NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
SCHOOL OF MEDICINE
Section of Social Medicine, Psychiatry and Neurology
Department of Hygiene, Epidemiology and Medical Statistics
Department Chair: Professor P. D. Lagiou

PhD THESIS

Development and implementation of Risk-Benefit Assessment methods to evaluate the replacement of dietary choices and their impact on public health in Greece.

Ermolaos Ververis
Chemist, MSc

ATHENS
September 2024

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ
Τομέας Κοινωνικής Ιατρικής, Ψυχιατρικής και Νευρολογίας
Εργαστήριο Υγιεινής, Επιδημιολογίας και Ιατρικής Στατιστικής
Διευθύντρια: Καθηγήτρια Π. Δ. Λάγιου

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Ανάπτυξη κι εφαρμογή μεθόδων εκτίμησης κινδύνου-οφέλους (Risk-Benefit Assessment) για την αξιολόγηση αντικατάστασης διατροφικών επιλογών και των επιπτώσεων τους στη δημόσια υγεία στην Ελλάδα.

Ερμόλαος Βερβέρης
Χημικός, MSc

ΑΘΗΝΑ
Σεπτέμβριος 2024

PhD THESIS

Development and implementation of Risk-Benefit Assessment methods to evaluate the replacement of dietary choices and their impact on public health in Greece.

CANDIDATE

Ermolaos Ververis

THREE-MEMBER ADVISORY COMMITTEE

Androniki Naska, Professor, School of Medicine, National and Kapodistrian University of Athens (supervisor)

Géraldine Boué, Associate Professor, Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering, Oniris/Secalim

Evangelia Samoli, Professor, School of Medicine, National and Kapodistrian University of Athens

TIMELINE

Assignment of the advisory committee: December 18, 2018

Assignment of the PhD topic: February 25, 2019

Submission of thesis: August 20, 2024

Examination: September 17, 2024

SEVEN-MEMBER EXAMINATION COMMITTEE

In addition to the members of the advisory committee:

Pagona D. Lagiou, Professor, School of Medicine, National and Kapodistrian University of Athens

Vasiliki Benetou, Professor, School of Medicine, National and Kapodistrian University of Athens

Gkikas Magiorkinis, Associate Professor, School of Medicine, National and Kapodistrian University of Athens

Charalampos Proestos, Professor, School of Chemistry, National and Kapodistrian University of Athens

OUTCOME OF THE EXAMINATION

Successful defence. Grade: Excellent (10)

Ιπποκρατικός Όρκος

Ορκίζομαι στον Απόλλωνα τον Ιατρό και στον Ασκληπιό και στην Υγεία και στην Πανάκεια και σ΄ όλους τους Θεούς επικαλούμενος την μαρτυρία τους, να τηρήσω πιστά κατά τη δύναμη και την κρίση μου αυτό τον όρκο και το συμβόλαιό μου αυτό. Να θεωρώ αυτόν που μου δίδαξε αυτή την τέχνη ίσο με τους γονείς μου και να μοιραστώ μαζί μου τα υπάρχοντά μου και τα χρήματά μου αν έχει ανάγκη φροντίδας. Να θεωρώ τους απογόνους του ίσους με τ΄ αδέλφια μου και να τους διδάξω την τέχνη αυτή αν θέλουν να τη μάθουν, χωρίς αμοιβή και συμβόλαιο και να μεταδώσω με παραγγελίες, οδηγίες και συμβουλές όλη την υπόλοιπη γνώση μου και στα παιδιά μου και στα παιδιά εκείνου με δίδαξε και στους άλλους μαθητές που έχουν κάνει γραπτή συμφωνία μαζί μου και σ΄ αυτούς που έχουν ορκισθεί στον ιατρικό νόμο και σε κανέναν άλλο και να θεραπεύω τους πάσχοντες κατά τη δύναμή μου και την κρίση μου χωρίς ποτέ, εκουσίως, να τους βλάψω ή να τους αδικήσω. Και να μη δώσω ποτέ σε κανένα, έστω κι αν μου το ζητήσει, θανατηφόρο φάρμακο, ούτε να δώσω ποτέ τέτοια συμβουλή. Ομοίως να μη δώσω ποτέ σε γυναίκα φάρμακο για ν΄ αποβάλει. Να διατηρήσω δε τη ζωή μου και την τέχνη μου καθαρή και αγνή. Και να μη χειρουργήσω πάσχοντες από λίθους αλλά ν΄ αφήσω την πράξη αυτή για τους ειδικούς. Και σ΄ όποια σπίτια κι αν μπω, να μπω για την ωφέλεια των πασχόντων αποφεύγοντας κάθε εκούσια αδικία και βλάβη και κάθε γενετήσια πράξη και με γυναίκες και με άνδρες, ελεύθερους και δούλους. Και ό,τι δω ή ακούσω κατά την άσκηση του επαγγέλματός μου, ή κι εκτός, για τη ζωή των ανθρώπων, που δεν πρέπει ποτέ να κοινοποιηθεί, να σιωπήσω και να το τηρήσω μυστικό. Αν τον όρκο μου αυτό τηρήσω πιστά και δεν τον αθετήσω, είθε ν΄ απολαύσω για πάντα την εκτίμηση όλων των ανθρώπων για τη ζωή μου και για την τέχνη μου, αν όμως παραβώ και αθετήσω τον όρκο μου να υποστώ τα αντίθετα από αυτά.

Hippocratic Oath

I swear by Apollo the physician, and Asclepius, and Hygeia and Panacea and all the gods and goddesses as my witnesses, that, according to my ability and judgement, I will keep this Oath and this contract. To hold him who taught me this art equally dear to me as my parents, to be a partner in life with him, and to fulfil his needs when required; to look upon his offspring as equals to my own siblings, and to teach them this art, if they shall wish to learn it, without fee or contract; and that by the set rules, lectures, and every other mode of instruction, I will impart a knowledge of the art to my own sons, and those of my teachers, and to students bound by this contract and having sworn this Oath to the law of medicine, but to no others. I will use those dietary regimens which will benefit my patients according to my greatest ability and judgement, and I will do no harm or injustice to them. I will not give a lethal drug to anyone if I am asked, nor will I advise such a plan; and similarly, I will not give a woman a pessary to cause an abortion. In purity and according to divine law will I carry out my life and my art. I will not use the knife, even upon those suffering from stones, but I will leave this to those who are trained in this craft. Into whatever homes I go, I will enter them for the benefit of the sick, avoiding any voluntary act of impropriety or corruption, including the seduction of women or men, whether they are free men or slaves. Whatever I see or hear in the lives of my patients, whether in connection with my professional practice or not, which ought not to be spoken of outside, I will keep secret, as considering all such things to be private. So long as I maintain this Oath faithfully and without corruption, may it be granted to me to partake of life fully and the practice of my art, gaining the respect of all men for all time. However, should I transgress this Oath and violate it, may the opposite be my fate.

CURRICULUM VITAE OF THE CANDIDATE

Personal Information

Name	Ermolaos Ververis
Nationality	Greek

Education

Dec 2018 - present Athens, Greece	Ph.D. candidate, School of Medicine, National and Kapodistrian University of Athens
2014 -2016 Helsinki, Finland	M.Sc. in Food Technology- Dairy Technology track , University of Helsinki
2014 - 2016 Copenhagen, Denmark	M.Sc. in Animal derived Food, University of Copenhagen
2013 - 2015 Thessaloniki, Greece	M.Sc. in Food Chemistry & Technology, Aristotle University of Thessaloniki
2008 - 2013 Thessaloniki, Greece	Chemistry Degree, Aristotle University of Thessaloniki

Professional Experience

Mar 2019 - present Parma, Italy	Scientific Officer, Nutrition & Food Innovation Unit, European Food Safety Authority (EFSA) - Safety Assessment of Novel Foods, Human Nutrition, Risk-Benefit Assessment
Aug 2018 - Feb 2019 Parma, Italy	Scientific Technical Assistant, Nutrition Unit, EFSA - Safety Assessment of Novel Foods, Human Nutrition
Dec 2017 - July 2018 Parma, Italy	Scientific Assistant, Nutrition Unit, EFSA, Parma, Italy - Safety Assessment of Novel Foods, Human Nutrition
Dec 2016 - Nov 2017 Parma, Italy	Trainee, Nutrition Unit - Safety Assessment of Novel Foods
May 2016 - Aug 2016 Christchurch, New Zealand	Researcher, Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University - Synergistic effect of various hydrocolloids on the physicochemical behaviour of chemically-acidified milk gels
Nov 2014 - Apr 2015 Helsinki, Finland	Researcher (M.Sc. Thesis Project), Department of Food and Environmental Sciences, Division of Food Technology, University of Helsinki - vitamin B12 in cheese, food GMMs, in situ food fortification, method development
July 2015 - Aug 2015 Copenhagen, Denmark	Researcher (Internship), Department of Food Science, University of Copenhagen - Development of dry-fermented sausages with reduced salt content and evaluation of sensory characteristics

Mar 2015 Copenhagen, Denmark	Panellist, Department of Food Science, University of Copenhagen - fish-sauce sensory trials
Sep 2013- Aug 2014 Thessaloniki, Greece	Researcher (M.Sc. Thesis Project), Laboratory of Food Chemistry & Technology, Aristotle University of Thessaloniki - oxidative stability of plant-derived “milks” with sesame seed oil bodies, method development
Sep 2012 - Dec 2012 Wageningen, the Netherlands	Intern, Top Institute Food & Nutrition/ Wageningen University Research Centre - Replacement of animal fats in spreads and soft cheeses
Sep 2011 - Jun 2012 Thessaloniki, Greece	Researcher (B.Sc. Thesis Project), Laboratory of Food Chemistry & Technology, Aristotle University of Thessaloniki - adulteration detection of plant-derived food supplements, method development
Jul 2011 - Aug 2011 Mytilene, Greece	Intern, General Chemical State Laboratory of Greece, Chemical Service of Aegean, Department of Chemical Services of Mytilene - laboratory accreditation by ISO/IEC 17025, regulatory controls of foods, beverages, drugs, water, electronic database creation
Dec 2009 - Feb 2010 Thessaloniki, Greece	Assistant, Aristotle University of Thessaloniki, Department of Chemistry, Library - Arrangement and classification of scientific publications

Training Highlights

Mar 2024 - present	Personal Leadership Program, EFSA, Parma, Italy
Jun 2024	ILSI Europe Webinar on Current Perspectives on Risk Benefit Assessment
Jun 2022	Day-to-day Negotiation, EFSA, Parma, Italy
Mar 2022	Agile SCRUM Training (project management)
Oct 2021	OECD Webinar on Animal Cell Culture for Food Production
Sep 2021	Communicating Uncertainty in Scientific Assessments, EFSA, Parma, Italy
Feb 2021	7th Cochrane Workshop “Introduction to Cochrane Methodology” Webinar, Athens, Greece
Feb 2021 - May 2021	Epidemiology-Research Methodology II, Department of Hygiene, Epidemiology & Medical Statistics, NKUA
Oct 2020 - Jan 2021	Epidemiology-Research Methodology I, Department of Hygiene, Epidemiology & Medical Statistics, NKUA
Oct 2020	How Science Achievements Reach People and Contribute to a Better Life, Communications and Media Workshop 2020, One Health EJP, BFSA and University of Surrey
Jun 2020	Algae-based Fish and Meat, European Algae Biomass Association (EABA)

Jun 2020	Summer School 2020, One Health, EFSA, Università di Parma, Università Cattolica del Sacro Cuore
Jul 2019	Parma Summer School 2019, “Risk-Benefit in Food Safety and Nutrition”, EFSA, Università di Parma, DTU, SFA
Nov 2018	Risk-Benefit Assessment of Foods: Methods for Quantifying Health Effects (PhD course), DTU, Lyngby, Denmark
Jun 2018	Webinar - Future Meat Alternatives
Mar 2016	Evaluation of Quality on Poultry Meat and Eggs, Lithuanian University of Health Sciences, Kaunas, Lithuania
Jan 2016 - Feb 2016	3rd Food Law Winter School, Wageningen University, Netherlands
Mar 2013	Workshop: "Chemist’s Role in Food Safety and Consumers’ Protection," Association of Greek Chemists, Thessaloniki
Nov 2011 - Dec 2011	4th National Conference on Food, Modern Approaches to Food Hygiene and Safety, Greek Veterinary Company, Thessaloniki

Distinctions & Scholarships

Sep 2018	Most-voted poster presentation by the public (EFSA 3rd Scientific Conference on Science, Food and Society in Parma, Italy)
Feb 2016	1st place at Labelling workshop, Food Law Winter School, Wageningen University, The Netherlands
Sep 2014 - Jun 2016	European Union Grant for participation in Erasmus Mundus
Sep 2013 - May 2015	State Scholarships Foundation (IKY) for postgraduate studies
Mar 2013	Delivering the academic oath at the graduation ceremony, as the graduate with the highest grade (Chemistry Department, Aristotle University of Thessaloniki)
2008 - 2009	State Scholarships Foundation (IKY) for academic performance
2006	Award in national essay competition on the occasion of completing 25 years of Greece’s integration in the European Union

Languages

Greek	Mother tongue
English	University of Michigan, Certificate of Proficiency in English, C2
German	Goethe-Institut, ZMP (Zentrale Mittelstufenprugung), C1
French	Greek Ministry of National Education and Religious Affairs. French Language Certification. LEVEL C1
Italian	Diploma di lingua italiana, livello B2

Membership To Scientific Societies

Association of Greek chemists

Mentorship

Greece and New Zealand: Provided mentorship to B.Sc. and M.Sc. students during their research activities, guiding them through their academic and research activities.

Italy: Mentored three trainees in the EFSA Novel Foods Team over a four-year period, as well as interim and new staff. Provided guidance and support in their professional development, daily tasks, and integration into the team.

Publications

2024

- **Ververis, E.,** Niforou, A., Poulsen, M., Pires, S. M., Federighi, M., Samoli, E., Naska, A., & Boué, G. (2024). Substituting red meat with insects in burgers: Estimating the public health impact using risk-benefit assessment. *Food and Chemical Toxicology*. https://doi.org/10.1016/j.fct.2024.114764
- EFSA Scientific Committee, More, S. J., Benford, D., Hougaard Bennekou, S., Bampidis, V., Bragard, C., Halldorsson, T. I., Hernández-Jerez, A. F., Koutsoumanis, K., Lambré, C., Machera, K., Mullins, E., Nielsen, S. S., Schlatter, J., Schrenk, D., Turck, D., Naska, A., Poulsen, M., Ranta, J., Sand, S., Wallace, H., Bastaki, M., Liem, D., Smith, A., **Ververis, E.**, Zamariola, G., & Younes, M. (2024). Guidance on risk–benefit assessment of foods. *EFSA Journal, 22*(7), e8875. https://doi.org/10.2903/j.efsa.2024.8875
- EFSA, Afonso, A. L.**,** Gelbmann, W., Germini, A., Fernández, E. N., Parrino, L., Precup, G., & **Ververis, E.** (2024). EFSA Scientific Colloquium 27: Cell Culture-derived Foods and Food Ingredients. *EFSA Supporting Publications*, 21(3), 8664E.
- Precup, G.**,** Marini, E., Zakidou, P., Beneventi, E., Consuelo, C., Fernández-Fraguas, C., García Ruiz, E., Laganaro, M., Magani, M., Mech, A., Noriega Fernández, E., Nuin Garciarena, I., Rodríguez Fernández, P., Roldán Torres, R., Rossi, A., Ruggeri, L., Suriano, F., **Ververis, E.**, Liu, Y., & Germini, A. (2024). Novel foods, food enzymes, and food additives derived from food by-products of plant or animal origin: principles and overview of the EFSA safety assessment. *Frontiers in Nutrition*, 11, 1390734. https://doi.org/10.3389/fnut.2024.1390734

2023

- Mendes, V.**,** Niforou, A., Kasdagli, M. I., **Ververis, E.**, & Naska, A. (2023). Intake of legumes and cardiovascular disease: A systematic review and dose–response meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases*, 33(1), 22-37. https://doi.org/10.1016/j.numecd.2022.10.006
- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens)**,** Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Aguilera-Gómez, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Siskos, A., Schlatter, J. R., van Loveren, H., Zakidou, P., **Ververis, E.**, & Knutsen, H. K. (2023). Scientific Opinion on the safety of UV-treated powder of whole yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 21(5), 8009, 32 pp. https://doi.org/10.2903/j.efsa.2023.8009
- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens)**,** Turck, D., Aguilera-Gómez, M., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf,

I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Prieto Maradona, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Siskos, A., Schlatter, J., van Loveren, H., Zakidou, P., Mendes, V., **Ververis, E.**, & Knutsen, H. K. (2023). Scientific Opinion on the safety of partially hydrolysed protein from spent barley (Hordeum vulgare) and rice (Oryza sativa) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 21(9), 8064, 28 pp. https://doi.org/10.2903/j.efsa.2023.8064

- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens)**,** Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Aguilera-Gómez, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Siskos, A., Schlatter, J., van Loveren, H., Muñoz González, A., Rossi, A., **Ververis, E.**, & Knutsen, H. K. (2023). Scientific Opinion on the safety of an ethanolic extract of the dried biomass of the microalga Phaeodactylum tricornutum as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 21(7), 8072, 27 pp. https://doi.org/10.2903/j.efsa.2023.8072
- Ackerl, R.**,** Mendes, V., Germini, A., McArdle, H. J., Neuhäuser-Berthold, M., Roldán-Torres, R., & **Ververis, E.** (2023). Assessment of Protein Quality in Novel Foods by the European Food Safety Authority: Methodology and Challenges. *Proceedings*, 91(1), 44. https://doi.org/10.3390/proceedings2023091044
- Nuin Garciarena, I.**, Albert**, O., **Ververis, E.**, Fernández Dumont, A., Laganaro, M., Muñoz González, A., Noriega Fernández, E., Precup, G., Roldán Torres, R., Rossi, A., Germini, A., & Kass, G. E. N. (2023). Approach and challenges in the toxicological and allergenicity risk assessment of novel proteins and their sources. *Toxicology Letters*, Volume 384, Supplement 1, Pages S296-S297. https://doi.org/10.1016/S0378-4274(23)00945-1
- Rossi, A.**,** Albert, O., Kouloura, E., Laganaro, M., Muñoz González, A., Noriega Fernández, E., Nuin Garciarena, I., Rivero Pino, F., Zakidou, P., **Ververis, E.**, Germini, A., & Kass, G. E. N. (2023). Data gaps in the risk assessment of Cannabidiol (CBD) as a Novel Food. *Toxicology Letters*, Volume 384, Supplement 1, Page S298. https://doi.org/10.1016/S0378-4274(23)00949-9

2022

- **Ververis, E.,** Boue, G., Poulsen, M., Pires, S. M., Niforou, A., Thomsen, S. T., Tesson, V., Federighi, M., & Naska, A. (2022). A systematic review of the nutrient composition, microbiological and toxicological profile of Acheta domesticus (house cricket). *Journal of Food Composition and Analysis*, *114*, 104859. https://doi.org/10.1016/j.jfca.2022.104859
- Boué, G., **Ververis**, E., Niforou, A., Federighi, M., Pires, S. M., Poulsen, M., Thomsen, S. T., & Naska, A. (2022). Risk–benefit assessment of foods: Development of a methodological framework for the harmonized selection of nutritional, microbiological, and toxicological components. *Frontiers in Nutrition, 9*, 951369. https://doi.org/10.3389/fnut.2022.951369
- Yann Devos**,** Maria Arena, Sean Ashe, Max Blanck, Edward Bray, Alessandro Broglia, Stef Bronzwaer, Angelo Cafaro, Elisa Corsini, Bruno Dujardin, Antonio Fernandez Dumont, Matilde Gomez Garcia, Ciro Gardi, Beatriz Guerra, George E. N. Kass, Angelo Maggiore, Laura Martino, Caroline Merten, Cinzia Percivaldi, Andras Szoradi, Silvia Valtueña Martinez, **Ermolaos Ververis**, Domagoj Vrbos, & Marta Hugas (2022). Addressing the need for safe, nutritious, and sustainable

food: Outcomes of the "ONE–Health, Environment & Society–Conference 2022". *Trends in Food Science & Technology*, 129, 164-178. https://doi.org/10.1016/j.tifs.2022.09.014

- Naska, A.**, Ververis, E.**, Niforou, A., Pires, S. M., Poulsen, M., Jakobsen, L. S., Becker, N., Lohmann, M., Tesson, V., & Federighi, M. (2022). Novel foods as red meat replacers–an insight using Risk Benefit Assessment methods (the NovRBA project). *EFSA Supporting Publications*, 19(5), 7316E.
- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens)**,** Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Schlatter, J., van Loveren, H., **Ververis, E.**, & Knutsen, H. K. (2022). Scientific Opinion on the safety of dried coffee husk (cascara) from Coffea arabica L. as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 20(2), 7085, 16 pp. https://doi.org/10.2903/j.efsa.2022.7085
- EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA panel), Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Schlatter, J. R., van Loveren, H., **Ververis**, E., & Knutsen, H. K. (2022). Scientific opinion on the safety of frozen and freeze-dried formulations of the lesser mealworm (Alphitobius diaperinus larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal, 20*(7), 7325. https://doi.org/10.2903/j.efsa.2022.7325

2021

- Boehm, E., Borzekowski, D., **Ververis**, E., Lohmann, M., & Böl, G.-F. (2021). Communicating food risk-benefit assessments: Edible insects as red meat replacers. *Frontiers in Nutrition*. https://doi.org/10.3389/fnut.2021.711127
- Thomsen, S. T., Assunção, R., Afonso, C., Boué, G., Cardoso, C., Cubadda, F., Garre, A., Kruisselbrink, J. W., Mantovani, A., Pitter, J. G., Poulsen, M., Verhagen, H., **Ververis**, E., van der Voet, H., Watzl, B., & Pires, S. M. (2021). Human health risk–benefit assessment of fish and other seafood: A scoping review. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2021.1915240
- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens**)**, Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Schlatter, J. R., van Loveren, H., **Ververis, E.**, & Knutsen, H. K. (2021). Scientific Opinion on the safety of dried yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19(1), 6343, 29 pp. https://doi.org/10.2903/j.efsa.2021.6343
- EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens)**,** Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Schlatter, J., van Loveren, H., **Ververis, E.**, & Knutsen, H. K. (2021). Scientific Opinion on the safety of frozen and dried formulations from whole yellow mealworm (Tenebrio molitor larva) as a

novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19(8), 6778, 30 pp. https://doi.org/10.2903/j.efsa.2021.6778

2020

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Participation in Conferences

Participation in and presentation to various conferences and scientific events including: AOAC international Midwest (2024, oral), 17th Hellenic Congress in Nutrition & Dietetics (2023, oral), 37th International Conference of the European Federation of Food Science and Technology EFFoST (2023, oral & poster), ILSI Annual Symposium (2023, oral), 10th International Conference on Nutrition &

Growth (2023, oral), International Burden of Disease Conference (2022, oral), 5th Chemistry Conference of Graduate, Postgraduate Students and PhD Candidates in Aristotle University of Thessaloniki (2022, oral), Ελληνικό Φόρουμ Επιστήμης και Τεχνολογίας Λιπιδίων (Greek Lipid Forum) (2021, oral), 35th EFFoST International Conference (2021, oral), 2nd LARAS: Latin American and the Caribbean Risk Assessment Symposium (2021, oral), 3rd International Conference on Food Contaminants ICFC (2019, oral), 13th Federation of European Nutrition Societies FENS (2019, poster)

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Abstract

Introduction

Food systems and dietary habits evolve in response to societal norms, individual preferences, health priorities, and environmental concerns. Growing awareness of the health and environmental impacts associated with red meat consumption has increased interest in novel, alternative protein sources, including edible insects. This study evaluates the potential of house cricket (*Acheta domesticus*) as a substitute for red meat using Risk-Benefit Assessment (RBA) methods. We developed a harmonized and standardized RBA methodological framework to assess the nutritional, microbiological, and toxicological profiles of house crickets and minced beef, as well as the health outcomes associated with their components. In addition, drawing on sociological aspects and public knowledge about these two types of food, we explored strategies to effectively communicate the study findings.

Materials and Methods

Using literature data and reports from European national food authorities, we initially selected an insect species with a nutrient profile and food technological prospects to replace red meat, as well as with high commercial potential in the EU market, i.e., house cricket. We conducted a comprehensive data compilation on dried and undried insect's forms, employing a systematic framework for data retrieval, extraction, and collation, creating new food composition tables for this novel food. A harmonized framework was developed to select the most relevant compositional components for RBA, considering nutrient content and hazard occurrence, health outcome severity, and public health implications. For health outcomes, meta-analyses were utilized reporting on associations with the intake of nutrients and toxicological elements. In microbiology, we used disease incidence and source attribution, as well as safety thresholds and exponential dose-response models for specific microbial agents. Using a probabilistic approach (through Monte Carlo simulations), we assessed the public health impact of substituting beef with cricket powder in burger patties, in the adult populations of Denmark, France, and Greece. To quantify the overall health impact, we used disability-adjusted life years (DALY) as a common metric, with the respective values retrieved from the Global Burden of Disease database. Communication strategies were developed through comprehensive literature reviews about risk perceptions, knowledge levels, and information needs of the public related to red meat consumption and entomophagy in Europe.

Results

The findings of our study indicate that house cricket powder may be a viable dietary alternative to red meat. However, the health impact of this alternative is contingent upon the quantity utilized and the specific formulation of the food product in question. The sodium content emerged as a critical factor influencing the overall health impact. While house cricket powder is generally safe, it is not always a healthier alternative to beef. The incorporation of cricket powder into burger patties in a considered manner could result in a positive health impact; however, further research is needed to address existing uncertainties and data gaps. The effective communication of RBA results must consider emotional and cognitive factors, utilise trusted information sources, and reflect cultural contexts to support informed dietary choices and decision-making.

Conclusions

The potential of house cricket as a meat substitute is promising when this novel food ingredient is incorporated thoughtfully into recipes and product development. Our findings and the developed methodological framework emphasise the importance of continuous research and the refinement of RBA methodologies. This is crucial for addressing emerging food safety and nutrition challenges, particularly in light of the prevailing dietary shift trends and the need for swift, informed, and science-based decision-making that takes into account both health risks and benefits.

Subject Area: Public Health, Risk-Benefit Assessment (RBA), Nutritional Epidemiology, Method Development, Food Composition Tables, Food Safety, Risk-Benefit Communication

Keywords: Red meat substitution, Red meat replacement, Alternative proteins, Novel Foods, Edible insects, Risk-Benefit Assessment (RBA), Dietary Shifts, Probabilistic Modelling

Περίληψη

Εισαγωγή

Τα διατροφικά συστήματα και οι διατροφικές συνήθειες εξελίσσονται ως απάντηση στα κοινωνικά πρότυπα, τις ατομικές προτιμήσεις, τις προτεραιότητες για την υγεία και τους περιβαλλοντικούς προβληματισμούς. Η αυξανόμενη ευαισθητοποίηση σχετικά με τις επιπτώσεις στην υγεία και το περιβάλλον που συνδέονται με την κατανάλωση κόκκινου κρέατος έχει αυξήσει το ενδιαφέρον για νέες, εναλλακτικές πηγές πρωτεϊνών, συμπεριλαμβανομένων των βρώσιμων εντόμων. Η παρούσα μελέτη αξιολογεί τις δυνατότητες του οικιακού γρύλου (*Acheta domesticus*) ως υποκατάστατο του κόκκινου κρέατος χρησιμοποιώντας την προσέγγιση αξιολόγησης επικινδυνότητας-οφέλους (RBA). Αναπτύξαμε ένα εναρμονισμένο και τυποποιημένο μεθοδολογικό πλαίσιο RBA για την αξιολόγηση των διατροφικών, μικροβιολογικών και τοξικολογικών προφίλ του οικιακού γρύλου και του μοσχαρίσιου κρέατος, καθώς και των υγειονομικών επιπτώσεων που συνδέονται με τα συστατικά τους. Επιπλέον, αξιοποιώντας κοινωνιολογικές πτυχές και τις γνώσεις του κοινού σχετικά με αυτούς τους δύο τύπους τροφίμων, διερευνήσαμε στρατηγικές για την αποτελεσματική επικοινωνία των ευρημάτων της μελέτης.

Υλικά και Μέθοδοι

Χρησιμοποιώντας βιβλιογραφικά δεδομένα και αναφορές από ευρωπαϊκές εθνικές αρχές τροφίμων, επιλέξαμε αρχικά ένα είδος εντόμου με διατροφικό προφίλ και τεχνολογικές προοπτικές για την αντικατάσταση του κόκκινου κρέατος, καθώς και με υψηλή εμπορική δυνατότητα στην αγορά της ΕΕ, δηλαδή τον οικιακό γρύλο. Πραγματοποιήσαμε μια εκτενή συλλογή δεδομένων για τις αποξηραμένες και μη αποξηραμένες μορφές του εντόμου, χρησιμοποιώντας ένα συστηματικό πλαίσιο για την ανάκτηση, εξαγωγή και ομαδοποίηση δεδομένων, δημιουργώντας νέους πίνακες σύνθεσης τροφίμων για αυτό το καινοφανές τρόφιμο. Αναπτύχθηκε ένα εναρμονισμένο πλαίσιο για την επιλογή των πιο συναφών συστατικών για την RBA, λαμβάνοντας υπόψη την περιεκτικότητα σε θρεπτικά συστατικά και παράγοντες κινδύνου, τη σοβαρότητα του αποτελέσματος για την υγεία και τις συνέπειες για τη δημόσια υγεία. Για τα αποτελέσματα υγείας, χρησιμοποιήθηκαν μετα-αναλύσεις για τα διατροφικά στοιχεία και τα στοιχεία τοξικολογικού χαρακτήρα. Στην μικροβιολογία, χρησιμοποιήσαμε την εμφάνιση νόσων και την απόδοση της πηγής, καθώς και κατώτατα όρια ασφαλείας και εκθετικά μοντέλα δόσης-απόκρισης για συγκεκριμένους μικροβιακούς παράγοντες. Χρησιμοποιώντας πιθανολογική προσέγγιση (μέσω προσομοιώσεων Monte Carlo) αξιολογήσαμε την επίπτωση στη δημόσια υγεία της υποκατάστασης του βόειου κρέατος με σκόνη γρύλου σε μπιφτέκια, στους ενήλικες πληθυσμούς της Δανίας, της Γαλλίας και της Ελλάδας. Για να ποσοτικοποιήσουμε τη συνολική επίπτωση στην υγεία, χρησιμοποιήσαμε το δείκτη των Σταθμισμένων Ετών Ζωής ως προς την Ανικανότητα (DALY) ως κοινό μετρικό σύστημα, με τις αντίστοιχες τιμές να έχουν ανακτηθεί από τη βάση δεδομένων Global Burden of Disease. Οι στρατηγικές επικοινωνίας αναπτύχθηκαν μέσω εκτενών βιβλιογραφικών ανασκοπήσεων σχετικά τις αντιλήψεις κινδύνου, τα επίπεδα γνώσεων και τις ανάγκες πληροφόρησης του κοινού αναφορικά με την κατανάλωση κόκκινου κρέατος και την εντομοφαγία στην Ευρώπη.

Αποτελέσματα

Τα ευρήματα της μελέτης μας δείχνουν ότι η σκόνη γρύλου μπορεί να αποτελέσει μια βιώσιμη διατροφική εναλλακτική λύση στο κόκκινο κρέας. Ωστόσο, ο αντίκτυπος αυτής της εναλλακτικής επιλογής στην υγεία εξαρτάται από την ποσότητα σκόνης που χρησιμοποιείται και την τελική σύνθεση του αναπτυγμένου προϊόντος τρόφιμου. Η περιεκτικότητα σε νάτριο αναδείχθηκε ως κρίσιμος παράγοντας που επηρεάζει την συνολική επίπτωση στην υγεία. Ενώ η σκόνη γρύλου σπιτιού είναι γενικά ασφαλής, δεν αποτελεί πάντα μια πιο υγιεινή εναλλακτική λύση για το βόειο κρέας. Η ενσωμάτωση σκόνης γρύλου σε μπιφτέκια με μελετημένο τρόπο θα μπορούσε να έχει θετικό αντίκτυπο στην υγεία. Ωστόσο, απαιτείται περαιτέρω έρευνα για την αντιμετώπιση των υφιστάμενων αβεβαιοτήτων και των κενών δεδομένων. Η αποτελεσματική επικοινωνία των ευρημάτων της RBA πρέπει να λαμβάνει υπόψη συναισθηματικούς και γνωστικούς παράγοντες, να χρησιμοποιεί αξιόπιστες πηγές πληροφόρησης και να αντανακλά τα πολιτισμικά πλαίσια για την υποστήριξη ενημερωμένων διατροφικών επιλογών και λήψης αποφάσεων.

Συμπεράσματα

Η προοπτική του γρύλου ως υποκατάστατο κρέατος είναι πολλά υποσχόμενη, όταν αυτό το καινοφανές συστατικό τροφίμων ενσωματώνεται προσεκτικά σε συνταγές και στην ανάπτυξη προϊόντων. Τα ευρήματά μας και το μεθοδολογικό πλαίσιο που αναπτύχθηκε υπογραμμίζουν τη σημαντικότητα της συνεχούς έρευνας και της βελτίωσης των μεθοδολογιών RBA. Αυτό έχει ζωτική σημασία για την αντιμετώπιση των αναδυόμενων προκλήσεων στον τομέα της ασφάλειας των τροφίμων και της διατροφής, ιδίως υπό το πρίσμα των επικρατουσών τάσεων διατροφικών μετατοπίσεων και της ανάγκης για ταχεία, ενημερωμένη και επιστημονικά τεκμηριωμένη λήψη αποφάσεων που λαμβάνουν υπόψη τόσο τους κινδύνους όσο και τα οφέλη για την υγεία.

Θεματική Περιοχή: Δημόσια Υγεία, Αξιολόγηση Κινδύνου-Οφέλους, Διατροφική Επιδημιολογία, Ανάπτυξη Μεθόδων, Πίνακες Σύνθεσης Τροφίμων, Ασφάλεια Τροφίμων, Επικοινωνία Επικινδυνότητας-Οφέλους

Λέξεις Κλειδιά: Υποκατάσταση Κόκκινου Κρέατος, Αντικατάσταση Κόκκινου Κρέατος, Εναλλακτικές Πρωτεΐνες, Καινοφανή Τρόφιμα, Βρώσιμα Έντομα, Εκτίμηση Κινδύνου-Οφέλους (RBA), Διατροφικές Μετατοπίσεις, Πιθανολογική Μοντελοποίηση

Publications Related to the Thesis

1. **Ververis, E.,** Niforou, A., Poulsen, M., Pires, S.M., Federighi, M., Samoli, E., Naska, A. and Boué, G., 2024. Substituting red meat with insects in burgers: estimating the public health impact using risk-benefit assessment. Food and Chemical *Toxicology*, p.114764.

   Abstract: In Western societies, reducing red meat consumption gained prominence due to health, environmental, and animal welfare considerations. We estimated the public health impact of substituting beef with house cricket (*Acheta domesticus*) in European diets (Denmark, France, and Greece) using the risk-benefit assessment (RBA) methodology, building upon the EFSA-funded NovRBA project. The overall health impact of substituting beef patties with insect powder-containing patties was found to be impacted by the amount of cricket powder incorporated in the patties. While using high amounts of cricket powder in meat substitutes may be safe, it does not inherently offer a healthier dietary option compared to beef. Adjustment of cricket powder levels is needed to yield a positive overall health impact. The main driver of the outcome is sodium, naturally present in substantial amounts in crickets. Moreover, the way that cricket powder is hydrated before being used for the production of patties (ratio of powder to water), influences the results. Our study highlighted that any consideration for dietary substitution should be multidimensional, considering nutritional, microbiological and toxicological aspects, and that the design of new food products in the framework of dietary shifts should consider both health risks and benefits associated with the food.

2. Boué, G., **Ververis, E.,** Niforou, A., Federighi, M., Pires, S.M., Poulsen, M., Thomsen, S.T. and Naska, A., 2022. Risk–Benefit assessment of foods: Development of a methodological framework for the harmonized selection of nutritional, microbiological, and toxicological components. *Frontiers in Nutrition*, 9, p.951369.

   Abstract: Investigating the impact of diet on public health using risk-benefit assessment (RBA) methods that simultaneously consider both beneficial and adverse health outcomes could be useful for shaping dietary policies and guidelines. In the field of food safety and nutrition, RBA is a relatively new approach facing methodological challenges and being subject to further developments. One of the methodological aspects calling for improvement is the selection of components to be considered in the assessment, currently based mainly on non-harmonized unstandardized experts' judgment. Our aim was to develop a harmonized, transparent, and documented methodological framework for selecting nutritional, microbiological, and toxicological RBA components. The approach was developed under the Novel foods as red meat replacers-an insight using Risk-Benefit Assessment methods

(NovRBA) case study, which attempted to estimate the overall health impact of replacing red meat with an edible insect species, *Acheta domesticus*. Starting from the compositional profiles of both food items, we created a "long list" of food components. By subsequently applying a series of predefined criteria, we proceeded from the "long" to the "short list." These criteria were established based on the occurrence and severity of health outcomes related to these components. For nutrition and microbiology, the occurrence of health outcomes was evaluated considering the presence of a component in the raw material, as well as the effect of processing on the respective component. Regarding toxicology, the presence and exposure relative to reference doses and the contribution to total exposure were considered. Severity was graded with the potential contribution to the background diet alongside bioavailability aspects (nutrition), the disability-adjusted life years per case of illness of each hazard (microbiology), and disease incidence in the population, potential fatality, and lifelong disability (toxicology). To develop the "final list" of components, the "short list" was refined by considering the availability and quality of data for a feasible inclusion in the RBA model. The methodology developed can be broadly used in food RBA, to guide and reinforce a harmonized selection of nutritional, microbiological, and toxicological components and will contribute to facilitating RBA implementation, enabling the generation of transparent, robust, and comparable outcomes.

3. **Ververis, E.,** Boue, G., Poulsen, M., Pires, S.M., Niforou, A., Thomsen, S.T., Tesson, V., Federighi, M. and Naska, A., 2022. A systematic review of the nutrient composition, microbiological and toxicological profile of *Acheta domesticus* (house cricket). *Journal of Food Composition and Analysis*, *114*, p.104859.

   Abstract: *Acheta domesticus* is an insect offering several nutritional and technological opportunities for the food industry. After a positive safety assessment as novel foods by the European Food Safety Authority, whole A. domesticus ingredients aspire to gain their share on consumers' plates. Through a systematic literature review, we describe the nutrient, microbiological, and toxicological profiles of undried and dried forms of A. domesticus. Both dried and undried forms contain a vast array of macro and micronutrients, with protein and minerals reported in considerable amounts in the dried forms. A heating step is the minimum requirement to meliorate the microbiological safety and stability of both forms. The toxicological profile of *A. domesticus* does not raise safety concerns per se, with the concentrations of contaminants in *A. domesticus* forms dependent on the contaminants' level in the insects' feed. Considerations of how to produce harmonized and robust compositional data on edible insects are discussed.

4. Boehm, E., Borzekowski, D., **Ververis, E.,** Lohmann, M. and Böl, G.F., 2021. Communicating food risk-benefit assessments: edible insects as red meat replacers. *Frontiers in Nutrition*, 8, p.749696.

   Abstract: Risk-benefit Assessment (RBA) is an emerging methodology in the area of Food and Nutrition that offers a simultaneous evaluation of both risks and benefits linked to dietary choices. Communication of such research to consumers may present a challenge due to the dual nature of RBA. We present a case study of a communication strategy developed for the NovRBA-project. The NovRBA-project (Novel foods as red meat replacers-an insight using Risk Benefit Assessment methods) performed a risk-benefit assessment to evaluate the overall health impact of substituting red meat (beef) by a novel food (house cricket), considering the microbial, toxicological and nutritional characteristics of the respective dietary choices. A literature review of risk perceptions and acceptance of beef and insects as food formed the basis of the communication strategy for the study's results, drawing on environmental and emotional as well as health-related motivations to consume or avoid either food and considering the sociodemographic characteristics of likely consumers. Challenges and future directions for consumer protection organizations communicating findings of risk-benefit analyses on food safety are discussed.

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Abbreviations

ANSES	Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail
BIOHAZ	EFSA Panel on Biological Hazards
BRAFO	Benefit-Risk Analysis for Foods
CHD	Coronary Heart Disease
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CVD	Cardiovascular Disease
DALY	Disability-Adjusted Life Years
DANSDA	Danish National Survey of Diet and Physical Activity
DRVs	Dietary Reference Values
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FAO/INFOODS	FAO International Network of Food Data Systems
FASFC	Federal Agency For The Safety of The Food Chain (Belgium)
GBD	Global Burden of Disease
GHG	Greenhouse Gas
GMO	Genetically Modified Organism
HACCP	Hazard Analysis Critical Control Point
HBGV	Health-Based Guidance Value
HFA-DB	European Health for All Database
IARC	International Agency for Research on Cancer
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IHME	Institute for Health Metrics And Evaluation
INCA3 survey	Third French Individual and National Food Consumption Survey
MCDA	Multi-Criteria Decision Analysis
MS	Member States
MUFA	Monounsaturated Fatty Acids
NDA	EFSA Panel on Nutrition, Novel Foods And Food Allergens

PICO	Patient/Population, Intervention, Comparison, Outcome
PBDE	Polybrominated Diphenyl Ethers
PIF	Potential Impact Fraction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	Polyunsaturated Fatty Acids
RBA	Risk-Benefit Assessment
ROBIS	A Risk Of Bias Assessment Tool for Systematic Reviews
RR	Relative Risk
RRalt	Relative Risk Alternative Scenario
RRref	Relative Risk Reference Scenario
SFA	Saturated Fatty Acids
spp	species pluralis (multiple species)
STEC	Shiga Toxin-Producing *Escherichia Coli*
STR	Serum Transferrin Receptor
TNF-α	Tumour Necrosis Factor α
TWI	Tolerable Weekly Intake
YLD	Years Lived with Disability
YLL	Years of Life Lost
WHO	World Health Organization

General Part

1. Introduction

1.1. Red meat consumption in the Western world

Red meat, referring to all mammalian muscle meat including, beef, pork, lamb, and veal, holds a prominent place in the dietary practices of several individuals in the Western world, driven by deep-rooted cultural, economic, and social traditions. This pronounced consumption is reflected in several European countries, the United States, Canada, and Australia, where red meat is a staple (WHO, 2023). Despite the growing trends toward vegetarian, plant-based and alternative diets (Hassoun et al., 2022), red meat remains integral to many Western diets, a preference mirrored in the substantial increase in meat consumption globally, often in amounts well beyond the national dietary recommendations (FAO, 2018). This rise can be attributed to increasing incomes, which correlate with higher meat consumption, indicating the persistent demand for red meat as a key dietary component (Libera et al., 2021). Other factors such as availability, convenience, preference for energy-dense and nutrient-rich food, habits and societal norms have a significant role too in shaping the current meat consumption patterns (Godfray et al., 2018).

The perception of red meat's role in diets varies significantly between developed and developing nations. In the Western world, red meat is often scrutinized for its association with non-communicable diseases (NCDs) such as cardiovascular diseases, type II diabetes, and certain types of cancer. Public health campaigns frequently highlight the health risks associated with high red meat consumption, advocating for moderation and the inclusion of alternative protein sources. Conversely, in developing countries, red meat is viewed as a valuable resource for combating malnutrition and enhancing food security (Adesogan et al., 2020). This dichotomy underscores the complex role red meat plays globally, where its benefits and risks are weighed differently based on regional economic and health contexts.

1.1.1. Health Risks associated with Red Meat Consumption

The consumption of red meat, particularly in its processed forms, has been associated with an increased risk of several chronic diseases, for excess intakes (Grosso et al., 2022; Libera et al., 2021; Zhang et al., 2023).

Studies indicate that diets high in red and processed meat can lead to elevated intake levels of cholesterol and saturated fats, contributing to an increased risk of ischemic heart disease (Papier et al., 2023) and stroke (Bernstein et al., 2012; de Medeiros et al., 2023; Micha et al., 2010). Moreover, consumption of both processed and unprocessed red meat has been associated with a higher incidence of cardiovascular disease (CVD), compared to diets lower in red meat (Shi et al., 2023).

In 2015, the International Agency for Research on Cancer (IARC) classified red meat as "probably carcinogenic to humans", based on evidence suggesting that daily consumption of 100 grams of red meat is associated with a 17% increased risk of developing various types of cancer (Bouvard et al., 2015). High red meat consumption is particularly associated with colorectal cancer (Larsson & Wolk, 2006), likely due to compounds such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) that form when meat is cooked at high temperatures (Chiavarini et al., 2017; Cross et al., 2010). Potential association with other types of cancer such as prostate (Cross et al., 2005; Sinha et al., 2009) and pancreatic (Anderson et al., 2002; Stolzenberg-Solomon et al., 2007) has been mentioned. High red meat intake has been strongly associated with metabolic disorders like type II diabetes (Feskens et al., 2013; Gu et al., 2023). The high levels of haem iron in red meat can induce oxidative stress and inflammation, leading to insulin resistance (White & Collinson, 2013). Furthermore, red meat often has a high caloric density, contributing to obesity, a significant risk factor for diabetes.

Red meat also poses microbiological safety concerns, accounting for a substantial amount of the total European foodborne outbreaks (de Oliveira Mota et al., 2020; EFSA & ECDC, 2018). Increased risk for *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) infections and outbreaks have been attributed to red meat (Omer et al., 2018). Recent changes in handling and consumption practices, such as longer storage before consumption and the increasing preference for raw meat, may present new challenges to the microbiological safety of meat products.

1.1.2. Health Benefits associated with Red Meat Consumption

Despite the risks, red meat can contribute positively to a balanced diet, when moderately consumed. Red meat is a dietary source of high-quality protein, containing a well-balanced array of essential amino acids, readily absorbed by the human body. Moreover, red meat is a source of essential micronutrients such as cyanocobalamin (vitamin B12), iron, and zinc (Cocking et al., 2020; De Smet & Vossen, 2016). Its iron, being in the haem form, is more readily absorbed by the body compared to non-haem iron found in plant-derived foods (van Wonderen et al., 2023), rendering red meat beneficial in preventing iron-deficiency anaemia, a common condition, especially among women and children (Czerwonka & Tokarz, 2017). Red meat also provides substantial amounts of zinc, crucial for immune function and wound healing (EFSA NDA Panel, 2015). Additionally, it contains B vitamins, particularly B12 (Gille & Schmid, 2015; Obeid et al., 2019), essential among others for nerve function and the production of red blood cells. Vitamin B12 deficiency can lead to serious health issues, including pernicious anaemia and neurological disorders (Green et al., 2017).

1.1.3. Environmental Impact of Red Meat Consumption

The environmental impact associated with red meat consumption is a critical issue, especially within the context of global climate change and sustainability challenges. Red meat production is resource-intensive, requiring substantial water, land, and feed (Godfray et al., 2018). The livestock sector accounts for about 14.5% of global greenhouse gas (GHG) emissions, with cattle being a major contributor (FAO, 2017). Methane, a potent GHG, is produced during digestion in ruminant animals like cattle and sheep (Beauchemin et al., 2020). High GHG emissions from livestock farming for red meat production and milk contribute to 55% of agricultural emissions globally (Romanello et al., 2022). Red meat production is also linked to deforestation and habitat destruction. Large areas of forests, notably in the Amazon, are cleared for pastureland or feed crops, leading to biodiversity loss and reduced carbon dioxide absorption (Sombroek & Higuchi, 2003). Moreover, wastewater from industrial livestock production contributes substantially to waterway pollution (Mallin & Cahoon, 2003). Additionally, the use of antibiotics and hormones in livestock farming raises concerns about environmental contamination and antibiotic-resistant bacteria (WHO, 2023). With the climate crisis intensifying, livestock producers may face challenges from rising temperatures and extreme weather conditions, affecting livelihoods and food security. This highlights the need for more evidence from diverse agrifood production systems (Dwivedi et al., 2017).

1.1.4. Red meat consumption and animal welfare/ethics considerations

Red meat consumption raises also animal welfare and ethical concerns. Industrial livestock farming often involves practices detrimental to animal well-being, such as overcrowding, lack of natural behaviours, and the use of growth hormones and antibiotics (Cozzi et al., 2009; Tucker et al., 2015). These practices can lead to physical and psychological stress in animals, raising ethical questions about humane treatment (Kumar et al., 2023). Ethical implications extend to issues of moral responsibility and sustainability (Broom, 2018). The industrial production of red meat challenges consumers and producers to consider the moral dimensions of their dietary choices (Croney & Swanson, 2023). While not all consumers share these concerns, the number of individuals who do is growing (European Commission, 2023). This debate is further complicated by cultural norms and economic factors influencing farming practices (Ahmed et al., 2023; Jerlström et al., 2022). Thus, discussions about red meat consumption and animal welfare require understanding ethical considerations alongside environmental and economic realities.

1.2. Novel protein sources as dietary alternatives to red meat

Due to growing awareness of the health risks, environmental impact, and ethical concerns associated with meat production and consumption, the reduction of meat consumption, particularly red meat, is a major driver for changing food systems in the Western world (Dagevos, 2021; Devos et al., 2022; Post, 2012). This shift is gaining ground among consumers, policymakers, and the food industry, leading to the emergence of dietary alternatives aspiring to serve as red meat substitutes (Ekmekcioglu et al., 2018; Onwezen et al., 2021). In the European Union, these alternatives include foods traditionally consumed by Europeans, such as pulses (Estell et al., 2021; Tacon et al., 2020; Thomsen et al., 2018), as well as novel, innovative foods and food ingredients derived from sources like algae, cell cultures, microorganisms, fungi, and insects (Hadi & Brightwell, 2021; Van der Spiegel et al., 2013; van der Weele et al., 2019).

Algae, including microalgae and macroalgae (seaweeds), are promising alternative protein sources due to their high growth rates, photosynthetic efficiency, low water consumption, and non-reliance on arable land (Fasolin et al., 2019). Microalgae have attracted attention for their potential in meat analogue[1] production due to their high protein content, exceeding in certain cases 60% in their dried forms (Mosibo et al., 2024; Severo et al., 2024). Macroalgae's protein content has been reported in lower ranges (Brien et al., 2022). Microalgae's growth rate surpasses that of conventional crops, highlighting their efficiency as a biomass resource (Van Krimpen et al., 2013). Despite these benefits, the industrial-scale cultivation of microalgae is currently unsustainable, and social acceptance poses a significant barrier to their integration into the food industry (Fu et al., 2021). Challenges in protein extraction, purification, and concentration remain, as environmental and species-specific factors affect protein content and the presence of toxic or allergenic compounds (Mosibo et al., 2024). Furthermore, protein quality of commonly used microalgal species like *Chlorella* sp. and *Arthrospira* sp. has been mentioned to be lower than that of beef, raising concerns about their nutritional adequacy as meat (Fu et al., 2021).

Cell culture-derived "meat", also known as "cultured meat", "lab-grown meat", or "cell-based meat", aims to represent an innovative protein source produced by cultivating animal cells in controlled environments such as bioreactors, mimicking the natural development of meat tissues (EFSA et al., 2024). This process combines tissue engineering and cell culture techniques, allowing to produce animal-derived foods by propagating animal cells without further impacting the animals, avoiding the need for traditional animal farming and slaughter (FAO & WHO, 2023). A significant milestone was

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[1] products devoid of meat yet designed to simulate its taste and texture

achieved a decade ago when academics presented a proof-of-concept for a beef-cell culture-derived burger (Post, 2014). This method not only addresses animal welfare concerns but also holds promises for reduced environmental impact, enhanced food security, and improved food safety. Additionally, similar approaches are being explored, although to a lesser extent, for producing cell culture-derived foods and ingredients from plant cells. In the European Union, if the source and cells used do not fall under the Genetically Modified Organism (GMO) regulatory framework, such cell culture-derived food products are classified as "novel foods" (EFSA et al., 2024).

Precision fermentation, a process that utilizes microorganisms to produce specific target products, including proteins, through controlled production systems, represents a promising approach for producing novel proteins to be used in the production of meat analogues. Engineered microorganisms are programmed to produce recombinant proteins via large-scale fermentation, potentially replacing animal-derived proteins. In the European Union, ingredients derived from precision fermentation require pre-market authorization under various regulatory frameworks (EFSA et al., 2024). This regulatory step ensures that these innovative products meet safety and quality standards before reaching consumers.

Mycoprotein is a single-cell protein-rich food derived from the aerobic fermentation of filamentous fungi such as *Monascus purpureus*, *Aspergillus oryzae*, *Paradendryphiella salina*, *Neurospora intermedia, Rhizopus oryzae*, and *Fusarium venenatum* (Majumder et al., 2024). Among the most studied filamentous fungi is *F. venenatum*, a fungus commonly found in soil (Kumar et al., 2017), and is compatible with large-scale fermentation systems (Gastaldello et al., 2022). Mycoprotein has been studied as an alternative to meat, due to its desirable fibrous structure and functional properties (Ahmad et al., 2022). Noteworthily, it has been reported that the carbon footprint of mycoprotein is ten-folds less than that of beef (Majumder et al., 2024). As the academic research and industrial efforts evolve, mycoprotein is well-positioned to potentially have a crucial role in providing sustainable and nutritious alternatives to meat (Khan et al., 2024), provided the absence of consumption-related food safety concerns.

Last but not least, among the alternative protein sources gaining attention, insects stand out as a novel, unconventional option for the Western world. Insects such as crickets, mealworms, and grasshoppers are highly valued in many cultures for their protein content, vitamins, and minerals (Lange & Nakamura, 2021). Compared to conventional livestock farming, insect farming presents a lower environmental impact, characterized by markedly reduced GHG emissions and resource consumption (Ros-Baro et al., 2022). This perspective sets the stage for understanding the growing interest and challenges associated with integrating insects and products thereof into mainstream food systems.

Additionally, while consumers increasingly prioritize animal welfare concerns in the context of traditional livestock farming, they do not exhibit yet the same level of consideration when evaluating the ethical implications of consuming insects (Delvendahl et al., 2022). The subsequent sections examine the status of entomophagy in the Western world and more specifically in the European Union (EU), describe safety concerns associated with insect consumption and explore potential health benefits.

1.2.1. Insects as a novel dietary source in the European Union

Entomophagy, i.e., the consumption of insects by humans, is prevalent in many cultures worldwide (Ramos-Elorduy, 2009) but remains relatively novel in Western countries (Collins et al., 2019; Sogari et al., 2019; Svanberg & Berggren, 2021). Within the EU, insects intended for human consumption are categorized as "novel foods" according to the provisions of Regulation (EU) 2283/2015 (Ververis et al., 2020). The European Commission and the EU Member States have already authorized to place on the EU market of specific insect-derived food ingredients, following positive safety assessments by the European Food Safety Authority (EFSA) (EFSA NDA Panel et al., 2023; EFSA NDA Panel et al., 2021b; EFSA NDA Panel et al., 2022b; EFSA NDA Panel et al., 2021c; EFSA NDA Panel et al., 2021d; EFSA NDA Panel et al., 2022a; EFSA NDA Panel et al., 2021a). In its assessments, the EFSA NDA Panel highlighted potential risks of allergic reactions due to sensitisation to insect proteins, cross-reactivity with crustaceans, and/or allergens from insect feed (e.g., soy, gluten) that may end up in the final product. It should be noted that in the EU, novel food authorizations apply to specific insect-derived ingredients rather than to insect species in general (Precup et al., 2022).

The Western world's slow acceptance of entomophagy is influenced by various factors, including food neophobia and social norms (Kröger et al., 2022). This hesitancy contrasts sharply with the widespread consumption of insects in Asia, Africa, and South America, where 1,600 to 2,000 edible insect species have been identified (Jongema, 2017; Precup et al., 2022; Van Itterbeeck & Pelozuelo, 2022). While wild harvesting currently dominates, accounting for over 90% of global insect consumption, insect farming is increasingly recognized for its potential economic and environmental benefits (Osimani et al., 2017; van Huis et al., 2013). Insects require two to ten times less agricultural land to produce one kilogram of protein compared to swine or cattle (De Vries & de Boer, 2010; Oonincx & De Boer, 2012). Their efficient feed conversion and low environmental impact make them a promising alternative protein source.

Efforts to industrialize insect farming are already underway in EU countries such as the Netherlands, France, Denmark, Italy, and Belgium. Key criteria for selecting insect species for mass rearing include nutrient profile, reproduction rate, ease of handling, and high feed conversion rates (Veldkamp et al., 2022). These criteria are crucial for ensuring the sustainability and profitability of insect farms, which require controlled zootechnical parameters such as temperature, ventilation, and lighting (Kooh et al., 2019).

At this point, it is essential to distinguish between occasional or individual consumption of insects and widespread consumption at the population level. The latter necessitates the establishment of mass rearing systems to efficiently produce animal proteins on a large scale, while simultaneously reducing costs and minimizing environmental impact (Kooh et al., 2019). As awareness of the environmental and nutritional benefits of insect consumption increases, acceptance in Western countries may grow, potentially reshaping food production and consumption patterns.

1.2.2. Health Risks Associated with Insect Consumption and Safety Assessments

Insects and products thereof can carry chemical, biological and physical hazards that could pose risks to human health (EFSA Scientific Committee, 2015; FAO, 2021). Such hazards may be present due to the insect species per se, but also due to the rearing practices including their feed, and to the post-harvesting processing. Unlike other livestock, insects are mostly consumed whole, necessitating stringent control over their farming and processing methods to reduce the risk of chemical or microbiological contamination from their feed or rearing materials. Such contamination can persist through the production chain due to the difficulty of decontaminating insects (Murefu et al., 2019). The hazards associated with insect consumption can vary based on whether insects are reared under controlled conditions or harvested from the wild (Garofalo et al., 2019; Grabowski & Klein, 2017; Stoops et al., 2016). While the consumption of raw or unprocessed insects is rare, the potential for contamination remains a concern, necessitating the establishment of insect-specific hygienic practices (Kooh et al., 2019).

Insects can be vectors for harmful microorganisms, especially under poor hygienic conditions (EFSA Scientific Committee, 2015; Kooh et al., 2019). Their microbiota includes microbes intrinsic to their life cycle or introduced during farming and processing (EFSA Scientific Committee, 2015) while post-harvest practices like starvation and rinsing have limited effects on this microbiota (Wynants et al., 2018).

Regarding biological hazards, insects, both farm-reared and wild-caught, can harbour bacteria from genera such as *Staphylococcus*, *Streptococcus*, and *Bacillus* (Amadi & Kiin-Kabari, 2016; Garofalo et al., 2019; Murefu et al., 2019; Vandeweyer et al., 2017a). Effective biosecurity measures are crucial to prevent contamination, particularly from pathogens like *Campylobacter* and *Salmonella*, often transmitted through contact with livestock (Belluco et al., 2013). Moreover, spore-forming bacteria like *Bacillus cereus* and *Clostridium* spp. can survive common processing methods (Kooh et al., 2020; Osimani et al., 2017; Vandeweyer et al., 2020). The risk of foodborne viruses, such as hepatitis A, hepatitis E, and norovirus, from edible insects is low but caution is needed to avoid introduction through substrates (Vandeweyer et al., 2020). Fungal contamination can cause food spoilage and produce harmful mycotoxins. Yeast and mould species, including *Aspergillus*, *Fusarium*, and *Penicillium*, have been found on edible insects (Kooh et al., 2019; Osimani et al., 2017; Rumpold & Schlüter, 2013; Schlüter et al., 2017). Additionally, insects can be vectors for parasites, potentially transmitting intestinal flukes and protozoan species like *Entamoeba histolytica* and *Giardia lamblia* (Belluco et al., 2013; Chai et al., 2009; Gałęcki & Sokół, 2019; Graczyk et al., 2005). Insects reared on contaminated substrates, such as poultry manure, can harbour coccidia parasites, necessitating appropriate processing steps (Gałęcki & Sokół, 2019; Van der Fels-Klerx et al., 2018).

Concerning chemical hazards, insects can accumulate substances of concern including mycotoxins, pesticides, heavy metals, and dioxins, posing risks when used as human food. While several mycotoxins have been detected in edible insects, their levels are generally not of public health concern (De Paepe et al., 2019). For instance, beauvericin and enniatins have been found in dried housefly larvae without posing health risks (Charlton et al., 2015). However, significant levels of aflatoxins were reported in mopane worms, stressing the need for proper handling and processing (Mpuchane et al., 2000; Mpuchane et al., 1996). Research indicates that insects may metabolize or excrete ingested mycotoxins, though species-specific metabolism routes and their toxicological impacts require further investigation. Pesticide residues from agricultural produce can accumulate in insects, with studies showing that yellow mealworms can process various chiral fungicides (Liu et al., 2013; Lv et al., 2014). Proper feeding controls at insect farms can minimize the presence of pesticides, and further research is needed on the degradation and biotransformation processes of pesticides in insects (Houbraken et al., 2016). Insects can accumulate toxic metals based on factors such as metal type, insect species, and environmental conditions (Charlton et al., 2015; EFSA Scientific Committee, 2015; Greenfield et al., 2014; Van der Fels-Klerx et al., 2016; Vijver et al., 2003; Zhang et al., 2009). Cadmium accumulation has been documented in black soldier flies and field crickets (Diener et al., 2015; Purschke et al., 2017). Similarly, lead and arsenic have been found in insects, raising safety concerns, especially considering chitin's ability to adsorb heavy metals (Anastopoulos et al., 2017; Bailey et al., 1999; Van der Fels-Klerx et al., 2016). Evaluations of maximum levels for metals like cadmium, lead, mercury, and arsenic are essential for safe consumption (EFSA Scientific Committee, 2015). Contaminants such as flame retardants, dioxins, mineral oil hydrocarbons, and histamine also pose risks. Studies have shown bioaccumulation of flame retardants like polybrominated diphenyl ethers (PBDE) in house crickets and various organic pollutants in edible insects from multiple countries (Gaylor et al., 2012; Poma et al., 2019). Although dioxin-accumulation data is limited, there is evidence of polychlorinated biphenyls (PCBs) accumulation in crickets (Paine et al., 1993). High levels of mineral oil hydrocarbons have been found in black soldier flies, and their native content needs further understanding (Van der Fels-Klerx et al., 2020). Histamine intoxication has been reported upon the consumption of fried insects in Thailand (Chomchai & Chomchai, 2018). Contamination from production and processing can introduce harmful compounds like PAHs and acrylamide, necessitating further evaluation of accumulation from processing when considering insects as food (Fernandez-Cassi et al., 2019).

Besides chemical contaminants, certain insect species may contain inherent substances of concern, such as antinutrients (e.g., phytic acid, quinones, cyanogenic glycosides, thiaminases, tannins, oxalates, saponins), which can inhibit the bioavailability of nutrients (ANSES, 2015; Belluco et al., 2013; Chakravorty et al., 2016; Dobermann et al., 2017; NVWA, 2014; Precup et al., 2022). It has been reported that these substances are present at low levels in many commonly consumed insects (Ekop et al., 2010; Shantibala et al., 2014). However, their intake can be detrimental to individuals with poor diets and nutrient deficiencies. Thiaminase, for instance, found in *Anaphe* spp., degrades thiamine (vitamin B1) and can lead to deficiency in susceptible individuals. In Nigeria, the consumption of roasted larvae of *Anaphe venata*, a common alternative protein source, has been associated with seasonal ataxia, a condition treatable with high doses of thiamine infusions (Moyo et al., 2014; Nishimune et al., 2000). Cyanogenic glycosides, which release hydrogen cyanide upon breakdown, have been found in wild-harvested and processed *Eulepida mashona* and edible stinkbugs (Musundire et al., 2016). In addition, as part of their defence mechanism, it has been reported that *Tenebrio molitor* adults can secrete chemical substances such as benzoquinones with potentially toxic effects (Attygalle et al., 1991; Brown et al., 1992; Ladisch et al., 1967). Such findings though refer to *T. molitor* adults (beetles), but not to their larvae. Thus, it is important that larvae are reared separately from adult insects (EFSA NDA Panel et al., 2021a).

Edible insects present allergenic risks via *de novo* sensitization to insect proteins, cross-reactivity with e.g., crustaceans, and allergens originating from insect feed. Insects, classified under the Hexapoda class within the subphylum Arthropoda, are sources of several known allergens, including tropomyosin, arginine kinase, and glutathione S-transferase (Binder et al., 2001; Galindo et al., 2001; Reese et al., 1999). Additionally, chitinases and chitin are recognized for their potential allergenicity (Zhao et al., 2015). Allergens from feed ingredients such as gluten and soy can also be present in the final product, as insects are consumed in their entirety, including their gastrointestinal tracts (Mancini et al., 2020).

Severe allergic reactions to yellow mealworms in individuals with allergy to crustaceans have been confirmed through double-blind, placebo-controlled food challenges (Broekman et al., 2016). Individuals allergic to shrimp may be at risk for similar reactions to mealworms and potentially other insects (Broekman et al., 2017). Known panallergens, including arginine kinase, tropomyosin, glyceraldehyde-3-phosphate dehydrogenase, hexamerin1B, sericin, and hemocyanin, are associated with cross-reactive allergies (Belluco et al., 2013; Leni et al., 2020; Phiriyangkul et al., 2015; Ribeiro et al., 2018; Srinroch et al., 2015). Allergic reactions via inhalation or skin contact have also been documented (Ganseman et al., 2023; Ganseman et al., 2022).

Processing techniques, such as enzymatic hydrolysis and thermal processing, are employed to reduce allergenicity in insect-derived food ingredients. These methods can alter allergens' structure, disrupt amino acid sequences, and degrade proteins into peptides. For instance, thermal processing has been shown to decrease the allergenicity of arginine kinase and enolase, while increasing that of glyceraldehyde-3-phosphate dehydrogenase in Bombay locusts (Phiriyangkul et al., 2015). However, the efficacy of these techniques is not guaranteed, as they may either reduce or increase allergenicity, potentially even introducing new allergens (Rivero-Pino et al., 2024). EFSA underscores the need for careful allergen management and appropriate labelling to address these risks (Rivero-Pino et al., 2024).

1.2.3. Health Benefits Associated with Insect Consumption

Interest in insect consumption from a nutritional perspective is driven by their protein content, amino acid profile, fatty acid composition, and levels of vitamins and minerals, as well as components like chitin. Additionally, ongoing research is exploring bioactive peptides within insect proteins, which may offer potential health benefits (Van Huis et al., 2021). However, there is currently no solid evidence associating insect consumption with health benefits in humans. Existing *in vitro* and cell-based assays cannot bridge the gap to *in vivo* outcomes, as their results are not readily extrapolated to human physiology. Furthermore, while animal studies can help elucidate potential mechanisms and modes of action, they cannot provide definitive evidence of health benefits (Kewuyemi et al., 2020; Lange & Nakamura, 2021; Nowakowski et al., 2022; Roos & Van Huis, 2017; Van Huis et al., 2021).

Various studies have investigated in animal models how insect and insect-derived products can impact different physiological parameters. Gessner et al. (2019) found that yellow mealworm meal lowered lipid levels in hyperlipidaemic rats, but the specific components responsible for the effect observed were not specified. Meyer et al. (2019) observed decreased lipid levels and altered phosphatidylcholine/phosphatidylethanolamine ratios in obese rats fed with yellow mealworms, though study limitations were noted. Islam and Yang (2017) found reduced *Salmonella* and *E. coli* counts in broiler chicks with insect diets. Park et al. (2020) reported antidiabetic effects of *Gryllus* *bimaculatus* powder in diabetic rats, and Ham et al. (2021) noted benefits of *T. molitor* larvae fermentate (*Saccharomyces cerevisiae*) in obese mice. Studies by Yu et al. (2019) and Borrelli et al. (2017) indicated changes in microbiota with insect diets, though associations to health benefits remain unclear. Gasco et al. (2021) and D'Antonio et al. (2021) reviewed insect impacts on immune responses, microbiota, and oxidative stress, concluding that while the evidence is promising, human trials are needed to confirm health benefits. These animal studies though cannot be considered solid evidence towards demonstrating entomophagy-related beneficial health outcomes in humans (Rivero-Pino et al., 2024).

Human studies on the health outcomes of entomophagy remain limited and primarily focus on potential benefits rather than safety aspects. The currently available human trials have investigated whether entomophagy could promote growth and influence iron status when added to complementary foods, modulate gut microbiota exerting prebiotic-like effects and provide amino acids similar to those of soya protein (Stull, 2021). Bauserman et al. (2015a) and their follow-up study (Bauserman et al., 2015b) assessed the acceptability and nutritional impact of caterpillar-containing cereals in infants. The studies demonstrated improved haemoglobin levels and reduced anaemia, though no effect on stunting was observed. Conversely, Konyole et al. (2019) found no significant impact on growth or iron status from consuming termites in infants. Kim et al. (2016) explored the inclusion of mealworms in hospital meals for postoperative patients. They suggested potential benefits based on improvements in anthropometric measures and blood test results, indicating that mealworms might offer nutritional advantages in specific clinical settings. Stull et al. (2018) conducted a double-blind, randomized crossover trial involving 20 healthy adults to examine the effects of daily cricket powder consumption on gut health. Their findings indicated that daily intake of 25 grams of whole cricket powder enhanced the growth of *Bifidobacterium animalis* and reduced systemic inflammation, as evidenced by decreased plasma Tumour Necrosis Factor α (TNF-α). This suggests potential benefits for gut health and inflammation, although the broader implications for long-term health require further study. Vangsoe et al. (2018a) explored the impact of insect protein supplementation on muscle performance in a cohort of 18 young men undergoing resistance training. Their chronic study found no significant differences in muscle hypertrophy or strength between those consuming insect protein and those given a carbohydrate control, indicating that insect protein might not offer distinct advantages over conventional protein sources for muscle development. A related study (Vangsoe et al., 2018b)compared the amino acid profiles of lesser mealworm, soy, and whey proteins, revealing similar amino acid blood concentrations but slower digestion of lesser mealworm protein, which could affect its efficacy in different physiological contexts. Melse-Boonstra et al. (2019) reported lower iron bioavailability from house crickets, potentially due to antinutritional factors. This highlights the need for further investigation into the nutrient absorption characteristics of different insect species. Hu et al. (2020) assessed the effects of compound Caoshi silkworm granules in conjunction with standard chronic obstructive pulmonary disease (COPD) medication. The study found improvements in respiratory symptom scores with the addition of insect granules, although lung function remained unchanged after three months. Hermans et al. (2021) conducted a parallel acute study comparing lesser mealworm protein to milk-derived protein in 24 young men. They observed lower peak levels of certain amino acids with insect protein compared to milk but found no difference in overall amino acid area under the curve or postprandial protein handling. This suggests that while insect protein may differ in amino acid peak levels, it does not significantly alter protein metabolism when compared to milk protein. Iron absorption from insect protein was specifically investigated by Mwangi et al. (2022) in a crossover acute study involving iron-depleted females. The study revealed that while haemoglobin and serum ferritin levels remained unchanged, fractional iron absorption was reduced with low phytate meals containing cricket powder compared to placebo. However, serum transferrin receptor (STR) levels increased with insect-containing meals, highlighting the complex interactions between phytate, iron, and insect protein. The impact of insect protein on appetite and satiety has also been explored.

Dai et al. (2022) compared cricket-derived protein and beef protein beverages, finding that cricket protein led to lower insulin levels and higher amino acid concentrations but did not significantly affect hunger, fullness, or energy intake compared to beef. Similarly, Miguéns-Gómez et al. (2020) studied the interactions of lesser mealworm protein with human intestine, ex vivo, and found that the insect protein educed ghrelin secretion in human colon and modulated duodenal and colonic entero-hormone release. Skotnicka et al. (2022) examined the effects of pancakes with varying levels of insect powder on hunger and satiety, noting that higher levels of cricket and lesser mealworm powder generally reduced hunger. Satiety was improved with higher insect powder levels, particularly in women, suggesting potential gender-specific responses to insect-based foods. Overall, while evidence on insect consumption’s effects is expanding, it remains insufficient to confirm health benefits definitively. Further human trials are needed, especially to investigate nutrient bioavailability, dietary chitin’s fate, and the activity of bioactive peptides (Stull, 2021).

1.3. Dietary substitutions

Dietary substitutions, involving the replacement of specific ingredients, foods, or entire dietary patterns, are a critical area of focus in nutritional practices. These substitutions address various factors including health requirements, ethical considerations, cultural preferences, and sustainability concerns. Such adjustments can range from single ingredient replacements to comprehensive dietary shifts, such as substituting red meat with alternative protein sources. The effectiveness and implications of these substitutions are significant areas of inquiry within nutritional epidemiology (Ibsen et al., 2021).

Historically, food substitution practices have been shaped by cultural traditions and regional resources availability. Societies have historically adapted their diets based on local resources and dietary needs. Currently, the trend towards food substitutions is growing, driven by motivations such as improving dietary health (e.g., reducing saturated fat or sugar intake), accommodating dietary restrictions (e.g., gluten intolerance), and pursuing more sustainable and ethically responsible eating habits (e.g., reducing meat consumption). These changes not only accommodate individual preferences but also enhance culinary diversity and support a varied diet.

Dietary substitutions are applicable across various dietary patterns, including vegetarianism, veganism, and gluten-free diets designed for individuals with celiac disease or gluten intolerance. Additionally, broader dietary patterns, such as the Mediterranean diet, which includes high consumption of fruits, vegetables and olive oil, or plant-based diets that minimize the consumption of animal products, are associated with a reduced risk of chronic diseases such as cardiovascular conditions and diabetes, compared to diets high in processed and red meat. This highlights the importance of informed dietary choices in preventive health strategies.

While dietary substitutions can enhance nutritional intake and reduce chronic disease risk, they may also present challenges, such as maintaining sensory qualities and ensuring a balanced nutrient profile. Further research is necessary to explore the long-term health impacts of food substitutions, their effectiveness across different dietary contexts, and their role in addressing global dietary trends. Ensuring food safety in the context of dietary substitutions is also crucial. Advancements in food science and technology facilitate these substitutions through innovative products such as meat alternatives, and nutrient-fortified foods. These innovations address evolving consumer preferences and dietary needs, making substitutions more feasible from a consumer’s perspective.

1.4. Risk-Benefit Assessment of foods

Traditionally, public health policies separate food safety, which deals with eliminating or managing hazards, from nutritional advice, which focuses on determining optimal nutrient levels and health-promoting dietary habits. However, consumers often face complex decisions that involve weighing both health risks and benefits, along with other factors like environmental and ethical concerns (Huang et al., 2022).

The integration of risk and benefit assessments in food consumption has advanced considerably, creating a robust framework to evaluate both potential hazards and health benefits associated with dietary intake. Historically, such assessments were carried out separately; however, the development of Risk-Benefit Assessment (RBA) has emerged to consolidate these processes into a unified approach (EFSA, 2006; EFSA Scientific Committee, 2010). RBA involves evaluating risks posed by the presence of hazards in foods and the benefits derived from dietary components (EFSA Scientific Committee, 2010). This methodology is inherently multidisciplinary, demanding expertise across various scientific fields, including chemistry, nutrition, toxicology, microbiology, epidemiology, and exposure evaluation. Additionally, skills in statistical modelling, data analysis, and uncertainty assessment are needed.

RBA is a core element of risk-benefit analysis, like traditional risk assessment models (EFSA Scientific Committee, 2010). It comprises three principal components: risk-benefit assessment, risk-benefit management, and risk-benefit communication (EFSA Scientific Committee, 2010). The primary objective of RBA is to rigorously characterize both the risks and benefits associated with the consumption of specific foods, dietary components, or dietary patterns. This involves the identification of hazardous and beneficial components, dose-response assessment, exposure evaluation, and risk and benefit characterization (EFSA Scientific Committee, 2010).

Unlike conventional toxicological and microbiological risk assessments, which primarily focus on hazard identification, RBA broadens its scope to include both adverse and beneficial health outcomes. This comprehensive approach acknowledges that the health outcomes of food consumption are influenced not only by individual hazards but also by the overall nutritional profile and dietary patterns. Consequently, RBA incorporates an additional step: the integration of risks and benefits to evaluate their combined impact on health (Boué et al., 2015; EFSA Scientific Committee, 2010).

Several frameworks have been proposed for conducting RBA, emphasizing a systematic approach that begins with problem formulation. This initial phase involves defining specific risk-benefit questions, identifying relevant foods or components, specifying the target population, and outlining scenarios for comparison (Nauta et al., 2018). Tiered approaches are frequently employed to enhance transparency and enable a progressive assessment from qualitative to quantitative methods, depending on data availability and the complexity of the issue (Nauta et al., 2018; Pires et al., 2019). Collaboration with risk-benefit managers ensures that assessments align with decision-making requirements and stakeholder expectations (Nauta et al., 2018; Pires et al., 2019).

According to the EFSA Scientific Committee (2010) Guidance, qualitative assessments offer valuable insights for policymakers and consumers by replying whether the risks clearly outweigh the benefits (or vice versa), without extensive numerical computations. Semi-quantitative or fully quantitative assessments, depending also on the quality and availability of data, can provide estimates of risks and benefits at relevant exposure levels, using common metrics. Using composite metrics, quantitative assessments can measure single net health impact values, such as changes in disease incidence or Disability-Adjusted Life Years (DALY), resulting from dietary modifications or food substitutions (EFSA Scientific Committee, 2010). DALY is a composite metric widely used in the food RBA field (Nauta et al., 2018). DALY combines Years of Life Lost due to premature death (YLL) and Years Lived with Disability (YLD), providing a comprehensive view of the impact of health conditions on a population. One DALY represents one year of perfect health (no disability) lost, reflecting both mortality and morbidity associated with diseases and health conditions, allowing for comparisons across different diseases, conditions, and populations (Devleesschauwer et al., 2014; Murray, 1994).

The RBA methodology has been in development for approximately 15 years, during which time both methods and data have significantly advanced, and substantial experience has been gained over the past decade (Boué et al., 2022a). RBA represents a significant advancement in assessing the complex interplay between food consumption, health risks, and benefits. By integrating scientific evaluations of risks and benefits, RBA supports informed decision-making and contributes to dietary recommendations that advance public health objectives (Membré et al., 2021).

1.4.1. A step-wise approach

The RBA methodology evolved from the traditional risk assessment framework, incorporating risk–benefit management and communication (EFSA Scientific Committee, 2010). Initiated by EFSA (EFSA, 2006; EFSA Scientific Committee, 2010), and further developed through several European projects (Alvito et al., 2019; Assunção et al., 2019; Boobis et al., 2013; Hart et al., 2013; Hoekstra et al., 2012; Naska et al., 2022; Pires et al., 2019; Tijhuis et al., 2012), RBA encompasses the four steps of risk assessment: hazard identification, hazard characterization, exposure assessment, and risk characterization, with an adapted approach to integrate both adverse and beneficial health outcomes related to nutrition, microbiology, and toxicology. Prior to performing an RBA, it is essential to define the risk–benefit question and corresponding exposure scenarios through continuous interaction between assessors and stakeholders, ensuring the assessment is tailored to specific population groups. The baseline scenario typically represents current or zero exposure to a dietary element, while alternative scenarios explore hypothetical consumer exposures (Hoekstra et al., 2012). Components and their associated health outcomes are then identified and selected for inclusion in the RBA, with each component assessed individually and, where feasible, its impact translated into a common metric for scenario comparison.

1.4.1.1. *Problem Formulation*

The RBA process begins with clearly defining the problem at hand. This involves identifying specific risk-benefit question(s) related to food, food components or dietary patterns. Stakeholders (e.g., policy makers, risk managers) define the scope of the assessment, including the target population, relevant food items or ingredients, and the scenarios for comparison. Problem formulation ensures that the assessment is aligned with the question(s) raised.

1.4.1.2. *Identification of hazards and beneficial components and linked health outcomes*

In this step, potential adverse health outcomes associated with the consumption of specific foods and/or their components are identified, including health risks from chemical contaminants, microbial hazards, and nutrient over-/ under-consumption. Simultaneously, beneficial impacts such as nutritional contributions that promote health are also considered, setting RBA apart from traditional risk assessments by addressing both health risks and benefits. A crucial task is to identify and prioritize the components and associated health outcomes for the RBA, ideally based on a systematic literature review to ensure high-quality data and robust evidence (Assunção et al., 2019). Historically, the selection of components and health outcomes in RBA has relied on non-standardized expert judgment across nutrition, microbiology, and toxicology, leading to inconsistencies. For example, a systematic review of 106 RBAs on fish and seafood revealed significant variability in component selection, even for similar foods (Thomsen et al., 2022). The choice of components can significantly impact RBA outcomes, underscoring the need for a standardized approach (Thomsen et al., 2022). This involves reporting identified components and health outcomes, developing methods to rank and prioritize them, and ensuring a justified, harmonized selection process. In microbiology, the selection and prioritization of hazards are guided by Hazard Analysis Critical Control Point (HACCP) principles, which include hazard analysis based on possible contamination, microorganism survival or proliferation, and severity of health consequences (Codex Alimentarius, 2020). Risk ranking strategies for biological hazards are well-established and applied (Swedish National Food Agency et al., 2018; Van der Fels-Klerx et al., 2018). Notably, ANSES extended these strategies to rank foods associated with biological and chemical hazards using multi-criteria decision analysis (MCDA) methods, which provide a harmonized approach with specific criteria for each field (ANSES, 2020a).

1.4.1.3. *Characterization of Adverse and Beneficial Effects*

Once identified, adverse and beneficial effects are characterized through rigorous scientific evaluation. This step involves gathering and analysing data to understand the (dose-response) relationships between foods(s) and/or food components and adverse or beneficial health effects. Methods include epidemiological studies, toxicological assessments, and nutritional analyses to characterise and quantify the magnitude and likelihood of health impacts.

1.4.1.4. Exposure Assessment

Exposure assessment determines the extent to which individuals or populations are exposed to the identified hazards or beneficial components through their diet. It involves estimating intake levels of specific foods or specific food components based on dietary surveys, consumption patterns, and food composition data. Accurate exposure assessment is crucial for assessing potential health risks and benefits associated with varying levels of food consumption.

1.4.1.5. Integration of Risks and Benefits

A pivotal step in RBA is integrating the characterized risks and benefits to evaluate their combined health impact. This integration may involve comparing various dietary scenarios based on associated health outcomes, using different qualitative, semi-quantitative or quantitative approaches. Such approaches may include evaluating exposure in relation to health-based guidance values (HBGV), employing common metrics (e.g., mortality rates), or using composite ones (e.g., DALY).

1.4.1.6. Communication of Findings

Effective communication of RBA findings is essential for translating scientific assessments into actionable insights for stakeholders. This step involves disseminating information to policymakers, health professionals, food producers, and the general public in a clear and transparent manner. It includes highlighting uncertainties, strengths of scientific evidence, and implications for dietary recommendations or regulatory measures.

1.4.1.7. Risk-Benefit Management

The final step involves integrating the outcomes of RBA into risk-benefit management decisions. This process considers scientific assessments alongside social, economic, and political factors to develop strategies that optimize health benefits while minimizing risks associated with food consumption. Risk-benefit managers play a crucial role in synthesizing RBA findings with broader policy goals and stakeholder perspectives.

1.4.2. Public Health Risk-Benefit Assessment of Food Substitutions

Most RBAs focus on the health impacts of changing consumption of a single food without considering overall dietary changes. Several RBA studies explore food component substitutions. The fortification of margarine with plant sterols was studied by Hoekstra et al. (2013). Verhagen et al. (2012) investigated replacing saturated fatty acids with mono-unsaturated fatty acids or carbohydrates, and sugar-sweetened beverages containing disaccharides with beverages containing artificial sweeteners in Europe, with similar previous studies in the Netherlands (Hendriksen et al., 2011) and in Norway (Husøy et al., 2008). Another RBA examined substituting sodium chloride with potassium chloride in Norway (Steffensen et al., 2018).

Investigating the substitution of whole foods using RBA is more complex, and, yet, relatively rare (Nauta et al., 2018). van der Voet et al. (2007) studied replacing red meat with fish in the Dutch diet, while another study assessed substituting red and processed meat with poultry, fish, or other foods in Nordic countries (Tetens, 2013). Hollander et al. (2019) investigated increasing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) intake by substituting meat with fish or walnuts in the Dutch diet. Studies on dairy and meat substitution with plant-based foods in the Netherlands used gram-by-gram approaches (Temme et al., 2013). Roodenburg et al. (2013) analyzed substituting non-compliant foods with those meeting health logo criteria, ensuring isocaloric substitution. More recently, substitution of red and processed meat by fish has been investigated by Thomsen et al. (2019); Thomsen et al. (2018). Assunção et al. (2021), evaluated isocaloric substitutions of breakfast and infant cereals in the diets of Portuguese children under 3 years of age.

Listing food substitution challenges in RBA, (Nauta et al., 2018) recommended discussing substitution and uncertainties, with specific substitutions within defined food groups included in assessments or scenario analyses. Approaches to modeling substitution vary, including isocaloric, gram-by-gram, or unspecified methods, depending on the foods and food groups involved.

1.5. Research Aim and Objectives

The present work aims to advance the implementation of a standardised RBA methodology, with applications in public health and nutrition. The developed methodology will be used to quantitatively evaluate the health impact on the Greek and other European populations resulting from substituting red meat with alternative dietary choices, particularly novel ones (edible insects). To achieve this research aim, the following objectives have been set:

- Identification of an edible insect species to be studied as a novel protein source for red meat substitution, considering its nutrient profile (e.g., protein content and micronutrients) and the quantity and quality of the available data.
- Determination of the compositional profile (i.e., nutrient, microbiological, and toxicological) of the selected novel protein source.

- Correlation of the compositional profiles of red meat and its replacer with health factors, and calculation of (a) the relative risk (estimated through meta-analyses) and (b) the population attributable risk.
- Establishment of a harmonized and transparent methodological framework for selecting the components from the areas of nutrition, microbiology, and toxicology to be considered in an RBA.
- Development of probabilistic RBA models to quantitatively estimate the public health impact of replacing red meat with the selected novel protein source (model evaluation via case study).
- Investigation of key factors (societal perceptions and knowledge aspects) for effective communication of RBA results regarding red meat and edible insect consumption.

Specific Part

2. Materials and methods

2.1. Selection of insect species

To gather relevant literature for identifying an insect species as potential replacement for red meat, a tiered approach was employed in terms of information sources, given the plethora of edible insect species reported worldwide. Initially, guidelines and report documents relevant to the safety of insects and their products as food, published by EU Member States (Austria, Belgium, Finland, France, Spain, the Netherlands) were screened (AECOSAN et al., 2018; ANSES, 2015; BMGF, 2017; EVIRA, 2018; FASFC, 2014; NVWA, 2014). Additionally, EFSA's publication, "Risk Profile Related to Production and Consumption of Insects as Food and Feed," provided examples of insect species that are commercially farmed both within and outside the European Union (EFSA Scientific Committee, 2015).

The selection of the insect species to be included in the study was based on two main criteria, detailed through the specific sub-criteria listed below. The first criterion was about the product's potential in the EU market, and the second one about the availability of scientific publications with comprehensive and reliable data on composition and related manufacturing processes Any additional relevant information that did not fit these categories was classified as "other."

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

- Sub-criterion 1: Prior consumption in EU countries
  Due to differing interpretations by EU Member States of Regulation (EC) No 258/97 of the European Parliament and the Council of 27 January 1997 concerning novel foods and novel food ingredients, certain EU MS have marketed and consumed food products consisting of or containing whole insects prior to the commencement of this project.
- Sub-criterion 2: Commercial potential in the EU
  The presence of insect-containing food products in the markets of some EU MS may indicate countries with experience in farming, processing insect species, and producing such food products. However, according to Regulation (EU) 2015/2283 of the European Parliament and 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 must receive authorization following a positive safety assessment by EFSA before being marketed. Consequently, insect species already produced and consumed in certain EU countries may have higher market potential.
- Sub-criterion 3: Food technological potential

Edible insects represent a relatively new scientific field that offers numerous opportunities for food innovation and research. Studies investigating the rheological, textural, and structural properties of insect preparations, as well as their use in fortifying and enhancing the nutritional and technological properties of other foodstuffs, have been reported. Insect species that have already been examined in this context may present better prospects in the Research & Development (R&D) food sector.

- Sub-criterion 4: Sensorial aspects: The availability of studies on the sensorial attributes of edible insects serves as an important indicator of the commercial potential of an insect species.

Criterion 2: The availability of scientific publications with comprehensive and reliable data on composition and related manufacturing processes

- Sub-criterion 1: Data on the insect's nutrient profile
  The presence of detailed and reliable scientific data on the nutritional composition of the insect species is essential for evaluating its potential as a food source.
- Sub-criterion 2: Data on the insect's microbiological characteristics
  Reliable data on the microbiological characteristics of the insect species are crucial for assessing its safety and suitability for human consumption.
- Sub-criterion 3: Data on the insect's toxicological profile

Information regarding the toxicological profile of the insect, including any compounds of potential concern, is necessary to ensure consumer safety and regulatory compliance.

2.2. Constructing the compositional profile of the selected insect species

(Ververis et al., Journal of Food Composition and Analysis, 2022, 114: 104859)

For the selected insect species, *Acheta domesticus*, data on its nutrient, microbiological, and toxicological profiles were gathered though a systematic literature review and standardized considering both dried and undried forms (Ververis et al., 2022). The methodology adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews (Page et al., 2021). Searches across four electronic bibliographic databases were conducted, employing the following combinations of search terms:

- **PubMed:** (*Acheta domesticus*) OR (Acheta domestica) OR (House cricket)
- **Science Direct:** ("*Acheta domesticus*") OR ("house cricket") OR ("*Acheta domestica*")
- **Scopus:** TITLE-ABS-KEY ((Acheta AND domesticus) OR (Acheta AND domestica) OR (house AND cricket))

- **Web of Science - all collections:** TOPIC: (("*Acheta domesticus*") OR ("house cricket") OR ("*Acheta domestica*"))

The final collection of articles was completed on November 1st, 2021. No restrictions regarding the publication year or language were imposed. After completing the searches, duplicates were removed. Additionally, the reference lists of the selected articles and the excluded review articles on edible insects were hand-searched to ensure no relevant publications were overlooked. Websites of relevant authorities and organizations, including the EFSA and the Food and Agriculture Organization (FAO), were reviewed.

2.2.1. Eligibility criteria

In line with the search protocol, only publications presenting original quantitative data in the areas of nutrition, microbiology, and toxicology for the following were considered eligible: (a) whole *A. domesticus* adults and/or late instar nymphs, and (b) various processed forms of these insects (e.g., whole boiled insects, whole dried insects, or powder from whole dried insects). Excluded from only qualitative composition data; (c) focused solely on compositional data of insect fractions (e.g., insects with offal removed or defatted insect powder); (d) involved crickets at developmental stages other than adults and late instar nymphs (e.g., eggs, pinheads); (e) pertained to insects as pests or insecticides; (f) involved feed-conversion studies; (g) were related to gut-loading studies; (h) were unrelated to the field of edible insects; and (i) did not present original data. Titles and abstracts of the identified studies were independently reviewed by two researchers to determine eligibility based on the outlined inclusion criteria. Any disagreements or conflicts were resolved through consultation with a third researcher (Ververis et al., 2022).

2.2.2. Extraction, collation, and standardization of data

The compositional data were collected and standardized in accordance with the guidelines provided by the European Food Information Resource (EuroFIR) for Food Composition Databases (Unwin et al., 2016). Two reviewers performed the data extraction using predesigned forms. Quantitative data concerning macronutrients (proximate parameters), fatty acid profiles (including total fatty acids, total polyunsaturated fatty acids (PUFA), total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), and the n-6/n-3 ratio), minerals, vitamins, minor lipid components, antinutrients, microbiological, and toxicological hazards (such as heavy metals and toxins) were systematically extracted and compiled. Additionally, the amino acid profile and the units used for these measurements were recorded.

Information about the samples, such as geographical origin, production processes (e.g., fasting, rinsing, killing method, heat treatment beyond killing/drying, and drying method where applicable), and the names of the analytical methods employed, as reported by the authors, was also extracted. Data concerning insects that were already deceased before the killing step were excluded from the analysis.

Proximate parameter values were standardized to grams per 100 grams of product (% w/w), while minerals, vitamins, and minor components were expressed in milligrams per kilogram of product. When possible, values reported on a dry matter basis were converted based on known moisture levels. If moisture levels were not provided and conversion was not feasible, these dry matter-based values were excluded from synthesis. It is important to note that "dry matter" refers to a state with zero moisture, whereas "dried form" may still contain some moisture. Microbiological profiles were reported in log cfu/g, and conversions from log cfu/g of dry matter to log cfu/g were performed when moisture levels were available. Toxicological profiles were expressed in milligrams per kilogram of product.

2.3. Constructing the compositional profile of red meat (minced beef)

Compositional data from EFSA databases, national food composition databases, and information from national food safety authorities were compiled and examined for beef. The nutrient profiles for minced beef, were sourced from the Danish (Frida, 2019) and French Food Composition Tables (ANSES, 2020b). Given the absence of specific composition data for the beef available to Greek consumers, the nutrient values from Denmark and France were employed for this analysis.

2.4. Selection and prioritisation of components

(Boué et al., Frontiers in Nutrition, 2022, 9:951369)

To ensure a standardized approach for component selection across nutrition, microbiology, and toxicology, a tiered, three-step method was employed. This strategy integrated principles from risk ranking, biological risk assessment, and the HACCP system, with modifications to accommodate nutritional and toxicological considerations.

Framework for Component Selection

The process involved creating three lists: "long," "short," and "final". The "long list" was compiled based on extensive literature review and included all potential components relevant to the RBA in each domain (nutrition, microbiology, and toxicology). This list was then refined and ranked to develop the "short list," which contained components prioritized for assessment based on their occurrence and severity. The "final list" included the components ultimately chosen for the RBA model. Components not included in the final list, despite being significant, were documented as sources of uncertainty in the assessment.

Component Selection Process

1. Literature Search and Initial Screening
- The long list was generated through a comprehensive literature search and included components from nutrients, microbiological and toxicological hazards.
- Each component's relevance was assessed based on the quality of evidence associating it with health outcomes and the differences in concentration levels between food items. Components with insufficient evidence or those not meeting specific criteria were excluded.

2. Short List Formation
- The components on the long list were reviewed based on data quality and availability to create the short list. Components were ranked using standardized criteria to determine their significance for further evaluation.

3. Final List Compilation
- The short list was further scrutinized to develop the final list, which included all components to be evaluated in the RBA. Components not included due to data limitations were noted as areas of uncertainty.

Detailed Component Evaluation

1. Nutrition
- Occurrence: The concentration of nutrients in raw materials and the effect of processing on these levels were evaluated. Components were scored on a scale of 1 to 3 based on their presence in samples and the impact of processing.
- Public Health Considerations: Factors such as inclusion in food-based dietary guidelines (FBDGs) and food fortification schemes were considered. Nutrients were scored based on their role in public health and their contribution to nutrient intake in the population.

2. Microbiology
- Occurrence: The presence of microbiological hazards in raw food and the effects of processing were assessed. Scoring was based on prevalence and the impact of manufacturing processes on hazard levels.

- Severity: Health outcomes associated with microbiological hazards were evaluated in terms of DALY. Components were ranked based on the severity of health outcomes.

3. Toxicology

- Occurrence: The concentration of chemical hazards was assessed relative to reference doses and total exposure. Components were scored based on their concentration in food and the contribution to overall exposure.
- Severity: The impact of chemical hazards was evaluated based on the severity of associated health outcomes. Components were scored on the basis of incidence, fatality, disability, and the disability weight of the disease.

General Ranking Calculation

The prioritization index for each component was determined by multiplying scores from two criteria: occurrence and impact on health outcomes. Each criterion included specific sub-criteria tailored to nutrition, microbiology, and toxicology. The final score for each component was calculated by combining scores from both criteria, ensuring that each aspect was equally weighted in the selection process.

Data Requirements for Final List

The final list comprised components selected for inclusion in the RBA, with the goal of quantifying health impacts using the DALY as a single metric. Essential data for each component included:

- Nutrients: Dose-response data, health outcome incidence, and food composition data.
- Microbiological Hazards: Exposure data, including prevalence and concentration in food, and source attribution.
- Toxicological Hazards: Dose-response relationships, health outcome data, and concentration levels.
- In the absence of necessary data, a component was excluded from the final list but noted as an uncertainty factor in the health impact assessment.

2.5. Risk-Benefit Assessment of substituting red meat by insects

(Ververis et al., Food and Chemical Toxicology, 2024, 114764)

The RBA followed the stepwise methodological approach illustrated in Figure 1, adapted from (Assunção et al., 2019; Boué et al., 2015; EFSA Scientific Committee, 2010).

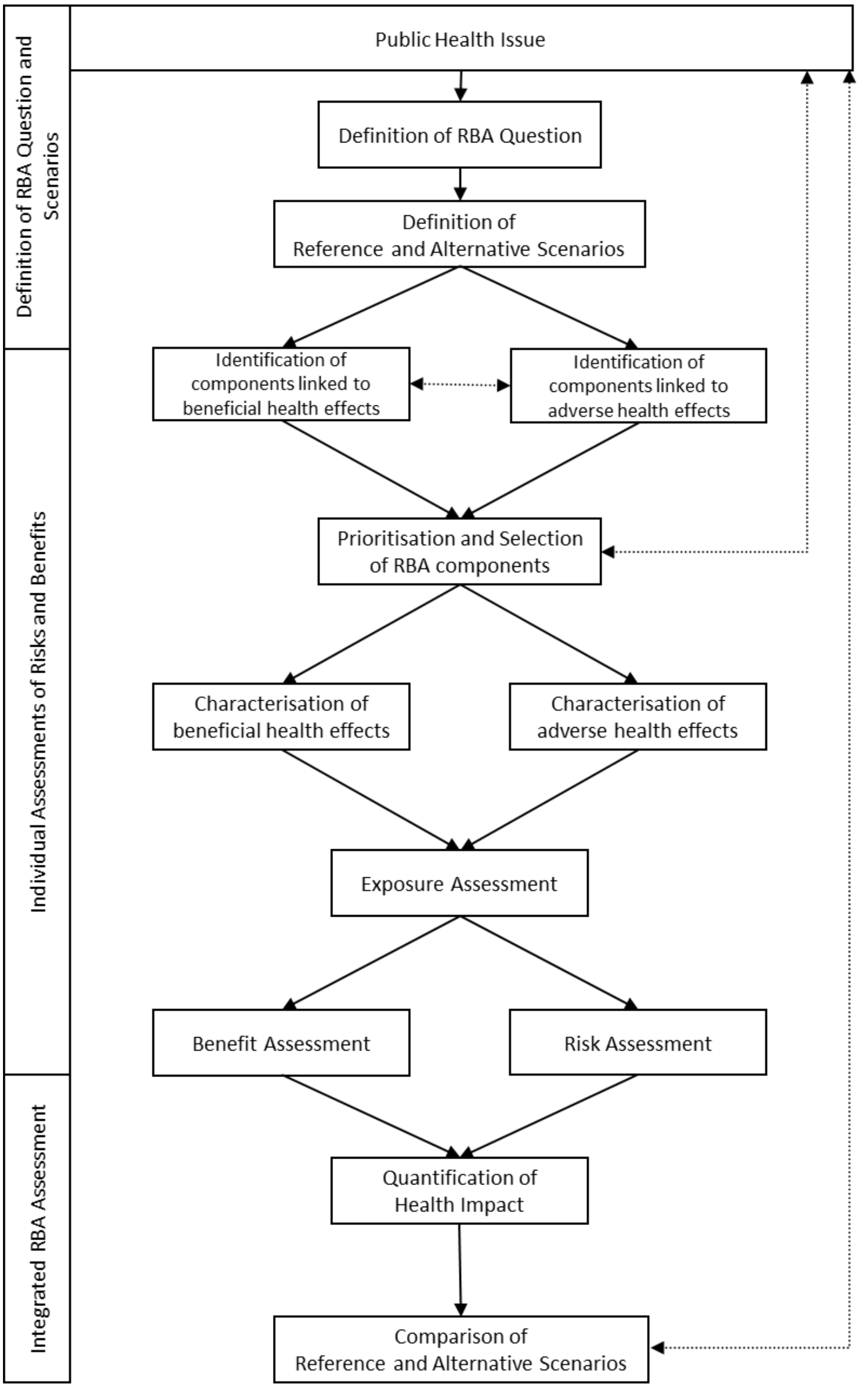

Figure 1 The implemented RBA stepwise methodological approach (Ververis et al., 2024).

2.5.1. Definition of RBA question

The RBA question was formulated based on previously described principles (EFSA Scientific Committee, 2010; Nauta et al., 2018). The main elements considered to define the RBA question were the definition of substitution and reference food commodities, the respective food recipes (Figure 2), the definition of the reference and substitution scenarios (theoretical), as well as the target population.

2.5.2. Definition of substitution and reference food commodities

The insect species *A. domesticus* was chosen (section 3.1). The powdered form of the insect was selected due to literature on consumer perceptions indicating that edible insects are more acceptable in Western societies when they are not visible (e.g., incorporated into other foodstuffs in powdered form). This preference is further supported by a recent literature review by van Huis and Rumpold (2023). In terms of red meat, beef was selected because it is widely consumed across all age groups in European countries and due to the significant environmental impact of cattle farming (Eshel et al., 2014; Poore & Nemecek, 2018; Saget et al., 2021). Minced beef in the form of burger patties was chosen to facilitate the inclusion of cricket powder in a product with a similar appearance.

2.5.3. Definition of the reference and substitution scenarios

To facilitate a realistic quantitative comparative approach, theoretical scenarios for burger patties were developed. It was assumed that 10% of the ingredients (such as herbs, spices, and vegetables, which are common across all scenarios) remained the same to capture variability in different recipe scenarios (both industrially-prepared and home-prepared patties). The scenarios are as follows:

- Reference scenario: 90% minced beef and 10% other ingredients.
- Substitution scenario A: 90% cricket "dough" and 10% other ingredients.
- Substitution scenario B: 45% minced beef, 45% cricket "dough" and 10% other ingredients.

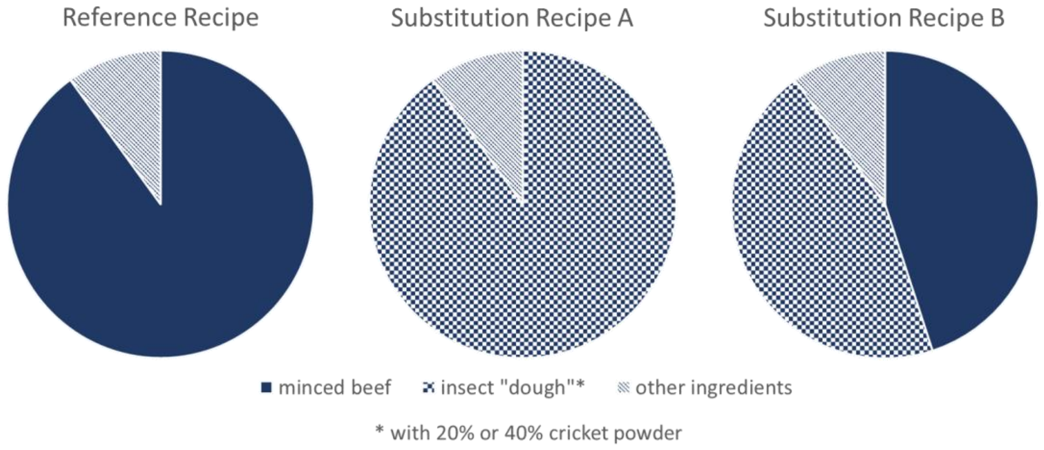

Figure 2 The recipes under investigation (Ververis et al., 2024).

The study explored two compositions for the "cricket dough." In the first composition, the hydrated powder was made up of 20% cricket powder and 80% water. In the second composition, it consisted of 40% cricket powder and 60% water. The final inclusion levels of cricket powder in the patties were designed to be close to or within the maximum permitted levels currently authorized in the European

Union [Commission Implementing Regulation (EU) 2017/2470], as assessed by the EFSA NDA Panel et al. (2021) which allows up to 16% and 50% cricket powder in meat preparations and meat analogues, respectively. Including the two different compositions of "cricket dough" in substitution scenarios A and B resulted in the following four substitution scenarios:

- Reference scenario: consumption of patties containing only minced beef.
- Substitution scenario (A1): minced beef in the patties is completely substituted by cricket "dough" consisting of 20% cricket powder and 80% water.
- Substitution scenario (B1): minced beef in the patties is partially (50%) substituted by cricket "dough" consisting of 20% cricket powder and 80% water.
- Substitution scenario (A2): minced beef in the patties is completely substituted by cricket "dough" consisting of 40% cricket powder and 60% water.
- Substitution scenario (B2): minced beef in the patties is partially (50%) substituted by cricket "dough" consisting of 40% cricket powder and 60% water.

The concentrations of the main ingredients for each scenario are presented in Table 1.

Table 1 Concentration of patty ingredients for each scenario (Ververis et al., 2024).

Ingredients (%)	**Scenario**				
	Reference	**A1** [a]	**B1** [a]	**A2** [b]	**B2** [b]
other ingredients	10	10	10	10	10
minced beef	90	0	45	0	45
cricket powder	0	18	9	36	18
water (from the "dough")	0	72	36	54	27

[a] "dough" cricket powder-to-water ratio= 20:80

[b] "dough" cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket "dough" and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket "dough" and 10% other ingredients

2.5.4. Target population

The general adult population (Denmark, France, Greece) was selected on the basis of research indicating that adults (and young adults in particular) may be more willing to eat insects as food (Naska et al., 2022). The decision to select the general adult population was to ensure the availability of individual food consumption data for this population subgroup in all three countries under investigation. The inclusion of Denmark, France and Greece in the study enables a comprehensive analysis by accounting for variations in e.g., consumption patterns, food compositions, burden of disease, and the geographic distribution of these countries across Southern, Western, and Northern Europe, thereby enhancing the generalizability and robustness of the findings, while allowing for the identification of potential differences with regard to the RBA outcome across diverse European contexts.

Considering the above-described elements, the RBA question was formed as follows:

“What would be the net health impact of partially or totally substituting the beef in burger patties with cricket powder in the adult populations of Denmark, France and Greece?”

2.5.5. Individual assessment of risks and benefits

2.5.5.1. Identification and selection of nutritional, microbiological, and toxicological components of minced beef and cricket powder

The methodology used for identifying and selecting nutrients, microbiological, and toxicological components related to the consumption of beef and cricket powder has been previously described in section 2.4 (Boué et al., 2022b). In summary, a systematic literature review with predefined inclusion and exclusion criteria was conducted in accordance with the PRISMA guidelines. This review was followed by the standardization of the extracted evidence to identify the components of oven-dried cricket powder, including nutrients, nutrient-related compounds, microbiological, and toxicological components (Ververis et al., 2022). The corresponding components of minced beef were identified using national food composition tables and databases concerning microbiological and chemical hazards (Naska et al., 2022).

The identified components were then ranked according to the methodological framework outlined by (Boué et al., 2022b) and selected for inclusion in the RBA model. The ranking and selection process took into account both the prevalence of each component in the food matrix and the severity of the associated health outcomes (“short list”). The final selection was based on the quality and availability of relevant data (“final list”).

2.5.6. Characterisation of beneficial and adverse health outcomes

For the selected components intended for inclusion in the RBA model, a comprehensive list of associated health outcomes was compiled to characterize both adverse and beneficial health outcomes. We solely focused on diseases (hard outcomes) taking into consideration summary reports of EU authorities (EFSA, 2017) and considering results from the literature search conducted for each component of interest. Regarding nutrition and toxicology, a bottom-up approach was followed. For each pair of component-hard outcome, the literature (PubMed) was screened to identify dose-response associations, with a preference to results of meta-analyses.

For example, the search string used for fibre was: (((((fiber OR fibre) AND (health*)) AND (diet* OR intak*)) AND (("2009/01/01"[Date - Publication]: "3000"[Date - Publication]))) AND (analys* [Title/Abstract])) AND (fibre [Title/Abstract] OR fibre [Title/Abstract]).

If no evidence of dose-response associations between the component and the disease was found, the pair could not be considered in the assessment. In nutrition, when multiple dose-response meta-analyses were available, preference was given to those with a lower risk of bias and a more recent publication date. The ROBIS tool (A Risk of Bias Assessment Tool for Systematic Reviews) (Whiting et al., 2016) was used to assess the risk of bias. This tool involves a three-phase process: evaluating relevance, identifying issues regarding bias in the review process, and assessing the overall risk of bias.

Briefly, the first phase is optional and involves the use of the PICO (Patient/Population, Intervention, Comparison, Outcome) framework or a similar one. Regarding the second phase, it includes evaluating the criteria for study eligibility, assessing the methods for identifying and selecting studies, investigating the data collection and study appraisal processes, as well as judging on the quality of synthesis and findings. The third phase investigates the risk of bias in the review. Both second and third phases include specific, predefined signalling questions that assist assessing potential bias-related concerns. The ROBIS assessment results are presented as “high risk of bias”, “low risk of bias”, or “unclear risk of bias”.

In microbiology, two distinct approaches were used to elaborate on related health outcomes. For beef patties, a top-to-bottom approach considered disease incidence, source attribution, and patties intake. Foodborne disease estimates were sourced from the World Health Organization (WHO) Global Burden of Disease (GBD) data for *Toxoplasma gondii* and *Salmonella* spp., and from French data for *Clostridium perfringens*. For cricket powder patties, a bottom-up approach utilized exposure data, applying threshold and exponential dose-response models for *B. cereus* and *C. perfringens*, respectively.

2.5.7. Exposure assessment

2.5.7.1. Concentrations of nutrients, microbiological and toxicological components

The value of nutrients, nutrient-related compounds, and components of toxicological concern was implemented with a uniform distribution spanning the range between minimum and maximum values obtained for both minced beef and cricket powder components. Regarding beef (derived both from grass and grain-fed cattle), we used the range of the macro and micronutrients reported in the national food composition databases of Denmark and France (section 2.3), and the numerical values in the probabilistic scenarios can be within these ranges (Naska et al., 2022). With regard to cricket powder, the respective component values were within the ranges reported for oven-dried crickets by Ververis et al. (2022) (section 2.2).

Concerning the selected microbiological components in the insect powder, the impact of heat-induced inactivation was estimated, taking into account a boiling step upon the production process of the cricket powder, as outlined in the work of Kooh et al. (2020). Subsequently, for non-inactivated microbiological hazards, a beta distribution was employed to implement the prevalence of potentially contaminated patties based on collected frequencies of contamination. The concentration of each hazard was modelled using a uniform distribution spanning the range between minimum and maximum concentrations.

2.5.7.2. Food consumption data

The respective beef patty intake data were retrieved from the Danish National Survey of Diet and Physical Activity (DANSDA) (Pedersen et al., 2015), the Third French Individual and National Food Consumption Survey (INCA3 survey) (ANSES, 2017) and the Hellenic National Nutrition and Health Survey (Magriplis et al., 2019). The overall daily intake (in g per day) among adult participants was estimated.

2.5.7.3. 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 cricket powder).

2.5.8. Risks and benefits characterisation

To evaluate individual risks and benefits, we utilized dose-response estimates in combination with the exposure assessment results. In the fields of nutrition and toxicology, we estimated relative risks (RR) of disease associated with the reference scenario (RR_{ref}) and alternative scenarios (RR_{alt}), both estimated on the basis of the same reference category of intake from the original epidemiological study using the log- linear slope and the following equations.

(i) $\beta = \ln RR_{lit.pert} / \text{dose}$

(ii) $RR_{ref} = \exp(\beta * \text{exposure}_{ref})$

(iii) $RR_{alt} = \exp(\beta * exposure_{alt})$

β: linear slope (calculated from literature data); **dose**: intake linked to a response (calculated from literature data); **$RR_{lit.\ pert}$**: the relative risk of disease associated with a food component. It is estimated through the implementation of a Pert distribution to model uncertainties, taking into consideration literature-derived point estimates as well as their lower and higher intervals (95% CI); **RR_{ref}:** the relative risk for reference scenario; **$exposure_{ref}$**: the mean intake of a component in the reference scenario; **RR_{alt}**: the relative risk for alternative scenario; **$exposure_{alt}$**: the mean intake of a component in the alternative scenario.

The yearly increase or decrease in number of cases was estimated by combining the current incidence rates per country with the Potential Impact Fraction (PIF), which represents the change in disease risk associated with an alternative scenario as compared to the reference scenario. Additionally, we considered the specific national frequency of patty consumption when determining the change in the number of cases which could be attributed to the alternative scenario.

(iv) $PIF = (RR_{alt}-RR_{ref})/RR_{ref}$

(v) $\Delta Ncases = (\%\ of\ population) * frequency_{patty} * PIF * incidence$

PIF: potential impact fraction; **%of population**: percentage of population at risk for the health outcome under study (e.g., % of males or % of females); **$frequency_{patty}$**: the country-specific likelihood to consume patty; **incidence**: the estimate of incidence derived through the implementation of a Pert distribution to model uncertainties, taking into consideration the incident values from GBD as well as their lower and higher intervals (95% CI);

In the field of toxicology, the incidence of disease associated with different exposures to inorganic arsenic (iAs) has been estimated on the basis of literature-derived average increase in population risk per µg iAs/day (mean slope) and the country-specific life expectancy.

In the field of microbiology, two distinct approaches were employed for the two food commodities, as described in the subsections below.

2.5.8.1. Top-to-bottom microbiological approach considering disease incidence and source attribution

For beef patties, we adopted a comprehensive top-to-bottom approach, as delineated in the methodology established by (de Oliveira Mota et al., 2020). This approach considered the current disease incidence, source attribution estimates, and proportion of beef consumed in the form of patties.

The calculation involved assessing the annual number of cases attributed to *C. perfringens*, *T. gondii* (including both congenital and acquired forms), and *Salmonella* spp. associated with beef consumption. For *T. gondii* and *Salmonella* spp., we relied on estimates from the WHO GBD data (Havelaar et al., 2015), for the European region. In the case of *C. perfringens*, we utilized estimates specific to France due to the unavailability of alternative sources. Furthermore, we determined the proportion of foodborne disease cases associated with beef for *T. gondii* and *Salmonella* spp. by referencing the WHO GBD Study estimates (Hoffmann et al., 2017) and, for *C. perfringens*, using data from France (Fosse et al., 2008). All these estimates were modelled using a beta distribution and specifically applied to patty consumption, accounting for the ratio of patties consumed within the beef category. These consumption ratios were obtained from national dietary surveys specific to each country.

(vi) $\Delta \text{Ncases} = -\text{incidence of infection} * \text{attribution\_proportion} * \text{ratio patty/beef} * (\%\ \text{beef}_{ref} - \%\ \text{beef}_{alt})$

incidence of infection: number of cases due to beef per year per 100,000 individuals estimated through the implementation of a Pert distribution considering the estimate, the lower and higher boundaries (95% CI); **attribution_proportion**: the proportion of foodborne infection attributed to the consumption of beef; **ratio patty/beef**: beef consumed in the form of patties out of total beef consumed; **% beef**: percentage of beef in patties of reference and alternative scenarios.

2.5.8.2. Bottom-up microbiological approach considering threshold and exponential dose-responses

In the case of cricket powder, we adopted a bottom-up approach. The approach relied on the estimated exposure values, incorporating a threshold dose-response model for *B. cereus* and an exponential dose-response model for *C. perfringens*. The threshold dose- response was expressed as either a concentration limit (EFSA BIOHAZ Panel, 2016) or an exposure limit (Duc et al., 2005). We used both limits to estimate the number of *B. cereus* cases, considering that each exceedance corresponds to a case. For *C. perfringens*, we calculated the probability of illness and multiplied it by the population size to obtain the number of cases.

2.5.9. Overall health impact quantification in DALY

The overall health impact for each substitution scenario was quantified using DALY as common metric. Data on estimates of DALY and incident rates of selected health outcomes were drawn upon the Global Burden of Disease (GBD) database (IHME, 2020), utilizing country-specific DALY wherever available.

Additionally, demographic data pertaining to the adult populations of the respective countries were sourced from the World Health Organization's European Health for All database (HFA-DB, 2022).

2.5.10. Computation method with uncertainty and variability consideration

The RBA model was developed using the @Risk® add-in software in Microsoft Excel version 7.6 (Palisade Corporation, Ithaca, NY, USA). Monte Carlo simulations were used to capture the uncertainty and the variability of the model inputs and parameters.

2.6. Communication aspects

(Boehm et al., Frontiers in Nutrition, 2021, 8:749696)

Comprehensive literature reviews were conducted to outline the risk perceptions, knowledge levels, and information needs of populations across Europe regarding red meat consumption and entomophagy. The Scopus electronic bibliographic database was searched using the following search strings:

- TITLE-ABS-KEY (accept* OR perc*) AND ("edible insects" OR entomophagy)
- TITLE-ABS-KEY (accept* OR perc*) AND ("red meat" OR beef OR pork).

The last collection of articles was completed on March 3rd, 2020. No limitations on publication year or language were applied. Only those publications that specifically addressed risk perception and associated theoretical constructs (pan-European relevance, population's state of knowledge, information requirements), as opposed to general perceptions of insects as food or red meat, were included. The inclusion or exclusion of publications was carried out regardless of the study design. (Boehm et al., 2021).

3. Results

3.1. Selection of insect species

Data were collected for the 24 insect species identified using the resources previously outlined. In total, 44 references were reviewed, and 51 pieces of evidence were retrieved from these references. *A. domesticus* (house cricket) and *T. molitor* larvae (yellow mealworm) met all selection criteria, achieving the highest scores.

Both *A. domesticus* and *T. molitor* are commonly farmed in some EU countries (Mlcek et al., 2014), and their breeding continues to date (Belluco et al., 2017; Caparros Megido et al., 2017; Vandeweyer et al., 2017b). Products containing these species are already consumed in some EU countries and have potential for EU-wide food production (Van der Spiegel et al., 2013). At the time of the selection, EFSA had positively assessed products from both species (EFSA NDA Panel et al., 2021c; EFSA NDA Panel et al., 2021d; EFSA NDA Panel et al., 2021a). Their sensorial attributes have been reported (Elhassan et al., 2019), and their technological properties as food ingredients have been characterized (Bußler et al., 2016; Ndiritu et al., 2017; Roncolini et al., 2019; Yi et al., 2013; Zhao et al., 2016; Zielińska et al., 2018).

A. domesticus and *T. molitor* have potential as meat substitutes. *A. domesticus* powder can replace up to 10% of lean meat/fat in meat emulsions without negative impacts on texture or cooking properties, while enhancing protein and micronutrient content (Kim et al., 2017). Similarly, *T. molitor* larvae can replace up to 10% of lean pork in frankfurters, maintaining sensory and structural characteristics (Choi et al., 2017). Both species have also been studied as protein fortification agents in bakery products (González et al., 2019; Osimani, Milanović, et al., 2018).

Nutrient composition for *A. domesticus* and *T. molitor* has been systematically reviewed (Fasolato et al., 2018; Payne et al., 2016a) and previously reported (Finke, 2002; Kouřimská & Adámková, 2016). Their protein quality (Bosch et al., 2014; Nowak et al., 2016; Zielińska et al., 2015), chitin content (Finke, 2007), and lipid profiles (Paul et al., 2017; Tzompa-Sosa et al., 2014) have been studied. Microbiological aspects during production, processing, and storage have been addressed (Caparros Megido et al., 2017; Fasolato et al., 2018; Garofalo et al., 2017; Grabowski & Klein, 2017b; Klunder et al., 2012; Stoops et al., 2017; Vandeweyer et al., 2017b). Additionally, the occurrence of hazardous chemical agents in products containing *A. domesticus* or *T. molitor* has been investigated (Poma et al., 2017).

A. domesticus is successfully reared on a large scale and sold for domestic consumption outside the EU, meeting the demands for export and domestic consumption (Hanboonsong et al., 2013; Morales-Ramos et al., 2013; Payne et al., 2016a). Notably, in Thailand - recognized as a leading country in the edible cricket industry (Halloran et al., 2016) - *A. domesticus* is among the most commonly mass-reared edible insect species. Local insect farmers in Thailand often prefer this species over other edible crickets (Hanboonsong et al., 2013). *T. molitor* has a shorter history of farming for food and feed uses, and its large-scale production is relatively recent (Payne et al., 2016b). *A. domesticus*, with a long history of mass-rearing in the United States, is among the cheapest insects to farm due to refined breeding practices (Hanboonsong et al., 2013; Morales-Ramos et al., 2013; Paoletti, 2005). *A. domesticus* also has advantages in rearing, as its substrate can be easily removed before harvesting, reducing undesirable substances and microbiological hazards (Fasolato et al., 2018).

A. domesticus offers superior taste and versatility as a food ingredient (House, 2018). Its taste profile and protein content can be manipulated through dietary adjustments (House, 2018). Nutritionally, *A. domesticus* has significantly higher vitamin B12 levels than *T. molitor* (5.4 µg per 100g vs 0.47 µg per 100g) (Kouřimská and Adámková, 2016). It also contains higher amounts of essential fatty acids and has a lower n-6/n-3 ratio fatty acid ratio (Paul et al., 2017), unlike *T. molitor* larvae (204.15 for *T. molitor* larvae vs 37.04 for *A. domesticus*). A high n-6/n-3 ratio has been associated with physiological disorders (Milićević et al., 2014). As a result, *A. domesticus* (house cricket) was selected.

3.2. Compositional profile of *A. domesticus*

(Ververis et al., Journal of Food Composition and Analysis, 2022, 114: 104859)

A total of 234 articles were assessed for eligibility, with 2 additional sources identified through grey literature and reference lists of included and excluded articles. While most of the screened publications were in English, some were in other languages such as French and German. From the potentially eligible studies, 63 articles met the inclusion criteria and were selected for data extraction. Among these, 50 provided original quantitative data on nutrient composition, 18 on microbiological parameters and 5 on compounds with potential toxicological relevance. These studies were published between 1970 and October 31, 2021. Some studies contained quantitative data across multiple areas. Most compositional data on *A. domesticus* adults and late instar nymphs were from articles published within the last five years, focusing primarily on nutrient profile characterization. Quantitative descriptions of the microbiological characteristics of *A. domesticus* were published from 2012 onwards.

Study characteristics

The included studies are detailed in Table 2. Forms of *A. domesticus* examined included raw, frozen, and thermally processed crickets, both whole and in powder form. Most cricket samples were produced in Europe (n = 29), followed by North and Central America (n = 9 and n = 1, respectively), Asia (n = 11), and Africa (n = 5). Eight studies did not report the origin of the samples. Over half of the selected studies provided data on dried insect forms, with freeze-drying (lyophilization) being the most common method (n = 23). Other methods included oven-drying (n = 16), toasting (n = 1), microwaving (n = 1), and solar-drying (n = 4), with some studies not specifying the drying method (n = 9). In 48 studies, information on whether the crickets underwent a fasting step was not provided. Freezing was the predominant insect-killing method reported (n = 27), followed by boiling (n = 3). Most studies analysed a small number of samples (below 3) or did not report the number. The studies examined macro and micronutrients, various microbiological parameters, and a few elements of toxicological

Table 2 Studies that fulfilled the inclusion criteria of the systematic review of the nutrient, microbiological and toxicological profiles of Acheta domesticus(Ververis et al., 2022)

Study (n=63)	**Scientific Areas**			***A. domesticus* forms analysed**												**Sample's Origin**
	Nutrition	**Microbiology**	**Toxicology**	**Raw**	**Frozen**	**Boiled**	**Autoclaved**	**Steamed**	**Oven-dried**	**Freeze-dried**	**Solar-dried**	**Microwave-dried**	**Spray-dried**	**Toasted**	**Dried (unspecifie**	
	N=50	**N=18**	**N=5**	**N=16**	**N=10**	**N=6**	**N=2**	**N=1**	**N=16**	**N=24**	**N=4**	**N=1**	**N=1**	**N=1**	**N=9**	
(Ayieko et al., 2016)	x				x						x					Kenya
(Barker, 1997)	x				x					x						United States
(Bassett et al., 2021)	x								x				x			nr
(Bawa et al., 2020a)	x				x											Thailand
(Bawa et al., 2020b)	x	x			x				x			x				Thailand
(Bbosa et al., 2019)	x			x												Uganda
(Belluco et al., 2016)		x		x					x							Italy
(Bernard et al., 1997)	x			x												nr
(Boulos et al., 2020)	x									x						Belgium, Switzerland
(Brogan et al., 2021)	x									x						Thailand
(Caparros Megido et al., 2017)		x			x	x	x			x						Belgium, The Netherlands
(Collavo et al., 2005)	x		x							x						United Kingdom
(EFSA NDA Panel, 2021c)	x	x	x			x				x						The Netherlands
(Fasolato et al., 2018)		x								x					X	nr
(Fernandez-Cassi et al., 2020)		x			x											Sweden
*(Finke, 2015)	x				x											United States
*(Finke, 2002)	x				x											United States
(Finke, 2007)	x				x											United States
(Fröhling et al., 2020)		x			x	x	x	x	x							Germany
(Garofalo et al., 2017)		x													X	The Netherlands

Study (n=63)	Scientific Areas			*A. domesticus* forms analysed												Sample's Origin
	Nutrition	Microbiology	Toxicology	Raw	Frozen	Boiled	Autoclaved	Steamed	Oven-dried	Freeze-dried	Solar-dried	Microwave-dried	Spray-dried	Toasted	Dried (unspecifie	
	N=50	N=18	N=5	N=16	N=10	N=6	N=2	N=1	N=16	N=24	N=4	N=1	N=1	N=1	N=9	
(Grabowski & Klein, 2017a)		x			x											nr
(Grabowski & Klein, 2017b)		x								x					X	nr
(Grabowski et al., 2008)	x				x											nr
(Kamau et al., 2018a)	x										x					Kenya
(Kamau et al., 2018b)		x									x					Kenya
*(Khatun et al., 2021)	x					x			x	x						Belgium
(Klunder et al., 2012)		x			x	x			x							Laos
(Kovitvadhi et al., 2019)	x								x							Thailand
(Kulma et al., 2019)	x									x						Czech Republic
(Laroche et al., 2019)	x														X	Canada
(Lipsitz & McFarlane, 1970)	x			x												Canada
(Lipsitz & McFarlane, 1971)	x			x												Canada
(Lucas-González et al., 2019)	x								x	x						Spain
(Messina et al., 2019)		x													X	The Netherlands
(Milanović et al., 2016)		x													X	Austria, Belgium, France, The Netherlands
(Nakagaki et al., 1987)	x		x						x							United States
(Nyangena et al., 2020)	x	x		x		x			x		x			x		Kenya
(Ochiai & Komiya, 2021)	x														X	Thailand

Study (n=63)	**Scientific Areas**			***A. domesticus* forms analysed**												**Sample's Origin**
	Nutrition	**Microbiology**	**Toxicology**	**Raw**	**Frozen**	**Boiled**	**Autoclaved**	**Steamed**	**Oven-dried**	**Freeze-dried**	**Solar-dried**	**Microwave-dried**	**Spray-dried**	**Toasted**	**Dried (unspecifie**	
	N=50	**N=18**	**N=5**	**N=16**	**N=10**	**N=6**	**N=2**	**N=1**	**N=16**	**N=24**	**N=4**	**N=1**	**N=1**	**N=1**	**N=9**	
(Okamoto et al., 2021)	**x**									**x**						Japan
(Oonincx et al., 2015)	**x**								**x**							The Netherlands
(Oonincx et al., 2019)	**x**									**x**						The Netherlands
(Osimani et al., 2017)	**x**	**x**													**X**	The Netherlands
(Osimani, Milanovic, et al., 2018)	**x**	**x**													**X**	Thailand
(Otero et al., 2020)	**x**									**x**						Spain
(Pastell et al., 2021)	**x**		**x**		**x**					**x**						Finland
(Pennino et al., 1991)	**x**				**x**											nr
(Poelaert et al., 2018)	**x**									**x**						Belgium
(Ramos-Elorduy et al., 2012)	**x**								**x**							Mexico
(Ritvanen et al., 2020)	**x**				**x**											Finland
(Sabolová et al., 2021)	**x**									**x**						Czech Republic
(Singh et al., 2020)	**x**								**x**							Thailand
(Sipponen et al., 2018)	**x**									**x**						The Netherlands
(Sorjonen et al., 2019)			**x**							**x**						Finland
(Tilami et al., 2020)	**x**			**x**												Czech Republic
(Tzompa-Sosa et al., 2014)	**x**									**x**						The Netherlands
(Tzompa-Sosa et al., 2019)	**x**				**x**											nr
(Tzompa-Sosa et al., 2021)	**x**									**x**						The Netherlands
(Udomsil et al., 2019)	**x**								**x**							Thailand

Study (n=63)	Scientific Areas			*A. domesticus* forms analysed												Sample's Origin
	Nutrition	Microbiology	Toxicology	Raw	Frozen	Boiled	Autoclaved	Steamed	Oven-dried	Freeze-dried	Solar-dried	Microwave-dried	Spray-dried	Toasted	Dried (unspecifie	
	N=50	N=18	N=5	N=16	N=10	N=6	N=2	N=1	N=16	N=24	N=4	N=1	N=1	N=1	N=9	
(Ugur et al., 2020)	x									x						Thailand
(Vandeweyer et al., 2017b)	x	x		x												Belgium The Netherlands
(Verheyen et al., 2018)	x								x							Belgium
(Wakayama et al., 1984)	x								x							United States
(Yi et al., 2013)	x				x					x						The Netherlands

nr: not reported; *: results on late instar nymphs concern. Among the included studies, 23 identified *A. domesticus* as "adults" with detailed growth timespan information. Fourteen publications reported using "adult" *A. domesticus* without specifying the exact age at harvest. Analysis of "later instar nymphs" was reported in 3 studies, while the remaining studies (n = 14) described the samples as "commercially available" or "*A. domesticus* powder."

Compositional profiling of *A. domesticus* forms

A total of 50 publications provided quantitative data on the nutrient profile of *A. domesticus*. Table 3 summarizes the minimum and maximum values of macronutrients, micronutrients, and other nutrient-relevant components. In undried crickets (e.g., raw, frozen, boiled, autoclaved), water is the main constituent (approx. 52–79%), followed by crude protein (approx. 13–25%) and crude fat (approx. 1.6–18%). The predominant analytical methods used were "loss on drying", the Kjeldahl method (with a nitrogen-to-protein conversion factor of 6.25), and the Soxhlet method, respectively. The ranges of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated fatty acids (SFA) varied among studies. The main SFA is palmitic acid C16:0 (~26% of total fatty acids), the main PUFA is linoleic acid C18:2n-6 (~35% of total fatty acids), and the main MUFA is oleic acid C18:1 n-9 (~24% of total fatty acids) (Tzompa-Sosa et al., 2021). The n-6/n-3 ratio ranges from approximately 12–19. Minor lipid components quantified in undried crickets include sterols, phospholipids, and free fatty acids. Carbohydrate content is reported either as total carbohydrates (including fibre) or as digestible carbohydrates (excluding fibre). Often, the carbohydrate content was calculated rather than determined analytically. Dietary fibre in undried crickets (approx. 1–4%) was predominantly determined using enzymatic-gravimetric methods, with acid detergent fibre (ADF) and neutral detergent fibre (NDF) levels also reported. Various studies retrieved the vitamin and mineral content of undried samples using different analytical methods.

For dried crickets (whole or in powder form), crude protein is the predominant macronutrient (approx. 42–75%), determined via the Kjeldahl method using nitrogen-to-protein conversion factors of 4.76, 5.09, 5.60, and 6.25. Crude fat content, quantified using methods such as Soxhlet (with various solvents), the Folch method, and High Hydrostatic Pressure Assisted Extraction (HHPAE), ranged from approx. 7.5–35%. The n-6 fatty acids are much more abundant than n-3 fatty acids, with the n-6/n-3 ratio reported to be low (n-6/n-3 = 2) in one study with experimental diets (Oonincx et al., 2019), compared to other studies (15–40). Similar to undried samples, carbohydrate content in dried crickets was determined via calculation, with digestible carbohydrates ranging from 2% to 16%. The fibre content in dried crickets ranged from approx. 4–10%, with moisture content varying from 0.6% to 9.5%. Predominant minerals in dried crickets include potassium (K), phosphorus (P), and sodium (Na). Levels of several antinutrients (e.g., oxalic acid, hydrogen cyanide, trypsin inhibitors) were also reported (Table 3). Nineteen publications provided analytical data on the amino acid profile of *A. domesticus*. Some studies reported the quantity of individual amino acids per sample weight (mg/g or g/kg) (Collavo et al., 2005; Finke, 2002; Nakagaki et al., 1987; Ritvanen et al., 2020), while others reported amounts in dry matter (g/100 g dry matter) (Brogan et al., 2021; Pastell et al., 2021; Udomsil et al., 2019). Other studies reported the amino acid profile per crude/true protein (g/100 g protein, mg/100 g protein, or mg/g protein) (Bbosa et al., 2019; Boulos et al., 2020; EFSA NDA Panel et al., 2021c; Khatun et al., 2021; Kulma et al., 2019; Nakagaki et al., 1987; Pastell et al., 2021; Poelaert et al., 2018; Ramos-Elorduy et al., 2012; Ritvanen et al., 2020; Yi et al., 2013). Two publications reported the percentage of individual amino acids out of the total amount of amino acids (Osimani et al., 2017; Osimani, Milanović, et al., 2018).

Table 3 Ranges of reported nutrients and other relevant components of undried and dried Acheta domesticus forms, on a product basis (Ververis et al., 2022)

Undried *A. domesticus* forms				**Dried *A. domesticus* forms**			
Proximate parameters (g/100g)	**Minerals (mg/kg)**	**Vitamins (mg/kg)**	**Other relevant parameters (g/100g)**	**Proximate parameters (g/100g)**	**Minerals (mg/kg)**	**Vitamins (mg/kg)**	**Other relevant parameters (g/100g)**
Moisture Content: 52.29 - 78.9	**Calcium**: 366 - 1402.5	**Biotin**: 0.21	**Total sterols:** 0.11	**Moisture Content:** 0.6 - 9.43	**Boron:** 1.3 - 3.0	**Biotin**: 0.99 - 1.12	**Cholesterol:** 0.1 - 0.44
Crude Protein: 13.1 - 24.9	**Chlorine**: 2210 - 2270	**Folic acid:** 1.07 - 1.50	**Cholesterol:** 0.09 - 0.56	**Crude Protein**: 41.8 - 75.2	**Calcium:** 730 - 3150.4	**Folic acid:** 1.42 - 1.99	**Phospholipids:** 3.52
Fat: 1.59 - 17.8	**Chromium**: 0.68 - 1.02	**Niacin:** 1.1 - 38.4	**Campesterol:** 0.003	**Fat**: 7.5 - 35	**Chromium:** 0.18 - 2.79	**Niacin:** n.d. - 45.1	**n-3:** 0.07
TFA: 4.19 -5.35	**Copper**: 5.1 - 9.2	**Pantothenic acid**: 20.3 - 26.3	**Sigmasterol**: 0.01	**TFA**: 12.88 - 24.8	**Copper:** 17.9 - 50.8	**Pantothenic acid**: 43.0 - 44.2	**n-6:** 1.05
SFA: 1.1 - 2.72	**Iodine**: 0.145 - 0.28	**Riboflavin:** n.d. - 17.4	**β-sitosterol**: 0.006	**SFA**: 4.63 - 7.63	**Iodine:** 0.4 -0.57	**Riboflavin:** 0.97 - 45.8	**n-6/n-3:** 2 - 40.9
MUFA: 0.76 - 2.61	**Iron**: 9.7 - 40.8	**Thiamin:** n.d. - 12.1	**Total phytosterols:** 0.11	**MUFA**: 3.22 - 3.88	**Iron:** 44.40 - 82.47	**Thiamin:** 2.4 - 16.6	**Chitin:** 6.1 - 8.34
PUFA: 0.45 - 1.53	**Magnesium**: 193 - 403	**Vitamin A (retinol):** n.d. - 0.23	**free fatty acids:** 0.12 - 4.05	**PUFA**: 1.36 - 6.76	**Magnesium**: 612 - 1279.75	**Vitamin A (retinol):** n.d. -0.40	**Phytic acid**: 0.1 - 0.14
Total Carbohydrates: 1.1 - 4.1	**Manganese**: 4.6 - 27.2	**Vitamin B12:** 0.01 - 20.4	**Phospholipids**: 1.5 - 2	**Total Carbohydrates:** 4.90 - 6.47	**Manganese:** 15.1 - 28	**Vitamin B12:** n.d. - 0.09	**Hydrogen cyanide**: <5 (mg/kg)
Digestible Carbohydrates: <0.5 - 6.11	**Molybdenum**: 0.17 - 0.40	**Vitamin B6:** n.d. - 2.3	**n-6/n-3:** 12.82 - 18.54	**Digestible Carbohydrates:** 2.09 - 15.96	**Molybdenum:** n.d. - 635	**Vitamin C:** 239	**Oxalic acid**: <100 (mg/kg)
Dietary fibre: 1.09 - 2.9	**Phosphorus**: 126.9 - 3105.3	**Vitamin C:** 18 - 92	**Chitin:** 1.14 - 2.08	**Dietary fibre**: 3.9 - 9.58	**Phosphorus:** 323.7 - 9117.11	**Vitamin E:** 36.8 - 3320.6	**Tannins**: 0.7
ADF: 1.78 - 3.2	**Potassium**: 2408.7 - 3999	**Vitamin E:** 11.5 - 151.3		**ADF:** 7.70 - 11.78	**Potassium:** 3653.55 - 12800	**α- tocopherol:** 0.93 - 2.16	**Total polyphenols**: 0.72 - 0.8
NDF: 3.6 - 6.8	**Selenium**: n.d. - 0.19	**Vitamin K:** 78.4		**NDF**: 20.59 - 30.64	**Selenium:** n.d. - 0.43	**β- tocopherol:** n.d.	**Trypsin inhibitor**: <0.5 (mg/kg)
Ash: 0.6 - 2.37	**Sodium**: 1110 - 3775.1	**β-carotene**: <2 - 2.5		**Ash:** 3 - 11.5	**Sodium:** 950.49 - 8633.4	**γ- tocopherol:** 2.26 - 3.32	
Energy (KJ/100g): 397.1 - 981.27	**Zinc**: n.d. -68			**Energy (KJ/100g):** 1995.18 - 2300	**Sulphur:** 0.59	**δ- tocopherol:** n.d.	
					Zinc: 21.79 - 240		

TFA: total fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ADF: acid detergent fibre; NDF: neutral detergent fibre; n-3: omega 3 fatty acids; n-6: omega 6 fatty acids;

In the field of microbiology, 18 publications were identified (Table 2). Ten reported microbiological data on undried *A. domesticus* forms and 14 on dried forms. undried forms investigated the microbiota of unprocessed insects, with Klunder et al. (2012) examining the effect of blanching on microbiota (total aerobic counts and *Enterobacteriaceae*). *Salmonella* spp. and *L. monocytogenes* were not detected in any undried samples. Minimum and maximum values of retrieved quantitative data are presented in Table 4.

Table 4 Summary of microbiological profiles of undried and dried Acheta domesticus forms (Ververis et al., 2022).

	Levels reported (log cfu/g)					
	Undried *A. domesticus* forms				**Dried *A. domesticus* forms** [3]	
Microbiological parameter	**Raw/Frozen** [1]		**Heat-treated, undried** [2]			
	min	**max**	**min**	**max**	**min**	**max**
Aerobic mesophilic total viable count	7.2	10.2	<1.0	10.1	0.8	8.8
Aerobic mesophilic spore forming bacteria	2.6	4.3	1.5	7.8	1.6	8.1
Lactic Acid Bacteria	6.1	8.1	<1.0	<1.0	n.d.	6.1
Bacilli	3.0	4.0	/	/	0.5	5.9
Bacillus cereus group	3.07	8.7	<1.0	/	<1.0	8.4
***Campylobacter* spp.**	/	/	n.d.	/	n.d.	/
Clostridium perfringens	/	8.6	<1	1.9	<1.0	1.6
***Clostridium perfringens* spores**	/	/	/	/	<2.0	<2.0
Clostridium spp.	/	/	/	/	<1.0	<1.0
Enterobacteriaceae	4.2	8.0	<1	>9	<1.0	5.6
Escherichia coli	/	/	n.d.	<1.0	n.d.	<1.0
Listeria monocytogenes	n.d.	/	n.d.	/	n.d.	n.d.
Pseudomonas aeruginosa	/	/	<1.0	/	<1.0	/
***Pseudomonas* spp.**	/	/	/	/	3.6	3.6
***Salmonella* spp.**	n.d.	+	n.d.	/	n.d.	+
Staphylococci	/	/	/	/	2.7	5.3
Staphylococcus aureus coagulase positive	<1.0	8.0	<1.0	2.9	<1.0	4.0
Sulphite-reducing clostridia	/	/	/	/	<1	3
Yeasts/moulds	4.44	7.2	<1.0	<1.6	<1	7
Yeasts	n.d.	5.2	/	/	<1	5.10
Moulds	2.5	4.5	/	/	<1	3.32

[1] With or without effect of rinsing and/or storage,

[2] Boiled, steamed, heated, or autoclaved,

[3] With or without effect of storage,

/=no value reported,

n.d.=not detected,

+ = present

Only a few publications provided quantitative data for constructing toxicological profiles of *A. domesticus* (Collavo et al., 2005; EFSA NDA Panel et al., 2021c; Nakagaki et al., 1987; Pastell et al., 2021; Sorjonen et al., 2019) (Table 2). Most data in Table 5 refer to dried forms, with low or below detection levels of contaminants. Heavy metals and trace elements in undried *A. domesticus* were analysed using ICP-AES (Collavo et al., 2005), examining the impact of four experimental diets on composition. Glycoalkaloids (α-solanine and α-chaconine) were analysed in crickets partly fed with potatoes (Sorjonen et al., 2019). Mycotoxin levels in dried forms were retrieved from the EFSA NDA Panel's safety assessment (EFSA NDA Panel et al., 2021c). Heavy metals and trace elements in dried forms were reported by EFSA NDA Panel et al. (2021c) and (Pastell et al., 2021), using ICP-MS and/or ICP-OES. Nakagaki et al. (1987) reported aluminium levels in dried crickets.

Table 5 Levels of components of toxicological concern in undried and dried Acheta domesticus forms, on a product basis (Ververis et al., 2022).

Undried *A. domesticus* forms	**Dried *A. domesticus* forms**		
Heavy metals and trace elements (mg/kg)	**Heavy metals and trace elements (mg/kg)**	**Mycotoxins (µg/kg)**	**Alkaloids (mg/kg)**
As: 0.01 - 0.08	As: <0.01 - 0.96	Aflatoxin B1: <0.1	α-solanine: 3.975 - 4.255
Ag: n.d.	Al: 34	Aflatoxin B2: <0.04	α-chaconine: 3.650 - 4.625
Al: 9.86 - 12.58	Cd: 0.015 - 0.026	Aflatoxin G1: <0.1	
B: 0.27 - 0.56	Co: <0.1 - 0.44	Aflatoxin G2: <0.06	
Be: 0.01 - 0.02	Hg: 0.038 - 0.041	Aflatoxins (Sum of B1, B2, G1, G2): <0.3	
Cd: 0.01 - 0.02	Ni: 0.14 - 0.62	Ochratoxin: <0.4	
Co: 0.01 - 0.02	Pb: <0.02 - 0.115	Nivalenol: < 20	
La: n.d. - 0.06		Deoxynivalenol: < 20	
Li: 0.01 - 0.04		Zearalenone: <10	
Ni: 0.13 - 0.32		T-2 and HT-2: < 20	
Pb: 0.06 - 0.2		Fumonisin B1: < 0.012	
Sb: n.d. - 0.83		Fumonisin B2: < 0.0049	
Sr: 0.71 - 1.25			
Te: n.d. 0.11			
Th: n.d. - 0.21			
Ti: 0.09 - 0.14			
V: 0.01			
Y: 0.01			
Zr: 0.04 - 0.06			

3.3. Selection of components

(Boué et al., Frontiers in Nutrition, 2022, 9:951369)
(Ververis et al., Food and Chemical Toxicology, 2024, 114764)

3.3.1. Long List of Components

The long list of components for *A. domesticus* was compiled based on a systematic review approach as described earlier (oven-dried crickets). Additionally, the EFSA opinion on the safety of frozen and dried *A. domesticus* as a novel food was taken into consideration (EFSA NDA Panel, 2021c). For minced beef, profiles were derived from key sources in each domain, including EFSA databases and national food composition tables. Since the Greek food composition database lacked relevant data, the Danish and French Food Composition Tables (section 2.3) were used to obtain information on minced beef composition. The lists for minced beef and cricket powder comprised 42 and 41 nutrients and nutrient-related components, respectively, along with 13 and 14 microbiological hazards, and 10 and 12 chemical hazards, respectively.

3.3.2. Short List of Components

The ranking of components was based on scores assigned to each sub-criterion previously outlined (section 2.4). For each nutrient, microbiological, and toxicological component, an index of prioritization was calculated by combining the scores for occurrence and severity. Each criterion was based on one, two, or three sub-criteria. In the domains of microbiology and toxicology, which are both related to food safety hazards, equal weight was given to occurrence and severity, resulting in an index of prioritization ranging from 1 to 9. The inclusion threshold for the short list in both domains was set at 2, reflecting primary public health concerns.

In nutrition, the nature of compounds differs significantly from hazards as they are inherent to the food. The prioritization index was similarly based on the multiplication of occurrence and severity criteria, with two and three sub-criteria applied, respectively, allowing for a broader scale necessary to rank 43 nutrients. This produced an index of prioritization ranging from 1 to 243 points, with a threshold of 108 applied to both food items to ensure equal consideration.

The short list of components for minced beef and cricket powder is detailed in Table 6. It includes 9 out of 44 and 10 out of 44 nutrients, 5 out of 13 and 6 out of 14 microbiological hazards, and 2 out of 11 and 1 out of 12 chemical hazards for minced beef and cricket powder, respectively.

Table 6 Components to be included in the RBA model (short list) (Ververis et al., 2024).

component	**Nutrition**															**Microbiology**								**Toxicology**	
	Calcium	Copper	Cyanocobalamin	Fibre	Iron	Magnesium	Niacin	Selenium	Sodium	Thiamin	Total omega 6-fatty acids	Total omega-3 fatty acids	Total saturated fatty acids	Vitamin D3	Zinc	*Bacillus cereus*	*Clostridium botulinum*	*Clostridium perfringens*	*Cronobacter sakazakii*	*Listeria monocytogenes*	*Salmonella* spp.	*Staphylococcus aureus*	*Toxoplasma gondii*	Arsenic (inorganic)	PAHs
Cricket powder	x	x		x	x	x		x	x		x	x			x	x	x	x	x	x	x	x		x	
Beef			x		x		x	x	x	x			x	x	x			x		x	x	x	x		X

3.3.3. Final List of Components

The final list (Table 7) comprises components from the short list that were feasible for quantitative assessment and relevant for integration into the Risk-Benefit Analysis (RBA) model. In the context of nutrition, nutrients identified in the short list for one food were also considered for the other food to evaluate changes in nutrient exposure due to substitution. This approach, however, was not extended to microbiology and toxicology, where the presence of a hazard indicates contamination, necessitating an independent evaluation for each food item.

The second selection step was based on the availability of dose-response data and DALY corresponding to health outcomes. The focus was on hard endpoints such as disease incidence, thereby excluding intermediate factors like blood pressure or markers of glucose metabolism or inflammation. DALY estimates per case were either directly obtained from reported values or calculated by dividing total DALY by incidence rates for specific diseases, utilizing data from the GBD database and European sources for microbiological hazards.

The final list of components for minced beef and cricket powder includes 7 out of 9 and 7 out of 10 nutrients, 3 out of 5 and 5 out of 6 microbial hazards, and 1 out of 2 and 0 out of 1 chemical hazards for minced beef and cricket powder, respectively. The selected nutrients include calcium, cyanocobalamin (vitamin B12), insoluble fibre, iron, magnesium, sodium, and zinc. For microbiological hazards, the list includes *B. cereus*, *C. perfringens*, *C. sakazakii*, *L. monocytogenes*, *Salmonella* spp., and *T. gondii*. Among toxicological hazards, only inorganic arsenic was included. Exclusions due to the lack of dose-response epidemiological data were copper and Clostridium botulinum. Niacin, thiamin, and vitamin D3, initially shortlisted based on beef, were omitted from the final selection due to the absence of corresponding data for oven-dried cricket powder, despite available literature on other forms of dried crickets (Ververis et al., 2022). This decision aimed to reduce uncertainty by avoiding extrapolation due to potential nutrient losses during thermal processing. Selenium was excluded because preliminary calculations indicated that the overall daily selenium intake would not exceed 60 µg/day in any alternative scenarios, a threshold below which selenium intake has been associated with an increased risk of type 2 diabetes (Vinceti et al., 2021). Additionally, polyunsaturated fatty acids (mainly n-6) and saturated fatty acids were not included due to ongoing scientific debates regarding their health impacts, particularly concerning inflammation, cardiovascular disease, and metabolic health. Further research is needed to clarify their optimal intake levels and health outcomes.

The exclusion of *Staphylococcus aureus* (enterotoxin) from the final list was based on the lack of data regarding its concentration and prevalence in cricket powder, coupled with its relatively low public health concern in minced beef patties (Pires et al., 2012). Similarly, polycyclic aromatic hydrocarbons (PAHs) were not considered, as it was assumed that both minced beef and insect-containing patties would undergo the same cooking method.

3.3.4. Identification of Associated Health Outcomes & Risk of bias

To estimate the total health impact of various food components, Figures 3, 4 and 5 illustrate the intricate nature of these evaluations, which can yield both positive and negative outcomes. In the domains of microbiology and toxicology, the analysis is limited to adverse health outcomes. Food substitution introduces a dual aspect: a reduction in risks associated with minced beef and an increase in risks associated with cricket powder, potentially involving the same hazard. Both foods contain nutrients at varying levels, influencing risks differently. Furthermore, the same nutrient can have both adverse and beneficial effects, depending on the intake levels.

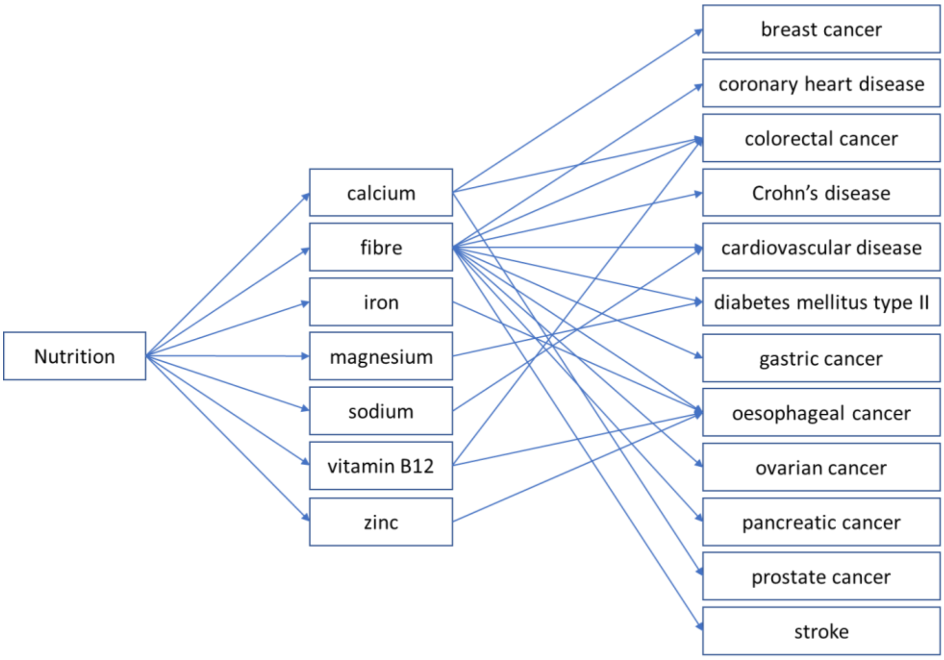

Figure 3 "Health-tree" – Nutrition (Ververis et al., 2024).

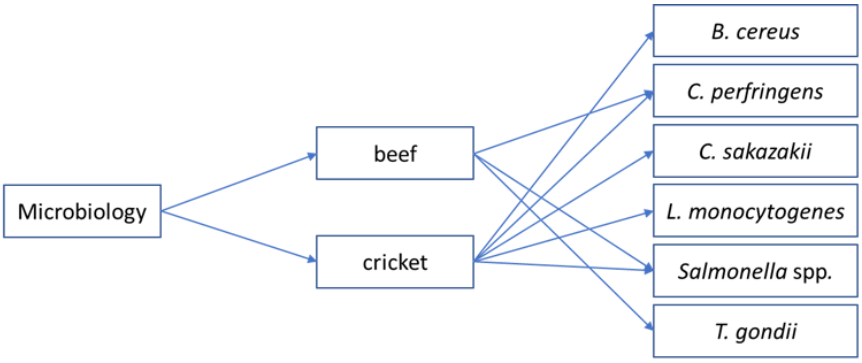

Figure 4 "Health-tree" – Microbiology (Ververis et al., 2024).

The health effect/outcome is an infection with these microbiological agents, with the possibility to lead to the symptom(s) detailed in Table 7.

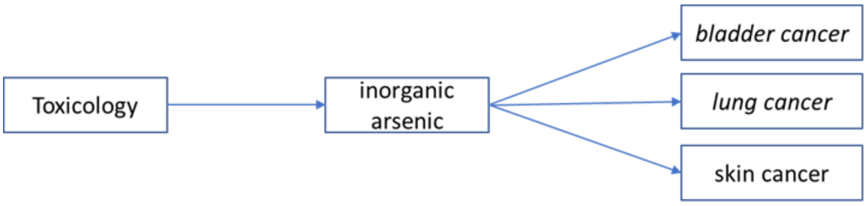

Figure 5 "Health-tree" – Toxicology (Ververis et al., 2024).

Table 7 Final selection of components to be included in the RBA model and associated health outcomes (Ververis et al., 2024).

component		**cricket powder**	**beef**	**health outcome(s)**	**Type and source of (dose-response) data**	**Risk of bias**
Nutrition	Calcium	**x**		Breast cancer	Meta-analysis of epidemiological studies (Hidayat et al., 2016)	low
				Prostate cancer	Meta-analysis of epidemiological studies (Aune et al., 2015)	high
				Colorectal cancer	Meta-analysis of epidemiological studies (Huang et al., 2020)	unclear/low
	Cyanocobalamin		**x**	Oesophageal cancer	Meta-analysis of epidemiological studies (Qiang et al., 2018)	high
				Colorectal cancer	Meta-analysis of epidemiological studies (Sun et al., 2016)	unclear
	Fibre	**x**		Coronary Heart Disease (CHD)	Meta-analysis of epidemiological studies (Reynolds et al., 2019)	low
				Colorectal cancer	Meta-analysis of epidemiological studies (Reynolds et al., 2019)	low
				Chron's disease	Meta-analysis of epidemiological studies (Liu et al., 2015)	high
				Cardiovascular Disease (CVD)	Meta-analysis of epidemiological studies (Threapleton et al., 2013b)	low
				Diabetes mellitus type II	Meta-analysis of epidemiological studies (Reynolds et al., 2019)	low
				Oesophageal cancer	Meta-analysis of epidemiological studies (Sun et al., 2017)	low
				Gastric cancer	Meta-analysis of epidemiological studies (Zhang et al., 2013b)	low
				Ovarian cancer	Meta-analysis of epidemiological studies (Zheng et al., 2018)	low
				Pancreatic cancer	Meta-analysis of epidemiological studies (Mao et al., 2017)	low
				Stroke	Meta-analysis of epidemiological studies (Zhang et al., 2013a)	low
				Breast cancer	Meta-analysis of epidemiological studies (Chen et al., 2016)	low
	Iron	**x**	**x**	Oesophageal cancer	Meta-analysis of epidemiological studies (Ma et al., 2018)	low
	Magnesium	**x**		Diabetes mellitus type II	Meta-analysis of epidemiological studies (Fang et al., 2016)	low
	Sodium	**x**	**x**	CVD	Meta-analysis of epidemiological studies (Wang et al., 2020)	low
	Zinc	**x**	**x**	Oesophageal cancer	Meta-analysis of epidemiological studies (Ma et al., 2018)	low
Microbiology	*Bacillus cereus*	**x**		Emetic symptoms (nausea, vomiting, discomfort, diarrhoea, and occasional abdominal pain); Diarrheal symptoms (watery diarrhoea, abdominal pains, occasional nausea)	Comparison with a threshold dose-response (Duc et al., 2005; EFSA BIOHAZ Panel, 2016)	n.a.
	Clostridium perfringens	**x**	**x**	Diarrhoea, severe stomach pain, nausea, vomiting, fever	Exponential dose-response (cricket powder) (Golden et al., 2009)	n.a.
					Source attribution (beef) (de Oliveira Mota et al., 2020)	n.a.

component		cricket powder	beef	health outcome(s)	Type and source of (dose-response) data	Risk of bias
	Cronobacter sakazakii	**x**		Abscesses, colonization, bacteraemia, osteomyelitis, pneumonia, urinary tract infections, ulcers	Calculation of heat treatment inactivation (cricket powder) (Kooh et al., 2019)	n.a.
	Listeria monocytogenes	**x**	**x**	Maternal neonatal forms [flu-like symptoms (fever, chills, back pain), miscarriage, death in utero, prematurity - neonatal infection); Non-maternal neonatal forms (septicaemia / bacteraemia, meningitis, meningoencephalitis, rhombencephalitis, brain abscess, local infections); Gastroenteric forms (fever, nausea, vomiting, diarrhoea)	Calculation of heat treatment inactivation (cricket powder) (Kooh et al., 2019)	n.a.
	Salmonella spp.	**x**	**x**	Non-typical Salmonellosis (Nausea, vomiting, Abdominal pain, Diarrhoea, Headache, Chills, Fever), Typhoid fevers (prolonged fever, intense headache, anorexia, constipation or diarrhoea, drowsiness, prostration during the day, insomnia at night, pinkish macules on flanks or chest)	Calculation of heat treatment inactivation (cricket powder) (Kooh et al., 2019)	n.a.
					Source attribution (beef) (de Oliveira Mota et al., 2020)	n.a.
	Toxoplasma gondii		**x**	Mild effects (cervical or occipital adenopathy, fever, myalgia, asthenia); Severe effects (pulmonary, neurological, or disseminated toxoplasmosis following contamination with virulent genotype); Ocular effects (chorioretinitis in variable locations progressing to spontaneous healing)	Source attribution (beef) (de Oliveira Mota et al., 2020)	n.a.
Toxicology	Arsenic (inorganic)	**x**		Bladder cancer	Slope factor for arsenic-related bladder cancer (Oberoi et al., 2014)	n.a.
				Lung cancer	Slope factor for arsenic-related lung cancer (Oberoi et al., 2014)	n.a.
				Skin cancer	Slope factor for arsenic-related skin cancer (Oberoi et al., 2014)	n.a.

n.a. not applicable

Table 7 presents a detailed summary of the health outcomes associated with the selected food components. It includes information on the sources of dose-response data and risk of bias assessments. Some components were specifically selected for cricket powder or beef, while others, such as iron, sodium, and *C. perfringens*, were included due to their relevance to both food items. Within the field of nutrition, most studies were determined to have a low risk of bias. However, two meta-analyses were identified as having a high risk of bias: one investigating the dose-response relationship between dietary calcium intake and prostate cancer (Aune et al., 2015), and the other examining cyanocobalamin intake and oesophageal cancer (Qiang et al., 2018).

3.4. Exposure Assessment of reference and alternative scenarios

Individual food consumption data, collected using the EFSA EU Menu methodology (Ioannidou et al., 2020), were utilized to calculate the cumulative distribution of minced beef patty intake for the adult populations of Denmark, France, and Greece (Figure 6). This analysis illustrates the variability in intake both among the three countries and within individuals.

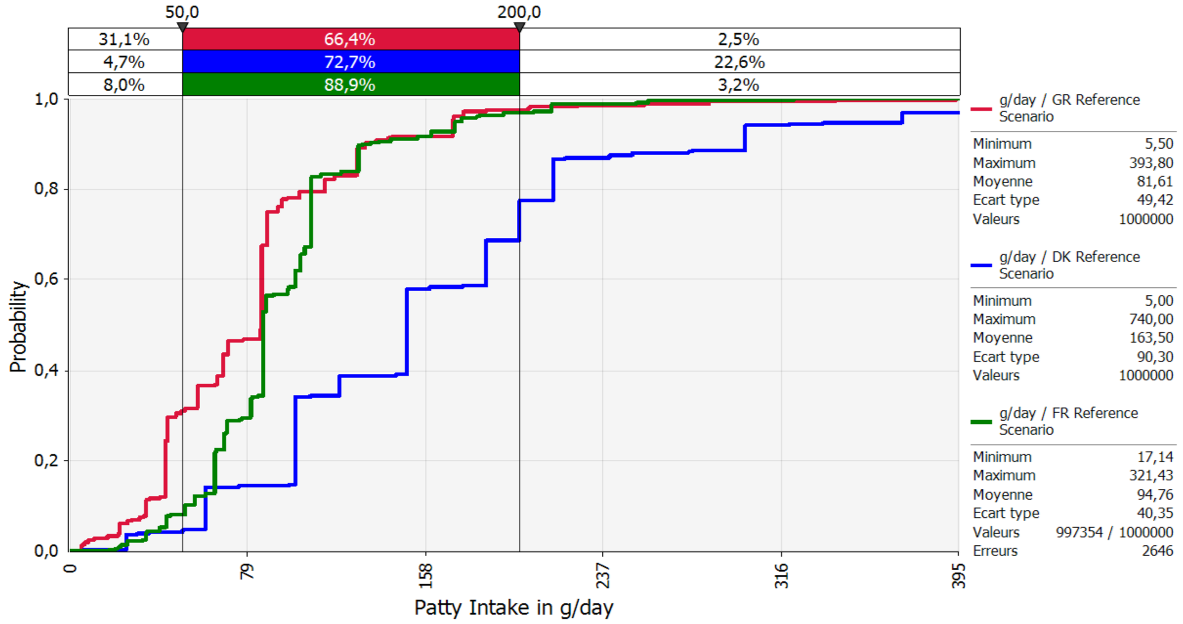

Figure 6 Cumulative distribution of current intake of minced beef patties (in grams per day) in Denmark (blue), France (green), and Greece (red) – created with @Risk® (Ververis et al., 2024).

Based on the current food consumption data and recipe information (Figure 2), cricket powder intake was estimated for four substitution scenarios in each country. The findings, presented in Table 8, reveal that Denmark had the highest median intake of minced beef patties (135 g/day), compared to nearly identical median intakes in France and Greece (~77 g/day).

Table 8 Intake of beef and cricket powder via the consumption of patties (Ververis et al., 2024).

Country	**Scenario**	**Beef (g/day)**			**Cricket powder (g/day)**		
		P2.5	**Median**	**P97.5**	**P2.5**	**Median**	**P97.5**
Denmark	Ref	22.5	135.0	387.0	0.0	0.0	0.0
	A1[a]	0.0	0.0	0.0	4.5	27.0	77.4
	B1[a]	11.3	67.5	193.5	2.3	13.5	38.7
	A2[b]	0.0	0.0	0.0	9.0	54.0	154.8
	B2[b]	11.3	67.5	193.5	4.5	27.0	77.4
France	Ref	30.9	77.1	192.9	0.0	0.0	0.0
	A1[a]	0.0	0.0	0.0	6.2	15.4	38.6
	B1[a]	15.4	38.6	96.4	3.1	7.7	19.3
	A2[b]	0.0	0.0	0.0	12.3	30.9	77.1
	B2[b]	15.4	38.6	96.4	6.2	15.4	38.6
Greece	Ref	7.4	76.6	183.1	0.0	0.0	0.0
	A1[a]	0.0	0.0	0.0	1.5	15.3	36.6
	B1[a]	3.7	38.3	91.5	0.7	7.7	18.3
	A2[b]	0.0	0.0	0.0	3.0	30.6	73.2
	B2[b]	3.7	38.3	91.5	1.5	15.3	36.6

[a] "dough" cricket powder-to-water ratio= 20:80

[b] "dough" cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket "dough" and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket "dough" and 10% other ingredients

Table 9 displays the daily exposure values for nutrients, nutrient-related components, and toxicological components included in the RBA model for all countries under both reference and alternative scenarios. Qualitatively, the trends in nutrient and component intake were consistent across all countries. Shifting from the reference scenario to any alternative scenario resulted in a substantial increase in calcium, fibre, magnesium, and inorganic arsenic intake. Conversely, vitamin B12 intake decreased across all substitution scenarios. Iron intake decreased in scenarios A1 and B1, while scenarios A2 and B2 saw a slight increase in iron levels compared to the reference scenario. Similarly, sodium and zinc intakes decreased in scenarios A1 and B1 but increased in scenarios A2 and B2 relative to the reference scenario.

Table 9 Daily exposure values of included nutrients, nutrient-related components, and components of toxicological concern for reference and alternative scenarios (Ververis et al., 2024).

Scenario	**Reference**			**A1**[a]			**B1**[a]			**A2**[b]			**B2**[b]		
minced beef (%)	90			0			45			0			45		
cricket powder (%)	0			18			9			36			18		
other ingredients (%)	10			10			10			10			10		
water (from the "dough")	0			72			36			54			27		
Percentile	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**
Denmark															
Calcium (mg/day)	2.52	13.67	45.03	8.49	48.67	132.47	5.49	31.68	86.26	16.97	97.34	264.94	9.74	56.22	152.12
Cyanocobalamin (µg/day)	0.49	2.53	7.93	0.02	0.10	0.26	0.25	1.31	4.08	0.03	0.19	0.51	0.26	1.35	4.18
Fibre (g/day)	0.00	0.00	0.00	0.35	1.89	5.52	0.18	0.94	2.76	0.71	3.78	11.03	0.35	1.89	5.52
Iron (mg/day)	0.62	3.20	9.69	0.31	1.67	4.87	0.46	2.53	7.16	0.62	3.33	9.74	0.60	3.42	9.52
Magnesium (mg/day)	4.79	27.47	74.67	5.30	30.30	82.66	4.94	29.04	78.01	10.59	60.60	165.32	7.59	44.42	118.86
Sodium (mg/day)	14.65	77.91	228.92	11.03	57.36	184.67	12.61	70.11	199.09	22.06	114.72	369.34	18.27	98.63	287.87
Zinc (mg/day)	1.08	5.99	16.72	0.83	4.66	12.89	0.93	5.42	14.65	1.66	9.33	25.78	1.34	7.79	21.02
Arsenic - inorganic (µg/day per Kg bw)	0.00	0.01	0.04	0.01	0.15	0.59	0.01	0.08	0.31	0.02	0.29	1.18	0.01	0.15	0.60
France															
Calcium (mg/day)	2.37	8.38	23.13	9.52	28.55	66.73	6.28	18.62	43.77	19.05	57.11	133.45	11.10	33.04	76.88
Cyanocobalamin (µg/day)	0.47	1.55	4.11	0.02	0.06	0.13	0.25	0.80	2.11	0.04	0.11	0.26	0.26	0.83	2.17
Fibre (g/day)	0.00	0.00	0.00	0.37	1.14	2.87	0.18	0.57	1.43	0.74	2.28	5.73	0.37	1.14	2.87
Iron (mg/day)	0.62	1.97	5.04	0.33	1.01	2.53	0.50	1.51	3.66	0.65	2.01	5.06	0.67	2.02	4.86
Magnesium (mg/day)	5.39	16.11	37.68	5.94	17.77	41.75	5.87	17.03	39.46	11.88	35.54	83.50	8.86	26.07	60.07
Sodium (mg/day)	15.29	47.15	118.59	10.28	35.13	94.94	13.71	41.86	101.99	20.56	70.27	189.88	19.19	59.47	147.77
Zinc (mg/day)	1.17	3.53	8.55	0.91	2.74	6.55	1.08	3.18	7.41	1.83	5.48	13.10	1.55	4.57	10.64
Arsenic - inorganic (µg/day per Kg bw)	0.00	0.01	0.02	0.01	0.10	0.34	0.01	0.05	0.18	0.01	0.20	0.67	0.01	0.10	0.35
Greece															
Calcium (mg/day)	0.92	6.54	23.08	3.02	24.04	65.38	1.97	15.82	42.77	6.04	48.07	130.76	3.47	27.97	74.95
Cyanocobalamin (µg/day)	0.17	1.23	4.11	0.01	0.05	0.12	0.09	0.64	2.11	0.01	0.10	0.24	0.09	0.67	2.18
Fibre (g/day)	0.00	0.00	0.00	0.13	0.94	2.86	0.06	0.47	1.43	0.26	1.88	5.72	0.13	0.94	2.86
Iron (mg/day)	0.22	1.60	5.06	0.11	0.83	2.53	0.16	1.26	3.63	0.22	1.66	5.06	0.22	1.70	4.80

Scenario	**Reference**			**A1**[a]			**B1**[a]			**A2**[b]			**B2**[b]		
minced beef (%)	90			0			45			0			45		
cricket powder (%)	0			18			9			36			18		
other ingredients (%)	10			10			10			10			10		
water **(from the "dough")**	0			72			36			54			27		
Percentile	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**	**P2.5**	**P50**	**P97.5**
Magnesium (mg/day)	1.71	13.57	36.90	1.88	14.96	40.91	1.76	14.69	37.82	3.77	29.91	81.83	2.69	22.27	57.82
Sodium (mg/day)	5.29	38.80	118.74	3.95	27.44	94.96	4.55	34.95	101.04	7.91	54.87	189.92	6.57	49.04	146.91
Zinc (mg/day)	0.38	2.94	8.47	0.29	2.29	6.48	0.33	2.71	7.20	0.59	4.59	12.95	0.48	3.90	10.33
Arsenic - inorganic (µg/day per Kg bw)	0.00	0.01	0.02	0.00	0.08	0.32	0.00	0.04	0.17	0.01	0.15	0.63	0.01	0.08	0.32
🟦	increase compared to the reference scenario														

[a] "dough" cricket powder-to-water ratio= 20:80

[b] "dough" cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket "dough" and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket "dough" and 10% other ingredients

The mean probability of infection associated with microbiological hazards from cricket powder consumption is detailed in Table 10, across scenarios A1, B1, A2, and B2. For *B. cereus* infection, the mean probability ranged from 0 to 4.7E-02, with the highest value observed in France for scenario A2. This broad range of uncertainty reflects the absence of a definitive dose-response relationship for *B. cereus*, while a specific dose-response relationship was used for *C. perfringens* (section 2.5.2). Scenario B1 exhibited the lowest probability of infection across all countries, followed by scenarios A1 and B2 (which were equivalent), with scenario A2 showing the highest probability. These infection probabilities correlate with the levels of cricket powder intake indicated in Table 8, highlighting that increased exposure to cricket powder is associated with a higher probability of illness.

Table 10 Mean probability of B. cereus C. perfringens infection associated with cricket powder consumption (Ververis et al., 2024).

	Probability of illness			
	Scenario A1	**Scenario B1**	**Scenario A2**	**Scenario B2**
Denmark				
B. cereus	[0.0E+00; 3.5E-02]	[0.0E+00; 3.3E-03]	[0.0E+00; 1.3E-01]	[0.0E+00; 3.5E-02]
C. perfringens	1.2E-08	5.8E-09	2.3E-08	1.2E-08
France				
B. cereus	[0.0E+00; 4.0E-03]	[0.0E+00; 4.3E-05]	[0.0E+00; 4.7E-02]	[0.0E+00; 4.0E-03]
C. perfringens	6.8E-09	3.4E-09	1.4E-08	6.8E-09
Greece				
B. cereus	[0.0E+00; 4.2E-03]	[0.0E+00; 2.1E-04]	[0.0E+00; 3.6E-02]	[0.0E+00; 4.2E-03]
C. perfringens	5.8E-09	2.9E-09	1.2E-08	5.8E-09

[a] “dough” cricket powder-to-water ratio= 20:80
[b] “dough” cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket “dough” and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket “dough” and 10% other ingredients

3.5. Overall health impact estimated

The overall health impact, quantified in DALY, for each substitution scenario is detailed in Table 11, considering national dietary intake variations across countries. The observed changes are predominantly due to nutritional and microbiological shifts resulting from the dietary substitution scenarios investigated. Transitioning from the reference scenario to alternative scenarios A1 or B1 yields a positive public health impact (ΔDALY < 0) in all countries, with scenario A1 proving to be more advantageous. Among the three countries, Greece exhibits the most favourable outcome (ΔDALY per 100,000 persons) under these scenarios. Conversely, transitioning to alternative scenarios A2 or B2 results in a negative public health impact (ΔDALY > 0) across all countries, with scenario A2 (where minced beef is fully substituted with cricket "dough" at an elevated cricket level) representing the

Table 11 Total ΔDALY per 100,000 person-years and per country's total population (Ververis et al., 2024).

	Scenario	A1[a]			B1[a]			A2[b]			B2[b]		
Ingredients	minced beef (%)	0			45			0			45		
	cricket powder (%)	18			9			36			18		
	other ingredients (%)	10			10			10			10		
	Water (from the "dough")	72			36			54			27		
	DALY	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**
per 100000 person-years	Nutrition	-74.95	-55.4	-98.38	-47.9	-32.35	-56.39	1189.81	342.24	505.91	247.28	107.08	170.28
	Toxicology	-0.03	-0.02	-0.01	-0.01	-0.01	-0.01	-0.05	-0.04	-0.03	-0.03	-0.02	-0.01
	Microbiology	-11.27	-11.73	-1.57	-5.53	-5.83	-0.77	-10.73	-11.44	-1.35	-4.99	-5.53	-0.55
	Total mean	-86.25	-67.15	-99.96	-53.45	-38.18	-57.17	1179.03	330.77	504.52	242.27	101.53	169.71
	Total P5	-104.37	-86.75	-128.01	-68.36	-51.08	-77.85	218.34	86.92	155.71	71.13	33.51	64.1
	Total P50	-85.23	-66.38	-99.87	-53.24	-38	-57.12	1178.33	330.59	504.2	242.1	101.46	169.6
	Total P95	-60.07	-43.3	-60.68	-33.55	-23.2	-32.37	4664	840.62	1159.8	563.85	198.56	312.61
per country's total population	Nutrition	-3617.15	-29375.07	-9027.92	-2311.77	-17153.83	-5174.52	57420.49	181483.3	46425.08	11933.75	56783.3	15625.98
	Toxicology	-1.27	-9.34	-1.31	-0.64	-4.67	-0.66	-2.65	-19.42	-2.73	-1.33	-9.71	-1.36
	Microbiology	-1034.53	-1076.65	-144.1	-507.81	-534.72	-70.74	-984.34	-1049.51	-124.13	-457.62	-507.58	-50.77
	Total mean	-4652.95	-30461.06	-9173.34	-2820.22	-17693.22	-5245.92	56433.5	180414.37	46298.23	11474.8	56266.01	15573.84
	Total P5	-6000.85	-38397.97	-11746.59	-3697.34	-23839.7	-7143.94	10061.39	51244.57	14288.92	3206.11	20309.51	5881.85
	Total P50	-4516.44	-30403.74	-9164.55	-2788.43	-17671.51	-5241.85	56400.95	180271.78	46268.61	11470.13	56219.72	15563.49
	Total P95	-3202.57	-18989.85	-5568.77	-1785.68	-10196.86	-2970.31	224625.52	450661.92	106430.77	27003.08	107636.22	28686.63
🟩		ΔDALY < 0 (beneficial public health impact)											
🟨		ΔDALY > 0 (detrimental public health impact)											

[a] "dough" cricket powder-to-water ratio= 20:80

[b] "dough" cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket "dough" and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket "dough" and 10% other ingredients

worst-case scenario. Denmark is identified as the most adversely affected among the three countries under this scenario.

3.6. The contribution of components to the overall health impact

Table 12 provides the mean percentage contribution of each component to the total change in Disability-Adjusted Life Years (ΔDALY) when transitioning from the reference scenario to the alternative scenarios A1, B1, A2, or B2. Sodium emerges as the predominant factor influencing the overall health impact, accounting for a mean contribution to the total ΔDALY ranging from 74.53% to 97.51% across the various scenarios. In contrast, fibre contributes between 1.33% and 9.38% to the total ΔDALY. Other components in the model have a substantially lower impact on ΔDALY compared to sodium. It is noteworthy that the contribution of reduced risks of salmonellosis varies significantly among scenarios, with percentages ranging from as low as 0.16% to as high as 13.12%.

To further elucidate the impact of sodium on the overall health outcome, we simulated the substitution scenarios excluding sodium and its related health outcomes from the RBA model. The results, presented in Table 13, show that the overall health impact of all substitution scenarios becomes positive across all countries when sodium is excluded. Under these conditions, the contributions of nutrition and microbiology to the mean total ΔDALY are of similar magnitude for France and Denmark. However, in Greece, the nutritional domain continues to play a dominant role in shaping the overall health impact.

Table 12 Mean percentage contribution of each component to the total ΔDALY when moving from the reference to the alternative scenarios (Ververis et al., 2024).

	A1[a]			**B1**[a]			**A2**[b]			**B2**[b]		
minced beef (%)	0			45			0			45		
cricket powder (%)	18			9			36			18		
other ingredients (%)	10			10			10			10		
water from the "dough" (%)	72			36			54			27		
	Denmark	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**
Calcium	0.08	0.07	0.04	0.07	0.06	0.03	0.01	0.03	0.02	0.04	0.05	0.02
Cyanocobalamin	1.05	0.69	0.44	1.03	0.71	0.38	0.07	0.12	0.08	0.2	0.22	0.11
Fibre	9.38	7.1	7.95	7.65	6.28	7	1.33	2.57	2.94	3.09	3.94	4.28
Iron	0.3	0.23	0.05	0.23	0.2	0.04	0	0	0	0	0	0
Magnesium	0.05	0.03	0.03	0.04	0.03	0.03	0.04	0.07	0.07	0.1	0.11	0.1
Sodium	75.8	74.53	89.84	80.03	77.47	91.04	97.51	93.89	96.54	94.08	90.55	94.9
Zinc	0.25	0.19	0.04	0.2	0.17	0.03	0.04	0.08	0.02	0.09	0.13	0.02
MICROBIOLOGY												
B. cereus	0.23	0.04	0.03	0.37	0.07	0.05	0.06	0.09	0.05	0.28	0.26	0.13
C. perfringens	0.66	0.88	0.49	0.53	0.77	0.43	0.05	0.16	0.09	0.11	0.24	0.13
Salmonella spp.	9.83	13.12	0.88	7.93	11.53	0.77	0.71	2.4	0.16	1.62	3.64	0.24
T. gondii	2.33	3.12	0.21	1.88	2.67	0.18	0.17	0.57	0.04	0.38	0.84	0.06
TOXICOLOGY												
Arsenic (inorganic)	0.03	0.03	0.01	0.02	0.02	0.01	0	0.01	0.01	0.01	0.01	0.01

[a] “dough” cricket powder-to-water ratio= 20:80

[b] “dough” cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket “dough” and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket “dough” and 10% other ingredients

Table 13 Total ΔDALY per 100,000 person-years and per country’s total population, with the effect of sodium excluded (Ververis et al., 2024).

		A1[a]			B1[a]			A2[b]			B2[b]		
	minced beef (%)	0			45			0			45		
	cricket powder (%)	18			9			36			18		
	other ingredients (%)	10			10			10			10		
	water from the "dough" (%)	72			36			54			27		
	DALY	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**	**Denmark**	**France**	**Greece**
per 100000 person-years	**Nutrition**	-7.09	-4.18	-7.58	-3.5	-2.07	-3.82	-16.75	-9.87	-16.01	-8.48	-4.96	-8.1
	Toxicology	-0.03	-0.02	-0.01	-0.01	-0.01	-0.01	-0.05	-0.04	-0.03	-0.03	-0.02	-0.01
	Microbiology	-11.27	-11.73	-1.57	-5.53	-5.83	-0.77	-10.73	-11.44	-1.35	-4.99	-5.53	-0.55
	Total mean	-18.39	-15.93	-9.16	-9.05	-7.9	-4.59	-27.54	-21.34	-17.39	-13.49	-10.51	-8.67
	Total P5	-31.99	-29.86	-10.79	-15.85	-14.43	-5.41	-41.24	-35.28	-19.93	-20.38	-17.04	-9.98
	Total P50	-16.88	-14.37	-9.15	-8.29	-7.22	-4.58	-26.08	-19.8	-17.41	-12.79	-9.84	-8.68
	Total P95	-10.01	-7.42	-7.7	-4.85	-3.76	-3.86	-18.89	-12.76	-14.98	-9.11	-6.33	-7.42
per country's total population	**Nutrition**	-342.39	-2218.46	-695.59	-168.88	-1096.35	-350.11	-808.53	-5234.23	-1469.09	-409.25	-2632.26	-743.17
	Toxicology	-1.27	-9.34	-1.31	-0.64	-4.67	-0.66	-2.65	-19.42	-2.73	-1.33	-9.71	-1.36
	Microbiology	-1034.53	-1076.65	-144.1	-507.81	-534.72	-70.74	-984.34	-1049.51	-124.13	-457.62	-507.58	-50.77
	Total mean	-1378.19	-3304.45	-841.01	-677.33	-1635.74	-421.51	-1795.52	-6303.16	-1595.94	-868.2	-3149.55	-795.3
	Total P5	-2623.46	-4619.97	-989.76	-1300.21	-2257.64	-496.4	-3043.99	-7711.38	-1828.54	-1494.79	-3823.79	-915.62
	Total P50	-1237.48	-3181.9	-839.32	-607.29	-1583.23	-420.74	-1657.27	-6213.77	-1597.4	-800.61	-3113.81	-796.33
	Total P95	-615.34	-2428.37	-706.78	-295.62	-1208.91	-354.06	-1024.19	-5250.11	-1374.35	-476.77	-2630.82	-681.32
🟩		ΔDALY < 0 (beneficial public health impact)											

[a] “dough” cricket powder-to-water ratio= 20:80

[b] “dough” cricket powder-to-water ratio= 40:60

- Reference scenario: 90% minced beef and 10% other ingredients
- Substitution scenario A: 90% cricket “dough” and 10% other ingredients
- Substitution scenario B: 45% minced beef, 45% cricket “dough” and 10% other ingredients

3.7. Communication Aspects

(Boehm et al., Frontiers in Nutrition, 2021, 8:749696)

3.7.1. Summary of Key Themes on Risk Perception Relating to Red Meat

The literature search yielded 332 unique references; 12 publications were identified as most relevant from the article abstracts and included in the literature review (Table 14).

Informational Engagement

People's attitudes towards red meat can be influenced by how they engage with information about it (Gaspar et al., 2016). Those who avoid information tend to have more favourable attitudes and feel more knowledgeable about red meat, while mandatory exposure to information tends to reduce these positive attitudes. Individuals who actively engage with food information, such as those with higher education or who use food labels, may perceive beef as safer. This suggests that how information is received and processed can affect attitudes and perceptions about red meat (Angulo & Gil, 2007). For instance, Polish consumers rely more on personal expertise and advice from family and friends rather than formal information sources like press articles or labels (Gutkowska et al., 2018). The importance of personal experience in determining the trustworthiness of information is also noted (Hornibrook et al., 2005).

Risk and Health Perceptions and Trust in Food Safety Standards

Concerns about beef safety, related to confidence in production standards and regulations, are significant barriers to meat consumption. These concerns vary depending on the sources of information and individuals' willingness to engage with risk-related or health information. In Poland, younger consumers view beef positively in terms of health (Gutkowska et al., 2018), whereas consumers from Germany, Spain, France, and the United Kingdom also see it as nutritious (Van Wezemael et al., 2010). However, awareness of the negative health impacts of beef, such as cancer and cardiovascular issues, seems low across Europe (Van Wezemael et al., 2010). Some studies show a general confidence in beef safety (Van Wezemael et al., 2011), while others, like a survey in Spain, reveal lower safety perceptions for beef and moderate ones for pork (Angulo & Gil, 2007). Perceptions of health risks may differ based on consumption patterns, preparation methods, and potential residues (Van Wezemael et al., 2010). Fluctuations in safety perceptions are influenced by media coverage and trust in safety regulations, varying over time and by country.

Table 14 Overview of selected studies on risk perception regarding red meat (Boehm et al., 2021)

Study	Study type	Location	Sample size	Key findings/topics
Angulo and Gil (2007)	Telephone survey	Spain	N = 650	Beef was perceived as one of the least safe food products, with higher risk perceptions of beef being associated with reduced overall confidence in food safety.
Branscheid et al. (2006)	Experiment	Germany	N = 200	An investigation of consumers' sensory ratings of sampled beef and lamb; however, risk perception regarding beef was not examined.
Branscheid (2012)	Literature review	n.a.	n.a.	A discussion of beef quality, focusing on the proportion of muscle to fatty tissue; however, risk perception regarding beef was not examined.
Dwan and Miles (2018)	Online survey	United Kingdom	N = 167	Participants who were more willing to accept the link between red meat and cancer exhibited more negative attitudes toward red meat, including perceptions of increased health risks and reduced benefits.
Gaspar et al. (2016)	Online experiment	United Kingdom, Belgium and Portugal	N = 174	Individuals with lower tendencies toward information avoidance exhibited less positive attitudes toward red meat and reported higher levels of perceived knowledge about it.
Gutkowska et al. (2018)	Survey	Poland	N = 1,004	The third most commonly cited reason for beef consumption was "it is healthy," while "due to health-related reasons" was the second least frequently mentioned.
Hornibrook et al. (2005)	Survey and interview	Ireland	N = 687	Risk perception was assessed solely in relation to purchasing decisions, with food safety identified as the most important factor. The avoidance of physical risks was rated as the top priority.
Schlup and Brunner (2018)	Questionnaire	Switzerland	N = 378	The perceived healthiness of meat negatively predicted participants' willingness to consume insects.
Schroeder et al. (2007)	Survey	Canada, the United States of America, Japan and Mexico	N = 4,005	The majority of respondents in Canada and the U.S.A. considered beef to be either very safe or somewhat safe. In contrast, most respondents in Japan and Mexico viewed beef as mostly safe or neither safe nor unsafe.
Van Wezemael et al. (2010)	Focus groups	France, Germany, the United Kingdom and Spain	$n_{groups} = 8$, $N_{participants} = 65$	Beef was generally perceived as healthful; however, participants anticipated both positive and negative health effects from its consumption.
Van Wezemael et al. (2010)	Focus groups	France, Germany, the United Kingdom and Spain	$n_{groups} = 8$ $N_{participants} = 65$	Participants encountered difficulties in evaluating the safety of beef and beef products.
Van Wezemael et al. (2011)	Online survey	France, Germany, Poland, Spain and the United Kingdom	N = 2,520	Consumers were overwhelmingly confident in the safety of the beef and beef products they purchased.

n.a.: not applicable

3.7.2. Summary of Key Themes on Risk Perception Relating to edible insects

The literature review examined 150 references, narrowing down to 33 key publications (Table 15). Key insights from these studies inform strategies for improving consumer acceptance of insect-based foods. The literature review on risk perception regarding insects as food identifies several key themes, focusing on disgust, familiarity, food neophobia, processing state, contextual information, cultural differences, social norms, and sociodemographic factors.

Disgust and Animal Reminder

Disgust is a significant barrier to insect consumption, particularly animal reminder disgust, which relates to reminders of an animal's origin. This type of disgust is more pronounced in women and can be mitigated by processing insects to reduce their "animalness." Evidence suggests that this form of disgust is distinct from general pathogen disgust, which is associated with fears of disease or contamination. Disgust toward eating insects does not primarily stem from concerns about potential infections or illness (Jensen & Lieberoth, 2019). Instead, animal reminder disgust, one of the primary domains of disgust along with core and contamination disgust (Olatunji et al., 2008), is a key factor influencing individuals' willingness to try insects. Research indicates that those with lower sensitivity to animal reminder disgust are better able to overcome their aversion, viewing cooked or processed insects as less "animal-like" and therefore more acceptable as food (Hamerman, 2016). This sensitivity to animal reminder disgust is notably higher in women compared to men, which may help explain the observed gender differences in the readiness to consume insect-based foods. Furthermore, the state of the insect food—whether processed or unprocessed—affects the level of disgust experienced. Processed insects are generally perceived as more acceptable than unprocessed ones, as processing reduces the sensory cues that trigger disgust. Animal reminder disgust may also enhance the perceived risk associated with insect-based foods, particularly when such foods are depicted in explicit images or descriptions (Hamerman, 2016). Thus, processing and presenting insects in a way that minimizes their animal-like characteristics could play a crucial role in improving their acceptance as a food source.

Familiarity and Food Neophobia

Past consumption experiences positively influence willingness to eat insects (Jensen & Lieberoth, 2019; Lensvelt & Steenbekkers, 2014; Verneau et al., 2016), although the context of such experiences (novelty vs. cultural habit) matters. Moreover, past consumption in case of foods containing whole insects is not helpful (Orsi et al., 2019). Food neophobia, a reluctance to(Verbeke, 2015) try new foods, is a strong deterrent to insect consumption (Gere et al., 2017; Jensen & Lieberoth, 2019) and purchase of insect-containing food products (Lombardi et al., 2019; Piha et al., 2018; Tan et al., 2016). This reluctance varies regionally, with central Europeans showing a stronger correlation between food neophobia and unwillingness to pay for insect-based foods than northern Europeans (Piha et al., 2018).

State of Insect-Based Foods (Processed vs. Unprocessed)

Insect-based foods are more acceptable in processed forms (Hartmann et al., 2015), especially to those new to consuming insects (Orsi et al., 2019). Familiarity with processed insect foods can increase willingness to try unprocessed forms later (Jensen & Lieberoth, 2019). Processing insects can also reduce animal reminder disgust and the novelty barrier, making them more palatable.

Contextual Information (Text, Images, and Sources)

The impact of contextual information on insect consumption is complex and influenced by the trustworthiness of the information source. Scientific researchers, government bodies, and trusted individuals are generally more persuasive (Lensvelt & Steenbekkers, 2014). Cultural differences also play a role; for instance, consumer organizations might be more trusted by Dutch participants compared to Australians (Lensvelt & Steenbekkers, 2014). Information highlighting social or individual benefits of insect consumption can positively influence willingness to try insect-based foods. Specifically, information emphasizing social benefits tends to have longer-lasting effects on the intention to consume, whereas individual health benefits more significantly impact willingness to pay (Lombardi et al., 2019; Verneau et al., 2016). However, the effects of contextual information on consumption behaviour and attitudes are not always straightforward. Some studies suggest that information provided may not significantly influence consumption choices or willingness to pay (Lensvelt & Steenbekkers, 2014; Manhartseder, 2014; Meixner & von Pfalzen, 2018). Moreover, explicit descriptions or images of insects can evoke disgust and deter consumption, though this effect can vary based on other contextual factors (Baker et al., 2016; Jensen & Lieberoth, 2019). Visual reminders of live animals and explicit descriptions that highlight the animal content may reduce purchase intent and consumption behaviours, particularly when combined with textual information that also evokes thoughts of the live animal (Baker et al., 2016).

Cultural Differences, Social Norms, and Contexts

Insect consumption is heavily influenced by cultural and social norms. Individuals are more likely to try insects if they perceive it as a common practice within their social circle (Jensen & Lieberoth, 2019). Cultural comparisons, such as lower willingness to consume insects in Germany compared to China, underline the importance of social and cultural contexts (Hartmann et al., 2015). Promoting positive social norms and sharing favourable experiences within social networks can enhance acceptance of insect-based foods.

Sociodemographic Characteristics

The reviewed studies show variability in sociodemographic criteria. Many studies used young, student populations, making it difficult to generalize findings across different age groups. Gender differences are notable, with men being more willing to consume insects than women (Hartmann & Siegrist, 2017), possibly due to men's lower animal reminder sensitivity (Hamerman, 2016). Regional differences also highlight the significant role of cultural and social contexts in shaping attitudes and behaviours toward insect consumption (Hartmann et al., 2015; Piha et al., 2018; Verneau et al., 2016).

Table 15 Overview of selected studies on risk perception regarding edible insects (Boehm et al., 2021)

Study	Study type	Location	Sample size	Key findings/topics
Baker et al. (2016)	Online experiments (3)	United States of America	$N_1 = 221$ $N_2 = 200$ $N_3 = 201$	Visual or descriptive information impacted risk perceptions and purchase intent.
Batat and Peter (2020)	Literature review	n.a.	n.a.	Development of a conceptual framework identifying key factors related to the acceptance and adoption of insect-based foods in Western food cultures.
Caparros Megido et al. (2016)	Online survey and experiment	Belgium	N = 79	Insect tasting sessions decreased food neophobia.
De Boer et al. (2013)	Online survey	the Netherlands	N = 1,083	The Dutch population showed a positive attitude toward a change to a diet with more environmentally friendly proteins, with the exception of insects.
DeFoliart (1999)	Literature review	n.a.	n.a.	Comparison of the perception and consumption of insects as traditional foods with the Western attitude toward edible insects.
Gere et al. (2017)	Online survey	Hungary	N = 400	Food neophobia was the main barrier to insect consumption.
Gmuer et al. (2016)	Online survey	Switzerland	N = 428	Disgust/uneasiness, inertia/dissatisfaction and positive emotional evaluations predicted willingness to eat insects.
Hamerman (2016)	Online survey	United States of America	N = 179	Different aspects of disgust reduced willingness to eat insects.
Hartmann et al. (2015)	Online survey	Germany China	$N_{DE} = 502$ $N_{CN} = 443$	Chinese participants rated insect-based foods more favorably than German participants. They also indicated greater willingness to eat the tested food products.
Hartmann and Siegrist (2016)	Experiment	Switzerland	N = 104	Exposure to processed insect products can increase consumers' willingness to consume unprocessed insects.
(Hartmann & Siegrist, 2017)	Literature review	n.a.	n.a.	Europeans 'willingness to consume insects was considered very low. Higher willingness was associated with male gender.
Jensen and Lieberoth (2019)	Online survey	Denmark	N = 189	Perceived social norms predicted the willingness to eat insects.
Kim et al. (2019)	Literature review	n.a.	n.a.	Entomophagy increases worldwide, despite its unfamiliarity to the consumers influenced by Western eating habits.
Lensvelt and Steenbekkers (2014)	Online survey	the Netherlands Australia	$N_{NL} = 134$ $N_{AU} = 75$	Information and providing the opportunity to try insect food positively influenced the attitude toward entomophagy.
Lombardi et al. (2019)	Experiment	Italy	N = 200	Food neophobia and beliefs and attitudes toward insects negatively affected the willingness to pay for insect-based products.
Mancini et al. (2019)	Literature review	n.a.	n.a.	Acceptability of edible insects in European countries was the topic of very few publications.
Manhartseder (2014)	Online survey	Austria	N = 164	There was no effect of type of information on the willingness to pay for insect-based food products.
Meixner and von Pfalzen (2018)	Online survey	Austria, Germany and Switzerland	N = 620	The consumption of insects was not perceived as particularly risky.

Study	Study type	Location	Sample size	Key findings/topics
Menozzi et al. (2017)	Online survey	Italy	N = 231	Beliefs in the positive effects on health and the environment positively impacted intention to consume insects-based foods. Disgust, incompatibility with local food culture, and lack of availability negatively impacted the intention.
Meyer-Rochow and Hakko (2018)	Experiment	Italy	N = 26	Insects were not easy to identify by taste alone.
Orsi et al. (2019)	Online survey	Germany	N = 393	Low willingness to try insects. Disgust and food neophobia were identified as one of the main barriers. Few participants perceived insects as unsafe.
Pambo et al. (2018)	Field experiment	Kenya	N = 432	Providing product information on insect-based products affected sensory evaluation of the products' sensory attributes.
Piha et al. (2018)	Online survey	Finland, Sweden, Germany and the Czech Republic	N = 887	Distinct types of knowledge and food neophobia affected willingness to buy, mediated by general attitudes.
Ruby et al. (2015)	Online survey	United States of America India	N_{USA} = 179 N_{IN} = 220	Perceived benefits of eating insects were related to nutrition and environmental sustainability, and the most common risks related to risk of disease and illness.
Schäfer et al. (2016)	Phone survey	Germany	N = 1,000	Insects as food and feed are known to a majority of the German population and they are rather seen as beneficial than as risky. The main reasons against insects as food are disgust and unfamiliarity.
Schösler et al. (2012)	Online survey	the Netherlands	N = 1,083	Meal formats, product familiarity, cooking skills, preferences for plant-based foods and motivational orientations toward food had in impact on the intention to prepare the presented meals at home.
Tan et al. (2016)	Experiment	the Netherlands	N = 103	Food appropriateness, but not the experienced sensory-liking, food neophobia or gender predicted willingness to eat unusual food among Dutch beef consumers
Tan et al. (2017a)	Experiment	the Netherlands	N = 100	Taste expectations were more negative when a food had never been tested before. Low willingness to eat was linked to food appropriateness more than the food's actual taste.
Tan et al. (2017b)	Experiment and online survey	the Netherlands	N_{exp} = 135 N_{onl} = 79	Appropriate product context improved expected sensory-liking and willingness to buy mealworm products.
Van Huis (2013)	Literature review	n.a.	n.a.	Focusing on ecological and economical aspects, the paper provides insights into the rearing of insects.
Van Huis (2016)	Conference proceeding	n.a.	n.a.	Discussion of research pathways to make insects a viable sector in food and agriculture.
Verbeke (2015)	Online survey	Belgium	N = 368	Food neophobia made the largest contribution to consumers' readiness to adopt insect substitution.
Verneau et al. (2016)	Experiment	Denmark Italy	N_{DK} = 141 N_{IT} = 141	Communication was effective on intention and behavior regarding the willingness to eat insect-based food.

n.a.: not applicable

4. Discussion

4.1. Red meat replacement by novel protein sources

The consumption of red meat in Western societies intertwines health benefits, associated risks, and considerable environmental impacts. Red meat is a vital source of protein and essential nutrients, but its excess intake is associated with increased risk of cardiovascular disease, type II diabetes, and specific types of cancer. Additionally, the production of red meat contributes significantly to greenhouse gas emissions and high resource use, necessitating a re-evaluation of dietary practices to mitigate these adverse effects. Adopting strategies to moderate red meat consumption, while supporting sustainable farming practices, is crucial for addressing both health and environmental concerns.

In contrast, certain insect species emerge as a promising alternative to traditional dietary sources, particularly in Europe. Although insect-based foods present potential hazards, such as contamination and allergenicity, these risks are being addressed through rigorous safety assessments and robust regulatory frameworks within the European Union. As Western societies begin to embrace entomophagy, overcoming cultural barriers and improving consumer awareness will be essential to fully capitalize on the benefits of insects as a sustainable and nutritious food source.

The exploration of novel dietary choices, including insects and lab-grown meats, mirrors changing consumer preferences and increased awareness of health and environmental issues. The shift towards plant-based diets, functional foods, and alternative proteins is a response to the health risks associated with high red meat consumption and reflects a broader societal push for more sustainable and ethical eating practices. These dietary transitions not only promise to reduce health risks but also address concerns related to animal welfare and environmental sustainability [reference]. From an industry perspective, the development of innovative dietary options offers significant opportunities for growth and diversification. The food industry is increasingly investing in novel foods that appeal to health-conscious consumers, such as plant-based proteins and cultured meats. Evaluating the risk-benefit profiles of these new foods can provide crucial insights for manufacturers, aid in navigating regulatory challenges, and enhance consumer acceptance (Ververis et al., 2024). Methodologically, integrating novel dietary choices into RBA presents both challenges and opportunities. The rapid advancement in food technologies necessitates new methodological approaches, including the integration of diverse data sources, managing uncertainties associated with emerging foods, and applying advanced modelling techniques to predict long-term health outcomes. By evolving RBA methodologies to include these novel dietary options, research can offer more comprehensive and dynamic assessments, leading to better-informed dietary guidelines and public health policies (Boué et al., 2022a).

4.2. Compositional profile of *A. domesticus* forms

A. domesticus has recently attracted attention as a potential food ingredient in Western diets, leading to a significant increase in research focused on its nutrient and microbiological profiles in both undried and dried forms. Despite this growing interest, there is a limited number of studies providing analytical data on potentially toxic endogenous or exogenous compounds in *A. domesticus*, whether in its dried or undried state. The compositional data presented here focus on two primary categories of *A. domesticus* products - “undried” and “dried” forms - representing the principal ways this insect is used in the food industry.

4.2.1. Nutrient profile of *A. domesticus* forms

Although *A. domesticus* is widely consumed outside Europe and countries such as Thailand are prominent in the edible cricket industry, our analysis reveals that most existing studies focus on *A. domesticus* products from Europe. An initial attempt to standardize nutrient composition data for various insect species was made a decade ago through the FAO/INFOODS Food Composition Database for Biodiversity (BioFoodComp) (Charrondière et al., 2013). This effort included data on the nutrient composition of *A. domesticus* from eight publications, reported on a raw weight basis following standardization. The BioFoodComp database was last updated in 2017 (version 4.0) (FAO/INFOODS, 2017), with no new data for *A. domesticus* added during this update. Similarly, Rumpold and Schlüter (2013) reviewed and reported on the nutrient composition of various insects, including *A. domesticus*, but their results were presented on a dry matter basis, which complicates conversion to a product basis (e.g., g/100 g of insect). Given current industry trends involving the consumption or use of insects in both undried and dried forms, having nutrient data for both raw (frozen/boiled) and dried (whole/powder) insect forms is essential. Reporting data on a dry matter basis presents practical challenges, as highlighted by Nowak et al. (2016), requiring additional conversions to a product basis. Our research compiled nutrient profile data for *A. domesticus* from 50 original studies.

The composition of *A. domesticus* varies significantly depending on rearing conditions, processing methods, and feed types. Additionally, it is important to note that the data collected were produced using analytical methods developed and validated for other food matrices, such as meat and meat products, cocoa products, and flour.

Protein content in dried *A. domesticus* is a major component but varies widely due to different nitrogen-to-protein conversion factors used across studies (EFSA NDA Panel et al., 2021a; Janssen et al., 2017). Recent advancements in analytical methods have suggested that traditional protein quantification methods may overestimate protein content by up to 25% due to the presence of non-protein nitrogen, such as chitin (EFSA NDA Panel et al., 2021b; EFSA NDA Panel et al., 2021c; EFSA NDA Panel et al., 2021d; EFSA NDA Panel et al., 2021a; Janssen et al., 2017; Ritvanen et al., 2020). The fatty acid profile of *A. domesticus* also shows variability, with n-6 polyunsaturated fatty acids (PUFAs) generally prevailing over n-3 PUFAs, although this ratio can be adjusted through feed manipulation (Oonincx et al., 2019; Pastell et al., 2021). It has been reported that a high n-6/n-3 ratio is detrimental to human health (Simopoulos, 2004), and that n-6 polyunsaturated fatty acids (PUFAs) may have different health outcomes compared to n-3 PUFAs, with elevated levels of n-6 PUFAs being particularly harmful (Hamley, 2017; Khandelwal et al., 2013). Mineral and vitamin levels in *A. domesticus* exhibit considerable variation, influenced by feed and processing conditions [Kouřimská and Adámková, 2016]. Okamoto et al. (2021)notably reported that the high levels of vitamin B12 found in house crickets might be inaccurate, as common detection methods do not differentiate between biologically active vitamin B12 and other corrinoid compounds, such as pseudo vitamin B12.

Among the minerals analysed in dried *A. domesticus*, it is important to highlight the relatively high sodium levels (up to approximately 8600 mg/kg), given the EFSA NDA Panel's recommended safe intake of 2.0 g sodium per day (EFSA NDA Panel et al., 2019). This high sodium content is consistent with reports that *A. domesticus* has a naturally high requirement for sodium (Luckey and Stone, 1968).

4.2.2. Microbiological profile of *A. domesticus* forms

Regardless of the form (whole or powder), insects are used and/or consumed alongside their digestive tract. Thus, by the end of the rearing process, insects can serve as reservoirs for a range of microorganisms (FAO, 2021), including pathogenic bacteria, yeast and moulds, viruses, and parasites (Kooh et al., 2019). Data on the microbial quality of raw and frozen crickets indicate high total mesophilic counts (7.2 to 10.2 log cfu/g) and significant levels of aerobic mesophilic spore-forming bacteria (2.6 to 4.4 cfu/g), lactic acid bacteria, *Enterobacteriaceae*, yeasts, and moulds. Pathogens such as *L. monocytogenes* and *Salmonella* spp. were not detected, though they are important to monitor (FASFC, 2014). *B. cereus*, a heat-resistant spore-forming pathogen, and coagulase-positive *S. aureus* were found in several studies (Bawa et al., 2020b; Belluco et al., 2016; Fernandez-Cassi et al., 2020; Fröhling et al., 2020; Grabowski & Klein, 2017b), even after some inactivation steps (Fasolato et al., 2018; Messina et al., 2019; Osimani et al., 2017). This highlights a significant food safety concern due to the difficulty of eliminating *B. cereus* spores with conventional heat treatments (Kooh et al., 2020). The impact of different rearing substrates and rinsing steps on microbial profiles has been minimal (Fernandez-Cassi et al., 2020), and the effect of fasting before killing remains unclear due to the lack of comparative studies (Bawa et al., 2020b; Belluco et al., 2016; Caparros Megido et al., 2017; Grabowski et al., 2008). Various processing methods, including heat treatments (autoclaving, steaming, boiling) and drying techniques (oven-drying, freeze-drying, etc.), were used, with boiling being essential for reducing spore-forming bacteria (FASFC, 2014; Kooh et al., 2020). However, more rigorous heat treatments are needed for better microbial reduction but may affect the nutritional and sensory qualities (Fröhling et al., 2020).

Drying alone may lower microbial loads but is insufficient for complete microorganism inactivation due to increased bacterial resistance as water activity decreases (Bourdoux et al., 2016). While powders are more stable due to low water activity, live microorganisms can survive and proliferate upon rehydration (Kooh et al., 2020). Therefore, ensuring the microbiological safety of *A. domesticus* powder requires comprehensive monitoring of raw insect quality, including total mesophilic bacteria, spore-forming bacteria*, B. cereus*, and *S. aureus*, combined with optimized heat treatments and strict hygiene practices throughout the production process.

4.2.3. Toxicological profile of *A. domesticus* forms

Fernandez-Cassi et al. (2019) attempted to create the risk profile of house crickets, but quantitative data on potentially toxic compounds were limited. Collavo et al. (2005) investigated heavy metals and trace elements in undried crickets fed various diets. This work primarily draws on EFSA's assessment of the safety of "Safety of frozen and dried formulations from whole house crickets (*A. domesticus*) as a Novel food pursuant to Regulation (EU) 2015/2283'' (EFSA NDA Panel et al., 2021c) and (Pastell et al., 2021). The EFSA NDA Panel emphasised that contaminant levels in dried *A. domesticus* are influenced by the contaminants present in their feed. (Pastell et al., 2021) found no significant differences in heavy metal levels among crickets fed different diets, although levels were similar across feeds. Heavy metal accumulation can vary by developmental stage and season (Janssen et al., 1993).Sorjonen et al. (2019) measured glycoalkaloid levels in crickets fed with potatoes, finding no accumulation, and the observed levels were deemed safe. Existing data indicate low contaminant levels in *A. domesticus*, with no significant toxicological concerns. Additionally, it is important to note that no evidence has been found indicating the presence of toxicologically concerning compounds that are endogenously produced in *A. domesticus*. This factor is crucial when evaluating the safety of products derived from insects (Ververis et al., 2020).

Studies on contaminants in products containing cricket powder and fried crickets (Köhler et al., 2019; Poma et al., 2017), offer limited insights into the toxicological profile of *A. domesticus* and may reflect processing impacts such as oil contamination or toxic compound formation from high temperatures (González-Gómez et al., 2021; Melgar-Lalanne et al., 2019).

4.3. Selection of components for the RBA

4.3.1. Methodological aspects

The identification, prioritization, and selection of components for inclusion in an RBA alongside associated health outcomes presents significant challenges. Historically, this selection has primarily relied on expert judgment without standardization, with qualitative justifications provided for their choices. Thomsen et al. (2022) illustrated this in a recent study, highlighting considerable differences in component selection among 106 RBA studies on fish and seafood. The outcome of an RBA is heavily influenced by the health impacts associated with the included components, meaning the inclusion or exclusion of relevant components can significantly alter the results.

To address these challenges, we developed a strategy for the transparent, reproducible, and harmonized selection of RBA components related to nutrition, microbiology, and toxicology. This strategy was tested though assessing the substitution of minced beef with cricket powder, revealing challenges related to food sources and data availability.

A three-step tiered approach was employed. The first step involved compiling a comprehensive "long" list of components for minced beef and cricket powder through an extensive literature review. This list included nutrients, nutrient-related components, microbiological agents, and toxicological compounds. In the second step, components were ranked and selected in each domain (nutrition, microbiology, and toxicology) using a harmonized strategy to create a "short" list. The final step resulted in a "final" list of components that were technically feasible and had available data for inclusion in the RBA. Components on the "short" list but not included in the "final" list were communicated with the results to highlight data gaps and limitations, which are critical for decision-making.

Several assumptions guided the method development. The scope was limited to a quantitative health impact comparison of defined scenarios using the DALY metric . A "food component-based approach" was adopted, focusing on associations between health outcomes and specific food components. The methodology assumed the inclusion of a boiling step in the cricket powder manufacturing process to reduce microbiological hazards (Kooh et al., 2020). Oven drying was considered for cricket powder production as it is the most common industrial method (FASFC, 2014). The developed approach can be integrated into the current methodological framework of RBA in food and nutrition. This structured and standardized methodology enhances the transparency and reproducibility of component selection, addressing historical challenges and improving the reliability of RBA outcomes. The developed strategy for RBA assessments at component level needs adaptation when including associations between health outcomes and consumption of a specific food commodity. In our case study, dose-response data on *A. domesticus*, a novel food, and health outcomes, were absent.

The prioritization index created, based on severity and occurrence, ensures equal importance is given to nutritional, microbiological, and toxicological components. This approach is crucial when comparing a well-studied dietary staple with a novel food that has many data gaps. For nutrition, the public health impact (severity) was weighted more heavily than occurrence (3/5 vs. 2/5), recognizing that nutrients are widely available, unlike contaminants. In microbiology and toxicology, severity and occurrence were equally weighted. This harmonized calculation method did not alter the final component list. The extensive list of nutrients, compared to contaminants, allowed for a more detailed ranking. Nutrient sub-criteria were multiplied, resulting in scores from 1 to 243, while contaminants scored between 1 and 9. This expanded nutrient ranking facilitated a thorough prioritization. The threshold for selecting components from the long list is informed by objective quantitative elements, enhancing transparency despite the subjective nature of the decision. While requiring additional time and research to justify sub-criteria scores, this method enhances the quality of decisions, identifies gaps, and provides a more objective evidence-based estimation of overall health impact.

4.3.2. DALY metric and feasibility constraints

In the field of food RBA, three main comparison strategies have been used for scenario comparison: HBGV, specific endpoints, and DALY (Pires et al., 2019). The most common approach compares consumer exposure levels to HBGV like the tolerable weekly intake (TWI) for toxicology and DRVs for nutrition. Although straightforward, this method treats all health outcomes with equal importance, neglecting severity differences. Furthermore, exceeding, or not meeting an HBGV doesn't necessarily correlate with a health outcome. The second approach, which evaluates changes in specific endpoints such as mortality rates, is also limited in its application for diverse health outcomes (Boué et al., 2022b).

Given the wide range of health outcomes in this study, we adopted the third strategy, which uses DALY to integrate both the quality and quantity of life lost due to disease (Boué et al., 2022b). Despite its complexity and need for detailed dose-response data, DALY provide a comprehensive metric being appealing to managers for comparing and ranking various risk management options because it encapsulates the entire complexity of the RBA issue into a single, straightforward figure. However, this requirement for detailed data restricted the list of components analysed, reducing it from 28 in the short list to 16 in the final list. The choice of DALY thus significantly influences the conclusions of an RBA study, as it depends on the availability of precise data for each "component-health outcome" relationship and corresponding DALY values per case.

4.3.3. The weight of evidence of “component(s)–health outcome(s)”

RBA can include various health outcomes with differing levels of scientific evidence for their association with specific food items, components or diets (Dorne et al., 2016). This evidence reflects the current understanding of the relationship between consuming a particular component and the resulting health outcome, termed the "biological knowledge of the day" (Hill, 1965). For example, health outcomes with well-documented biological mechanisms in humans are supported by stronger evidence than those suggested only by animal or in vitro studies. The evidence levels can vary among nutrients, microbiological, and chemical hazards. This was accounted for by selecting health outcomes from dose–response studies with strong evidence, but a clearer incorporation could be achieved using narrative descriptions as in BRAFO tables (Verhagen et al., 2012). Alternatively, a quantitative approach could consider the "probability of causation" based on expert evaluations, as proposed by Trasande et al. (2016).

As noted, the availability and quality of evidence can influence the selection of components and health outcomes, and consequently, the conduct of an RBA, potentially affecting the final outcome. This highlights the importance of exploring the use of alternative types of dose-response data, beyond meta-analyses, which are often regarded as the “gold standard” for aggregating evidence from multiple studies in epidemiology and other scientific fields. Meta-analyses combine the results of multiple studies, increasing statistical power and providing a more comprehensive estimate of effect size or association of interest, and can also help mitigate limitations such as small sample sizes or methodological biases in individual studies. However, other types of studies could also be considered to help fill evidence gaps, with careful consideration of their inherent strengths and limitations. As proposed in the recently updated EFSA Guidance on RBA of foods (EFSA Scientific Committee et al., 2024), approaches such as benchmark dose modelling (EFSA Scientific Committee et al., 2022), weight of evidence (EFSA Scientific Committee et al., 2017a) and considerations of biological relevance (EFSA Scientific Committee et al., 2017b) can be also applied in future efforts to integrate in RBA models different types of health effects, beyond just health outcomes, while ensuring the relevance, robustness, and accuracy of the evidence used.

4.4. Risk-Benefit Assessment outcome

Updated RBA methodologies were used to evaluate the public health impact of substituting red meat, a common dietary staple in Europe, with insect-derived alternatives. Specifically, our RBA focused on replacing beef with cricket powder in burger patties for adult populations in Denmark, France, and Greece. This research is pertinent in the context of evolving dietary patterns driven by health, environmental, and ethical considerations. The findings indicate that the health impacts of this dietary replacement vary depending on recipe formulations and the hydration percentage of cricket powder used, with sodium emerging as a key factor influencing the results. The study underscores that public health outcomes span a continuum rather than fitting into binary categories (Ververis et al., 2024).

The selection of burger patties and the formulation of reference and alternative recipes aimed to mirror common food preparation practices and explore extreme scenarios. Integrating insect-derived ingredients into Western diets is crucial, as consumer acceptance often depends on factors like familiarity, palatability, and food neophobia (Boehm et al., 2021). By considering two different hydration levels of cricket powder, 20% and 40%, we gained additional insights into how food processing and recipe formulation affect public health (Ververis et al., 2024).

A comprehensive RBA approach was employed, considering nutrients, microbiological, and toxicological hazards in minced beef and cricket powder. Using extensive compositional profiling (Naska et al., 2022; Ververis et al., 2022) and applying the methodological framework we developed on the harmonized selection and prioritization of food components (Boué et al., 2022b), we created a harmonized list of components for the RBA model. The novel nature of cricket powder posed challenges due to scattered and non-readily comparable data on toxicological and microbiological agents and nutrients (Ververis et al., 2022; Ververis et al., 2024).

Each selected component was paired with at least one health outcome, and systematic literature reviews and risk of bias assessments provided a robust foundation for evaluating the holistic health implications of the dietary substitution. The overall health impact was expressed using the common metric of DALY (Naska et al., 2022; Ververis et al., 2024).

The results indicate that substituting beef patties with insect patties can have beneficial or adverse health impacts, depending on the recipe and hydration percentage of the cricket powder. Shifting from the reference scenario to substitution scenarios A1 or B1 resulted in positive public health outcomes, while scenarios A2 and B2 did not offer beneficial alternatives. In scenarios where the health impact was not favourable, sodium in the cricket powder was the main contributing factor (Ververis et al., 2024). Sodium is an essential micronutrient for cricket growth and survival and is inherently present in cricket powder (Luckey and Stone, 1968). The production of meat preparations and meat analogues, whether domestic or industrial, may involve added sodium due to the use of salt in their preparation. Our study assumed that 10% of the patty ingredients, including any added salt, remained constant across all scenarios, thus isolating the comparison to minced beef and cricket powder as raw materials(Ververis et al., 2024).

In conclusion, the findings emphasize the importance of recipe formulation and ingredient composition in determining the health impact of substituting red meat with novel alternatives.

4.4.1. The importance of the recipe and the food comparators

While cricket powder contains significant sodium levels, it is possible to design recipes, such as scenarios A1 and B1, that result in lower overall sodium intake by using a 20% cricket powder content in the insect dough mixture. By excluding sodium and its related health outcomes from the RBA model (Table 13), our results indicated a positive health impact for all substitution scenarios across Denmark, France, and Greece (Ververis et al., 2024). These findings emphasize the need to create new food products with reduced salt content, aligning with salt-reduction strategies in the literature (Marakis et al., 2023). Novel ingredients like cricket powder can help achieve these goals, highlighting the importance of exploring dietary modifications for crickets to minimize sodium accumulation. Cricket powder, regulated under Regulation (EU) 2015/2283, must be incorporated into food products within EU-permitted levels. Our study examined "meat analogues" (A1, A2) and "meat preparations" (B1, B2). For meat analogues, the maximum EU permitted level is 50%; we explored 18% (A1) and 36% (A2). For meat preparations, the permitted level is 16%; we investigated 9% (B1) and 18% (B2), with B2 slightly exceeding the EU limit (Ververis et al., 2024). Food technological parameters are also critical. Studies have shown that up to 10% cricket powder can be used in emulsified meat products (Kim et al., 2017). Further research (Cavalheiro et al., 2023; Han et al., 2023) revealed technological limitations in hybrid meat sausages with higher cricket powder levels. Similar studies with other insect-derived powders, such as yellow mealworm and silkworm pupae, have reported comparable findings (Choi et al., 2017; Kim et al., 2016).

4.4.2. Relevant components not included in the RBA model

Copper and *Clostridium botulinum* were excluded from the model due to a lack of dose-response data for copper and insufficient data on *Clostridium botulinum*. Niacin, thiamin, and vitamin D3 were omitted because comprehensive data specific to oven-dried cricket powder were not available, and extrapolation from other dried cricket forms could introduce uncertainty. Polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) were excluded due to ongoing debates about their health outcomes. Although dried *A. domesticus* is high in n-6 PUFAs, substituting beef-derived saturated fats with cricket-derived PUFAs was not included in the RBA model because of data limitations and controversy (Naska et al., 2022; Ververis et al., 2024). The inclusion or exclusion of components can significantly affect the RBA outcome, making transparent documentation of all decisions and actions essential to ensure the assessment's completeness of RBA applications (Boué et al., 2022b; Naska et al., 2022; Ververis et al., 2024).

4.4.3. Strengths and Limitations

Current literature on the health impact of substituting red meat with insects is limited. Orkusz (2021) compared different meats and insect species based on nutrient composition, while Naska et al. (2022) provided preliminary results on RBA for novel proteins. Prior RBAs have focused on substituting red meat with other staples like fish (Thomsen et al., 2019; Thomsen et al., 2018) and pulses (Fabricius et al., 2021), mainly within the Danish diet. This study significantly advances the field of RBA and dietary assessment by comprehensively evaluating the health impacts of replacing red meat with edible insects. Unlike previous simplistic approaches, our study incorporates diverse data types (nutrition, microbiology, toxicology), various food formulations, and individual dietary intakes. We employed a probabilistic model, aligning with current RBA methodologies (Pires et al., 2019), which enhances the robustness and reliability of our findings. Our RBA model is versatile, allowing updates and incorporating new data, with a transparent and harmonized selection of components and health outcomes (Boué et al., 2022a; Boué et al., 2022b). However, the study has limitations. Assumptions, such as component distributions and prevalence of *C. perfringens* and *B. cereus* in crickets, introduce some uncertainty. The lack of dose-response data for certain components highlights the need for further research. Additionally, the component-based approach, necessitated by the novelty of the comparator, may affect some assumptions. For instance, the health outcomes of chitin (the primary fibre in *A. domesticus*) might differ from other dietary fibres, and matrix effects were not considered due to the novelty of cricket powder. Furthermore, although we assumed equivalent PAH levels in beef and insect patties, actual levels may vary due to matrix effects. Allergenicity was also excluded from the RBA model, although integrating allergenicity remains challenging in risk assessments of novel proteins (Fernandez et al., 2021; Verhoeckx et al., 2020; Ververis et al., 2020). Moreover, it should be noted that the WHO database HFA-DB provided demographic information on the size of the population of individuals aged 15 years and over, whereas the WHO GBD data refer to individuals aged 20 years and over. Though, given that the chronic diseases examined in this study are particularly pertinent to older adults, we anticipate that this discrepancy is unlikely to substantially affect our findings.

4.5. Social science and Knowledge insights for effective RBA communication

Proper communication of the RBA outcomes is crucial for facilitating dialogue between assessors and managers and providing dietary guidance to the public. The dual nature of RBA, which involves evaluating both risks and benefits, adds complexity to communicating its results (Boehm et al., 2021). Social science research can offer insights into these challenges, essential for developing an effective communication strategy. Communicating the outcome of this RBA study must consider the complex interplay of cognitive and emotional factors associated with red meat and edible insects.

The framework development is based on understanding how risk-benefit communication interacts with cognitive and emotional processes related to red meat and insect consumption. As there is limited research on communicating both risks and benefits simultaneously, the framework relies on expectations derived from existing literature on consumer behaviour and risk perception. The strategy must be adaptable considering the outcome of the RBA, to address all different scenarios that lead to a positive health impact overall (partial or total replacement).

4.5.1. Communication Principles

1. **Avoiding Negative Emotional Triggers**: For promoting insect consumption, it is crucial to avoid triggering disgust associated with animals. Visual imagery and text that evoke associations with live animals should be minimized. Instead, communication should use neutral language and avoid animal references, focusing on transparency about insect content in food.
2. **Collective Framing of Benefits**: Emphasizing the health benefits of insect consumption for vulnerable groups, such as those at risk from meat consumption or suffering from nutrient deficiencies, can be effective. Highlighting collective benefits and normalizing insect consumption could help overcome social stigma and encourage acceptance.
3. **Trusted Information Sources**: Engaging consumers in settings where they can interact with trusted organizations, such as government agencies or scientific institutions, can facilitate deeper engagement with the information. These settings might include information sessions or tasting events hosted by non-commercial, reputable organizations.
4. **Social and Cultural Context**: Social settings, such as family gatherings or friend groups, may foster positive discussions about the risks and benefits of insect consumption. Such environments can reduce emotional barriers and enhance social acceptability. Events that encourage exploratory behaviour and scientific engagement, such as science fairs or university outreach, can also promote acceptance.

5. **Transparency and Safety**: Unlike red meat, insects are less established in Western diets, making regulatory assurances less impactful. Thus, transparent communication about food safety for insects might have a smaller effect compared to meat. Focusing on reducing emotional barriers, such as food neophobia, and providing settings that support open discussion can help in making informed choices.

6. **Neutral and Comprehensive Messaging for Red Meat**: For red meat, which is well-established in European diets, a balanced presentation of both risks and benefits from trusted sources is essential. Cultural aspects and emotional barriers play a lesser role compared to insects. Clear, unbiased information about long-term health risks and benefits is crucial for informed decision-making.

4.5.2. Challenges and Limitations

Methodological differences and varied reporting standards among the studies reviewed hindered the ability to draw clear comparisons and discern trends. Additionally, the novelty of edible insects compared to red meat introduced emotional and cognitive factors that have been less extensively studied. Discrepancies between behavioural intentions and actual consumption further complicated strategy design, highlighting the need for approaches that address both attitudes and behaviours. Furthermore, inconsistencies in health perceptions related to red meat and insects were observed. For instance, conflicting views on the safety of beef and diverse cultural attitudes towards insects complicate the development of a cohesive communication strategy.

4.6. Conclusions

Food systems and diets continuously evolve, retaining some traditional elements while integrating new practices. These transformations can be driven by factors such as societal norms, individual preferences, health priorities, and environmental concerns. Increased awareness of the health risks and environmental impacts associated with red meat consumption has prompted interest in alternative protein sources, including insects.

Following the selection of *A. domesticus* (house cricket) as a promising insect for the EU food industry, we conducted a comprehensive, standardised compilation of its nutrient, microbiological, and toxicological profiles. This compilation, covering both dried and undried forms of the selected insect species, utilised a systematic methodological framework for data retrieval, extraction, and collation. We compared the compositional profiles of dried *A. domesticus* with those of minced beef from national food composition tables and databases of national food authorities, and we used the comparison results to develop a harmonized, transparent methodological framework for selecting nutritional, microbiological, and toxicological components when conducting an RBA. To develop this methodological framework, we used predefined criteria considering nutrient and hazard occurrence, health outcome severity, and public health implications. Given the lack of dose-response evidence linking *A. domesticus* consumption to health outcomes, whether beneficial or adverse, we employed a component-oriented RBA. Our approach methodologically treated *A. domesticus* powder (novel food) in a manner similar to beef, a staple food.

By integrating this methodology in RBA, and using a probabilistic approach, we assessed the health impact of partially or fully substituting beef with cricket powder in burger patties for adult populations in Denmark, France, and Greece. Our findings indicate that while house cricket powder has potential as a red meat substitute, the overall health impact depends on the amount used and the specific recipe, with sodium being the main driver of the results. High levels of cricket powder may be safe but are not always a healthier alternative compared to beef. Nonetheless, carefully designed incorporation of cricket powder into the burger patties can lead to positive overall health impact. Our study offers valuable insights for developing meat alternatives and hybrid products, highlighting at the same time areas requiring further research. Results should be interpreted cautiously, acknowledging uncertainties and data gaps. The same caution shall apply when RBA results are communicated to the public, considering the complex interplay of cognitive and emotional factors associated with the two food commodities we studied.

Dietary RBA can have a crucial role in informing and shaping evidence-based dietary guidelines, policies, and consumer choices. As our understanding of nutrition and food safety evolves, so must the RBA methodological and implementation framework. Ongoing research and advancements in assessment techniques are essential to address emerging challenges and support dietary patterns that enhance long-term health and well-being.

Effectively communicating RBA results must consider emotional and cognitive factors. It is vital to address emotional barriers, utilize trusted information sources, and reflect on cultural contexts. Tailoring communication to meet the specific needs of different audiences is essential for promoting informed and healthy consumption choices, as well as insightful dietary decision-making.

4.6.1. Future studies

Compiling the nutrient, microbiological, and toxicological profiles of *A. domesticus* forms was accompanied with challenges, primarily due to data heterogeneity and varying data quality across studies. The systematic review and data compilation identified data gaps that need to be addressed through original research to enhance the completeness and robustness of the compositional profiling of these insect forms. In line with the recommendations by Finglas et al. (2014) for improving food composition databases, increasing the geographical coverage of analysed samples could establish a more reliable, complete, and robust compositional profile for this species. Such considerations are essential when compiling the compositional profiles of novel foods and food ingredients, whether using existing literature data or generating new data.

During our research, we came across the fact that Greece lacks a comprehensive national food composition database comparable in scope and detail to those available in other European countries. However, past and ongoing efforts, with a more limited scope, exist (Katidi et al., 2021). Developing a Greek food composition database is crucial for advancing nutrition research, supporting public health initiatives, and enabling accurate dietary assessments. Such a database would facilitate research within the country both at academic and at industrial level, assist in better-informed food policy decisions and promote consistency in nutritional information (Delgado et al., 2021) within Greece and across Europe.

Additionally, a public health-related challenge that emerged from this study is the potential allergenicity associated with cricket powder consumption. Integrating allergenicity concerns into risk assessments of novel proteins remains a complex issue (Fernandez et al., 2021; Verhoeckx et al., 2020; Ververis et al., 2020). However, it is crucial to incorporate allergenicity aspects into RBA, especially when dealing with novel and alternative protein sources. Notably, food allergies already pose a significant epidemiological challenge, affecting up to 10% of the global population, with an increasing prevalence (Loh & Tang, 2018).

Finding and implementing a common metric that accounts for the health impact of allergenic reactions, including both acute and chronic effects, presents a significant challenge. Current allergy-related DALY estimates are limited to a few major allergenic foods, such as peanuts (Jakobsen et al., 2021). Other composite metrics such as the QALY (Quality-Adjusted Life Years), with potential uses in the RBA and MCDA fields, depending on the scope of the assessment, are being used in the field of food allergies (Fanning et al., 2021; Fong et al., 2022).

Furthermore, the impact of processing on the allergenicity of novel protein sources should be investigated, particularly for edible insects. Processing can affect the allergenic potential of proteins, and in some cases, processing might be necessary before incorporating these proteins into other products. Such insights could inform RBA, especially if they could be applied in a predictive manner, mimicking that of predictive food microbiology. This information would be useful in RBA cases comparing different food processing methods.

Our RBA was conducted on a food component rather than a food basis, due to the limited dose-response evidence available regarding human consumption of crickets, a novel food. Human trials should be conducted to investigate, and even establish, the potential health outcomes of entomophagy.

Finally, RBA approaches can be utilized to address complex food-related issues in an even more holistic manner, focusing on all potential health impacts to support decision-making. From a One Health perspective, the interconnections between human, animal, and environmental health are so integral that they cannot be considered in isolation. Thus, human health should not be viewed as the sole objective in a socially, environmentally, and economically sustainable agrifood system. A comprehensive evaluation of alternative protein sources should incorporate multiple factors such as greenhouse gas emissions, resource use, processing, pollution, waste management, and packaging. The balance or imbalance among these factors will determine the overall risks and benefits related to sustainability. Therefore, developing research projects and implementing tools that adopt a holistic approach to risk-benefit assessment, integrating sustainable practices from farm to fork, is crucial. RBA provides a foundation for integrating health and sustainability within a unified framework, facilitating evidence-based MCDA. Such approaches, while ensuring consumer health protection, also promote environmental sustainability by systematically comparing and ranking alternatives. It is important to emphasize that the development and implementation of these approaches will be significantly influenced by factors such as data quality, availability, interoperability, openness, and integration, both within and across countries.

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