of nutrients, microbial hazards and compounds of toxicological concern prioritised in the short list, we performed systematic literature searches to identify health outcomes associated with these components (i.e. building the health trees) and we further searched for recent evidence on corresponding dose-response associations estimated through meta-analytic methods. This process was limited by the fact that we could not obtain dose-response data for all the ‘component-health outcome’ pairs (Table 8). Thus, copper and phosphorus were not included in the final list of nutrients because of lack of dose-response data. Another source of information that had an impact on model building were the DALYs associated with health-related conditions, since DALY values were not available for some of the health outcomes of interest (Table 8). Although the associations listed in Table 8 are reported as of “limited evidence” in the World Cancer Research Fund Report on *Diet, Nutrition, Physical Activity and Cancer: a Global Perspective* (WCRF, 2018), these components should be seen as key priorities to reinforce this RBA.
Table 8: Components and health outcomes that were not quantified in this RBA

<table>
<thead>
<tr>
<th>Components (not prioritised because of lack of dose-response data)</th>
<th>Nutrition</th>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, Phosphorus</td>
<td></td>
<td>Clostridium botulinum</td>
</tr>
<tr>
<td>Health outcomes (not considered because of lack of information on DALYs)</td>
<td>Colorectal adenoma (calcium, fiber)</td>
<td>Diverticular disease (fiber)</td>
</tr>
<tr>
<td></td>
<td>Gestational diabetes mellitus (iron)</td>
<td>Metabolic syndrome (calcium, magnesium)</td>
</tr>
</tbody>
</table>

The currently available RBA models largely build on linear associations between dietary exposures and related hazards. An additional complication that we had to confront is the fact that some diet-disease associations were curvilinear. The consideration of such relationships required a specific approach that relied on the calculation of the expected change in the intake, generated by the substitution scenario under evaluation, together with estimations on the background intake of the populations under study.

The WHO European Health for All database and the data generated through the GBD Study were used to obtain information on population size, disease incidence and DALY values. Our decision to refer to adults above 18 years of age was challenged by the fact that the WHO database provides data for individuals aged 15 years and over whereas the GBD Study data refer to individuals aged 20 years and over. Nonetheless, given the fact that the chronic diseases considered in this analysis are relevant for older adults we would not expect this discrepancy to have a large impact on our findings.

Another issue identified in previous RBA relates to overlapping health-effects (e.g. all-cause mortality and CVD mortality). To address this in our approach, we have solely considered incidence-based indices, excluding mortality (overall or cause-specific). Moreover, allergenicity was not considered in the presently reported RBA model. Such considerations are particularly challenging considering the fact that there is no standard procedure for an allergenicity risk assessment.

3.6.3. Challenges related to model development

We have performed a RBA based on components (nutrients, microbiological hazards and compounds of toxicological concern) of the food items under study, primarily because of the lack of data on associations between *A. domesticus* intake and health outcomes. This necessary approach produced additional challenges given the uncertainties behind the nutrient data which is largely dependent on the quality of food composition tables, as well as our sometimes limited knowledge on nutrient losses occurring during cooking. Through this approach we could also not use published literature on associations between beef intake (as a food item) and health outcomes. The limited data were an additional obstacle in running a probabilistic approach. Furthermore, our scenarios had to make use of assumptions which could have been restricted if more robust "recipe" data were available.

An additional strong but necessary assumption behind the substitution of minced beef meat with an *A. domesticus* powder product is that the background diet of the populations under study remains unchanged. Qualitatively, the relative contribution of the different nutrients towards prudent dietary choices was
considered in the selection process (Section 2.6.1), but the quantity in the background dietary intake was not included in the model. This model parameter, if considered, would undoubtedly increase the complexity of the model and could alternatively be addressed through the evaluation of dietary patterns instead of specific food components or single food items.

3.7. Communicating the findings of the RBA case-study to substitute beef meat with *A. domesticus* powder products

In a general effort to promote the consumption of edible insects, one should note that any communication must avoid triggering animal reminder disgust or increasing risk perception by evoking associations with live animals (either through visual or text information). Furthermore, introducing individuals to insect-based foods in processed form may increase the possibility of consuming insects in unprocessed form in the future. Descriptions should be emotionally neutral and avoid animal references considering, however, the need for transparent and robust evidence. Product safety affects perceptions and attitude regarding red meat consumption and this factor seems to contribute to a far lesser degree to insect consumption due to the fact that insects are less established as a food source. Emotional barriers such as food neophobia play a disproportionately larger role. Highlighting health benefits to vulnerable groups and potential indirect benefits of increasing the perceived social acceptability of insects may result in stable, long-term, positive effects on consumption behaviour. At the end, establishing trusted sources of information about both foods is key to supporting consumption choices reflecting risks as well as benefits.

The communication process of the RBA case study should comprise a participatory dialogue with different stakeholder groups including managers, administrators, consumers, the industry, consumer organisations, and media representatives. The following communication strategy is aimed to guide the promotion of health benefits of edible insects over red meat consumption:

I. Provide understandable scientific evidence about insect consumption to policy makers

In order to provide science-based advice to policy makers with regards to promoting insect consumption, a scientific dialogue between politics and science should be initiated. The scientific findings of the NovRBA study should be prepared in an “application-oriented” short and easily understandable summary supported by visualizations. This should be accompanied by a transparent long version of the study findings, in which policy makers can read in more detail on individual aspects of the study. The long version should be easy to understand and include a transparent description of the applied methodology. In addition, practical recommendations for action could be drafted based on the scientific knowledge, but should clearly be separated from the scientific findings.

II. Create opportunities for consumers to engage in-depth with information about insect consumption

Organising participatory events like information or tasting events for consumers provide opportunities to engage in-depth with information about the relative risks and benefits of insect consumption. They can foster positive experiences, facilitate information-based attitudes towards edible insects and improve the social acceptability of insect consumption. Accompanied by scientists, government and consumer protection organisations they provide a trusted setting – especially compared to events and information by organisations or individuals with commercial interests. Similarly, presenting the RBA case study results in exploratory contexts such as science-based fairs (e.g., the International Green Week in Berlin, Germany or the Food Expo in Athens, Greece) or university events promotes the willingness to engage with scientific information in a playful manner. Government agencies as well as non-profit organisations should provide information about engagement opportunities to individuals through the organisation’s communication channels including their newsletter and social media channels (i.e., Instagram and Twitter).
III. Make use of multipliers who enjoy a high level of trust

In addition to consumers, communication should also be targeted at potential multipliers. Prospective consumer groups such as early adopters can aid the promotion of novel food products. Multipliers who enjoy a high level of trust (e.g., journalists, food bloggers or influencer) can amplify perceived social acceptability and normalise insect consumption. These usually know their audience very good and therefore enable targeted communication strategies that include key messages of the RBA case study and are adjusted to individual consumer needs. For example, when promoting insect diets to vulnerable groups (e.g., individuals at risk from meat consumption, individuals suffering from nutrient deficiency), key messages should highlight the consumption health benefits. When reaching out to multipliers, their preferred communication channels should be used including social media (e.g., Instagram, Twitter, or YouTube), and print media (e.g., information booklets). For example, a very descriptive format for communicating and sharing personal experiences are videos. These can be created by multipliers and further distributed across various social media channels. Showing first-hand consumer experiences of insect consumption, one of the most trusted informational sources, they can aid overcoming emotional barriers. Consumer experiences can for example address taste and appearance of insect food among other factors like the comparatively low price and environmental impact.

IV. Establish informational exchange with trusted sources

Trusted sources such as consumer protection organisations are key to facilitate informed consumption choices. While multipliers might set the ground for the first contact with insect foods by sharing consumer-to-consumer consumption experiences, they are unlikely to raise awareness about long-term health benefits that are not immediately observed. Here, stakeholder groups such as consumer protection and non-profit organisations can be trusted sources with regard to neutral and transparent information about long-term health risks and benefits of insect consumption. For instance, insects can be a source of protein, vitamins and minerals. In comparison to some meat products they also have lower concentrations of cholesterol alongside a favourable n-3/n-6-fatty acid ratio. To increase transparency, trusted stakeholder groups should also raise awareness about scientific uncertainty. For example, when considering the contents of nutrients, vitamins and minerals, the amount consumed must be taken into account. Currently, the majority of products available in some European markets contain only small amounts of insects. In addition, communication of trusted stakeholder groups should be addressed to specific risk perceptions, such as the likelihood of having allergic reactions, heavy metal intake or the transmission of zoonoses. Moreover, communication of trusted sources should take into account emotional barriers that may prevent engagement with informational considerations regarding consumption choices. For example, given the relative novelty of edible insects and the absence of insect food crises compared to the meat industry, consumers’ decisions on insect consumption might be less influenced by transparent information about food safety, i.e., the fact that edible insects offered in the EU exclusively come from controlled breeding (i.e., consumers therefore do not have to fear the use of wild-caught grasshoppers). However, consumer organisations should deliver neutral and transparent information about long-term health risks and benefits of insect consumption. Information about the RBA case study results and public perceptions of edible insects should be conveyed to these stakeholder groups through participatory events.

V. Inform industry about animal reminder disgust

Packaging design and design interventions strongly affect consumers’ decisions on insect consumption. Organising informational or participatory events for manufacturers of insect-derived products and non-profit organizations (representing the interests of the insect production sector) provide an opportunity not only to convey the results of the RBA case study but specifically public risk-benefit perceptions of edible insects. A specific focus should be put on informing that communication relating to insect consumption must avoid triggering animal reminder disgust or increasing risk perception by evoking associations with live animals (through visual or text information). Specifically, animal-shaped visual
imagery (including abstract imagery such as found in logos) should be avoided. Text descriptions should be emotionally neutral and avoid animal references. However, given the importance of trust in informational sources when weighting information received, it is imperative to maintain transparency on the insect content of food.

4. Conclusions

We have applied a risk-benefit assessment approach that allowed for the evaluation of the public health impact of substituting a product including beef meat with an edible insect powder (novel food) in three different EU countries, Greece, France and Denmark. Due to the more favorable nutrient content and the microbiological impact, the expected change in DALYs when moving from the reference scenario to the alternative scenario was estimated to be around 8,753 DALYs (per 100,000) saved in Greece, 6,572 DALYs (per 100,000) saved in Denmark and 21,972 DALYs (per 100,000) saved in France. These results should however be interpreted with caution given that several limitations in the availability of compositional data, in the identification of dose-response associations for the "component-health outcome" pairs identified and in the model development may have impaired the generated results.

The work done in the framework of the NovRBA project can be used for updating methodological approaches that have been developed and are currently widely followed in the field. Moreover, our meetings on model building have been followed by a risk communicator, whose objective was to translate science to a common language to be perceived by stakeholders and the general public. Through addressing all RBA components from: (a) defining the RBA scenario, (b) identifying the profiles of foods under study, (c) building the related health trees, (d) gathering reliable data on dose-response associations and (e) proposing communication strategies, the presently reported case-study can prove a useful tool for risk assessors, risk managers and policy makers, responsible to define dietary advice for the public.

The NovRBA project brought together three different disciplines involved in a risk benefit assessment, i.e. food microbiology, toxicology and nutritional epidemiology. Experienced researchers in RBA and field-specific experts interacted and cooperated to face the challenges emerging, identify sources of uncertainty and finally to have the latter integrated in the model. In doing so, project participants transferred knowledge from their specific discipline which lead to building a documented, tailored process for prioritising model components through the application of standard criteria. The NovRBA project offered to experts of three core disciplines the opportunity to interact so as to build new capacity in performing food-related risk benefit assessments. Hence, the competences gained in the framework of the NovRBA project can be used to update the methodological approach followed in the field.

The work performed within the NovRBA project formed the basis for the doctoral dissertation of a PhD student of NKUA, as this was foreseen in the project’s proposal. The exchange of knowledge among the project partners allowed the PhD student to develop further his knowledge in the fields of food composition, risk assessment and nutrition, and to acquire new knowledge in the fields of predictive microbiology, RBA model implementation and nutritional epidemiology. Within the framework of his ongoing PhD thesis, the NovRBA model will be further tested and adjusted in order to evaluate the substitution impact of other foodstuffs via RBA.

4.1. Impact assessment

The NovRBA project aimed to advance methods and procedures followed in previous assessments of risks and benefits related to dietary substitutions. A priority in the method development was to increase transparency in the decision-making process and enhance harmonization in the selection of model components. Within this context, the identification of the nutritional, microbiological and toxicological profile
of the novel food relied on a systematic review of the literature following a protocol based on PRISMA guidelines. Hence, the food profiling did not depend on experts’ judgments about possibly important food components and the potential impact of this standardized process was the formulation of a detailed, well-documented list of food constituents which provided a concrete background for the subsequent selection of model components. The process was however resource-demanding as three participants were fully engaged in the initial screening of related databases and the data extraction, and two of them repeated the process in the course of the project to check for additional evidence being made available. Furthermore, and as a consequence, an uncommonly large number of food components and health outcomes were considered in the RBA model.

The work undertaken in the NovRBA project in relation to compiling the full profile of A. domesticus constitutes the first attempt to collect data on the insect’s nutrient, microbiological, and toxicological profile, performed under a common systematic methodological framework. Through this process, several knowledge gaps and inconsistencies were identified. Scarcity of data (limited sources); methodological and quality issues of the studies included; heterogeneity introduced due to different samples analyzed and analytical methods performed; and, underreporting of factors that may affect the compositional, microbiological, and toxicological profile of the insect such as rearing conditions and feed were some of the challenges encountered that can have an impact on our current knowledge regarding insects, i.e. novel foods that have recently been authorized for introduction in the European market.

An essential step in the model development includes the selection of nutrients, microbiological agents and chemical hazards that will be considered. The project Consortium developed and tested a harmonized approach which builds on a scoring system that combines occurrence data and severity of disease and which has previously been applied in the field of microbiology. The criteria were tailored to address public health priorities, including the importance of a particular nutrient in the diet as well as in the dietary goals of the population under study. The application of this approach requires the collaboration of different disciplines, including food microbiologists, toxicologists and nutritional epidemiologists. The obvious potential impact relates to transparency in the selection of model components and the ability to replicate the process. Moreover, this approach addresses aspects of the populations’ background diet and can counterbalance the rather strong but necessary assumption that all other dietary intakes remain absolutely unchanged.

According to our findings, around 8,753 DALYs (per 100,000 population) will be saved in Greece, 6,572 DALYs (per 100,000 population) in Denmark and 21,972 DALYs (per 100,000 population) in France, if minced beef in patties was fully substituted with the A. domesticus powder product considered in this assessment. This potential impact is primarily due to the beneficial effects of the subsequent changes in nutrient intakes and exposure to microbiological agents. Among nutrients, the expected reduction in sodium intake which is anticipated to accompany the substitution of beef meat with A. domesticus powder is mainly responsible for the beneficial impact. This observation needs to be taken into consideration when food technologists decide on potential taste enhancers to be added to insect products.

Next to its scientific achievements, the NovRBA project planted the seeds for creating an environment in which risk assessors from various disciplines (food product characterization, epidemiology, microbiology, toxicology) interact with risk communicators to enhance consumers’ understanding and ultimately gain their trust.

Overall, the NovRBA project has advanced previous assessments in the field through progressing specific steps of the process towards tailored and harmonized criteria that have the potential to be adapted to other foods and risk-benefit questions, food consumption scenarios, countries and populations. It also allowed for the inclusion of different components, while ensuring that assessments are done in a documented, standardized approach. Such harmonization will be crucial for the comparison of results across questions and countries.
4.2. Capacity building

With the aim to ensure transparency, the NovRBA project participants built on the methodological approach developed under the RiskBenefit4EU project, an EFSA-supported partnering grant and further enhanced their capacities in:

- planning and implementing a common systematic methodological framework to review the literature in order to collect data on the profile of foods and components thereof that are to be considered in the assessment
- setting standardized criteria for the selection of model components. The NovRBA project developed a strategy to prioritise model components in a transparent, documented and harmonized manner for the selection of components and health outcomes related to microbiology, toxicology and nutrition and
- critically appraising the evidence from meta-analyses of epidemiological studies, using standardized tools to assess the risk of bias. Notably, in past assessments, these selections were based on experts’ judgement, reporting a qualitative description of their choices.

These critical steps can be adopted by other RBA activities. Overall, the work done in the framework of the NovRBA project can be used for updating methodological approaches that have been developed and are currently widely followed in the field.

5. Recommendations

Through the presently reported case-study the NovRBA project Consortium identified limitations in the data retrieved and gaps of knowledge that contribute to uncertainty inherent in the assessment. Related recommendations are listed below and could serve as priorities for generating enhanced pieces of evidence that will further advance future RBA assessments.

- Production of original and standardised compositional data both on novel foods, as well as on dietary staples (such as beef meat), that follow food composition tables’ standards
- Necessity for more high quality dose-response meta-analyses addressing a larger variety of nutrients
- Enhanced understanding of DALYs and identification of additional composite metrics to assess health impacts
- Integration of sociological issues and sustainability indicators for allowing food and nutrition RBA to evolve towards “multiple-criteria decision analysis”.
- Possibility to include in the model exposure/intake data collected through a pan-European harmonised methodology such as that followed in the EFSA EU-Menu survey. Our attempt to get access to these raw data was hampered by impediments imposed by a national data provider who cancelled the process.

Furthermore, an attempt to communicate the findings of a risk benefit assessment of actions to promote public health should rely on a participatory dialogue with different stakeholder groups including managers, administrators, consumers and consumer organisations, the industry and media representatives.
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>A.domesticus</td>
<td>Acheta domesticus</td>
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<tr>
<td>B. cereus</td>
<td>Bacillus cereus</td>
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<tr>
<td>C. perfringens</td>
<td>Clostridium perfringens</td>
</tr>
<tr>
<td>L.monocytogenes</td>
<td>Listeria monocytogenes</td>
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<tr>
<td>CFU</td>
<td>Colony-forming unit</td>
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<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
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<tr>
<td>CI (95%)</td>
<td>95% Confidence Interval</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DALYs</td>
<td>Disability-Adjusted Life Years</td>
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<tr>
<td>DANSDA</td>
<td>Danish National Survey of Diet and Physical Activity</td>
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<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation of the United Nations</td>
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<tr>
<td>GBD</td>
<td>Global Burden of Disease</td>
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<tr>
<td>HBGV</td>
<td>Health Based Guidance Value</td>
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<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>INCA3</td>
<td>Third French Individual and National Food Consumption Survey</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>LOQ</td>
<td>Limit of quantitation</td>
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<td>MS</td>
<td>Member States</td>
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<td>MetS</td>
<td>Metabolic Syndrome</td>
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<td>MoE</td>
<td>Margin of exposure</td>
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<td>PIF</td>
<td>Potential Impact Fraction</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated Fatty Acids</td>
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<td>RBA</td>
<td>Risk-Benefit Assessment</td>
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<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
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<tr>
<td>ROBIS</td>
<td>Risk of Bias Assessment Tool for Systematic Reviews</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>SFA</td>
<td>Saturated Fatty Acids</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>YLD</td>
<td>Years of healthy life lost due to disability</td>
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<tr>
<td>YLL</td>
<td>Years of life lost due to premature mortality</td>
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Appendices

A.1. Nutrition-related health trees

![Nutrition-related health trees diagram]