SAFE SEAFOOD GUIDE FOR INDUSTRY:
EMERGING CHEMICAL CONTAMINANTS IN SEAFOOD

JANUARY 2017
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SAFE SEAFOOD – INDUSTRY GUIDE

Seafood is widely recognised as a high quality and healthy food source. European Union (EU) legislation ensures that food placed on the market in Europe is safe to eat and does not contain contaminants at levels that could threaten human health. However, new seafood health risks in the form of priority contaminants have arisen, for which limited information is available and for which no maximum limits have yet been set by authorities.

In response to these emerging risks, research has been committed to understanding these risks in relation to seafood consumption. ECsafeSEAFOOD, a European Union-funded research project dedicated to assessing potential risks of seafood consumption and ways of minimising them, was one such initiative. ECsafeSEAFOOD has developed this guide for the seafood industry based on scientific evidence from the project, to increase seafood safety for consumers and reduce human health risk. This guide aims to assist the seafood industry, from harvesters and producers through to wholesalers, distribution, retailers and analytical laboratories, in assessing health risks associated with seafood provision.

The guide represents the ECsafeSEAFOOD project’s current understanding of this topic with a particular focus on non-regulated chemical contaminants of emerging concern. Alternative approaches can be used if they still satisfy the requirements of the applicable statutes and regulations.

CONSUMER ATTITUDES AND SEAFOOD

SEAFOOD HEALTH BENEFITS

Recent results from the European Food Safety Authority (EFSA, 2014) indicate that seafood has many health benefits:

• Seafood is an excellent source of energy and protein, containing all essential amino acids humans need;
• Seafood is a valuable source of essential nutrients (iodine, selenium, calcium and vitamins A and D), as well as minerals and trace elements (zinc, phosphorus, iron and copper), which are required by the body for a variety of functions;
• Seafood is the main source of omega-3 fatty acids, which is required for normal growth, to support immunity, and to improve cardiovascular and brain health;
• The consumption of seafood, particularly fatty fish, lowers the risk of mortality from coronary heart disease;
• Consumption of one to two servings of seafood per week and up to three to four servings per week during pregnancy has been associated with better functional outcomes of neurodevelopment in children. However, during pregnancy, consumption of seafood species with a high mercury content (e.g. tuna, swordfish) should be limited and replaced with species low in methylmercury (e.g. sardines, anchovies, mackerel, trout);

• Seafood is a component of dietary patterns associated with good health.

Most European food-based dietary guidelines recommend two servings of seafood per week for the general population. This ensures the provision of key nutrients, especially omega-3, but also vitamin D, iodine and selenium.
CONSUMER PERCEPTION OF BENEFITS AND RISKS OF SEAFOOD CONSUMPTION

Consumer perceptions related to seafood and human health were assessed in five European countries (Spain, Portugal, Ireland, Belgium, Italy). Of the 2,917 consulted consumers:

- 72% considered eating seafood to be healthy despite the possible risks;
- 74% were aware that eating fatty seafood rich in omega-3 fatty acids lowers their risk of coronary heart diseases;
- 42% reported concern about the safety of consuming seafood, being mainly worried about heavy metals contamination (48%), plastic residues (41%), pharmaceuticals (34%), antibiotics and hormones (33%);
- Finfish are perceived as the healthiest seafood, of high quality, nutritious and safer than meat, while farmed fish was considered less appealing, but more affordable;
- European consumers also showed a strong interest in information about seafood safety, especially regarding shelf life, origin, date of capture, contaminant level, quality mark, safety guarantee, additives used and wild versus farmed fish;
- Consumers have more confidence in information provided by organisations that perform controls on the safety of seafood and less in governments, seafood industry and processors;
- European consumers trust physicians, doctors and other healthcare professionals the most in relation to information providers on seafood, followed by consumers’ organisations and scientists;
- The main information channels that consumers use to acquire information about seafood safety are a range of media, including the press and the internet.

More detailed information can be found in the scientific paper by Jacobs et al. (2015).
NEW METHODS TO DETECT PRIORITY CONTAMINANTS IN SEAFOOD

Pharmaceuticals and Endocrine Disruptors

Four novel methods were developed for the analysis of pharmaceuticals and endocrine-disrupting compounds in seafood within the ECsafeSEAFOOD project:

<table>
<thead>
<tr>
<th>Method</th>
<th>Type of sample</th>
<th>Family</th>
<th>Compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHPLC-MS/MS to detect pharmaceuticals</td>
<td>Fish</td>
<td>β-blockers, psychiatric drugs, antiplatelet agent, analgesics/anti-inflammatories, diuretic, anhidromintic, and asthma drugs</td>
<td>atenolol, carazolol, metoprolol, nadolol, propanolol, sotalol, carbamazepine, citalopram, diazepam, 10,11-epoxy-carbamazepine, 2-hydroxy-carbamazepine, lorazepam, sertraline, venlafaxine, clopidrogel, codeine, diclofenac, hydrochlorothiazide, levamisole, salbutamol</td>
<td>Huerta et al. 2013</td>
</tr>
<tr>
<td>UHPLC-MS/MS to detect pharmaceuticals</td>
<td>Bivalves</td>
<td>Antibiotics, psychiatric drugs, analgesics, anti-inflammatories, tranquilizers, calcium channel blockers, diuretic, and prostatic hyperplasia</td>
<td>ronidazole, metronidazole, dimetridazole, sulfamethoxazole, N-acetyl-sulfamethoxazole, azithromycin, erythromycin, venlafaxine, o-demethylvenlafaxine, carbamazepine, 10,11-epoxy-carbamazepine, 2-hydroxy-carbamazepine, citalopram, alprazolam, codeine, phenzone, propyphenazona, piroxicam, azapam, azaperol, diltiazem, hydrochlorothiazide, tamsulosin and some of their major metabolites</td>
<td>Alvarez-Muñoz et al. 2015</td>
</tr>
<tr>
<td>QuEChERS and LC-MS for antibiotics</td>
<td>Fish, clams and mussels</td>
<td>Antibiotics</td>
<td>azithromycin, clarithromycin, roxithromycin, spiramycin, tilmicosin, tylosin, tetracycline, clindamycin, lincomycin, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfadiazine, sulfapyridine, sulfisomidin, sulfisoxazole, N-acetyl sulfadiazine, N-acetylsulfamerazine, N-acetylsulfamethazine, metronidazole, metronidazole-OH, trimethoprim, chloramphenicol</td>
<td>Serra-Compte et al. 2016</td>
</tr>
<tr>
<td>QuEChERS and UPLC-MS/MS for endocrine disruptors</td>
<td>Fish</td>
<td>Antibiotics</td>
<td>1H-benzo triazole, caffeine, progesterone, levonorgestrel, tolytriazole, tris(2-chlo roethyle) phosphate, tris(2-butoxyethyl) phosphate, tris(2-chloroisopropyl) phosphate, estrone, 17β-estradiol, estradiol, 17α-ethynylestradiol, estrone-3-sulfate, bisphenol A, triclosan, methylparaben, ethylparaben, propylparaben, benzylparaben</td>
<td>Jakimska et al. 2013</td>
</tr>
</tbody>
</table>
Biosensors: Biotoxins, Antibiotics and Brominated Flame Retardants

Biosensors are analytical devices or units capable of providing results in a rapid, cost-effective, and environmentally friendly way, complementing standard analytical methods for environmental and food applications. Biosensors may become a feasible alternative to animal experimentation in bioassays for certain marine biotoxins.

The ECsafeSEAFOOD project developed biosensors for different targets such as marine biotoxins (tetrodotoxins, azaspiracids), antibiotics (sulphonamides) and brominated flame retardants (tetrabromobisphenol-A). These new tools have been validated with real seafood samples and results have been compared with reference methods. Results show that these new simple and easy-to-use tools are suitable for the detection of target analytes (contaminants) with a sensitivity below regulatory levels in seafood. These biosensors are highly valuable since they are suitable for routine testing, first screening of samples and field analysis. Due to their characteristics, they can be easily implemented in industrial processes providing relevant information about the quality and safety control systems of the seafood industry.

In the case of certain biotoxins where there are no easily applicable detection methods, the fisheries and aquaculture industry should rely on evaluation of these toxins in fish by the relevant control authority (regulatory agencies, government bodies, food safety authorities) and strictly follow current regulations that may apply to the commercialisation of the different species of fish in their area. The fisheries and aquaculture industry should collaborate with the control authorities to strictly implement the recommended practices.

• For more details and additional information about the developed assay suited to screening for azaspiracids in shellfish samples, a group of biotoxins that cause food poisoning in humans, see the Samdal et al. 2015 publication.
• For more details and additional information about the developed versatile, dependent, and robust method for the determination of Tetrodotoxin (TTX) in pufferfish and trumpet shellfish, see the Reverte et al. 2015 publication.

Electrode array connected to a multiplexed potentiostat for the detection of azaspiracids in mussels.

Microtiter wells of a colorimetric mELISA for the detection of tetrodotoxins in pufferfish.

Industrial Recommendations:

1. Biosensors are complementary tools to the confirmatory methods used for the screening of contaminants in seafood samples such as chromatography coupled to mass spectrometry. Biosensors can be easily used by the seafood processing industry and help to increase their own controls as a part of the Hazard Assessment and Critical Control Points (HACCP). Positive results (presence of contamination) may be confirmed by confirmatory methods.

The newly developed ECsafeSEAFOOD biosensor prototypes are validated and ready to be applied along the whole seafood chain: producers, processors, distributors and retailers. The next step is the integration of all components into a final marketable design to commercialise the biosensors. Biosensor developers and manufacturers are invited to contact Alejandro Barranco (abarranco@azti.es) and Mònica Càmpas (monica.campas@irta.cat) for more information.
MITIGATING CONTAMINATION

EFFECTS OF ORIGIN AND BIOLOGY ON CONTAMINANT LEVELS

A wide range of environmental pollutants have been observed in a variety of seafood species. The types and levels of pollutants observed depend heavily on the site of origin; that is, how and to what extent the area is affected by human activities and by the biology of the seafood species.

• Estuaries of large rivers, especially those in industrial and densely populated coastal areas are in general more polluted;
• Open seas and oceans far away from human activities generally contain lower levels of contaminants;
• Fish higher in the food chain (e.g. tuna) are more susceptible to the bioaccumulation of pollutants such as methylmercury and PCBs;
• Larger and older specimens from the same location contain higher levels of contaminants (i.e. tuna specimens of larger sizes show higher methylmercury levels);
• Species with high lipid content contain higher levels of lipophilic contaminants, such as PCBs and flame retardants;
• Hydrophilic contaminants (e.g. pharmaceuticals) do not accumulate to very high levels in fish tissues. The levels of these contaminants in seafood are less affected by size (age) and food chain effects due to faster rates of uptake;
• Selenium-rich seafood (e.g. oysters, mussels, octopus) not only prevents methylmercury toxicity, but can also rapidly reverse some of its severe neurotoxic symptoms.

When the effects of origin and seafood biology are compared in terms of levels of contamination, it can be said that origin has the highest influence, since:
• in highly polluted areas all seafood species can be affected;
• in low polluted areas all seafood species contain very low levels of pollutants (with a few exceptions, like mercury in large and old predatory fish).

INDUSTRY RECOMMENDATIONS:

The current seafood consumption pattern in Europe indicates that consumers are most exposed to methylmercury and pentabromodiphenyl 99 (PBDE99, flame retardant), which occur at higher levels in larger sized specimens and in species occupying higher trophic levels. Yet, seafood in Europe generally has low levels of other contaminants, posing no risk to human health. The highest levels of contaminants in seafood are usually observed in seafood from estuaries of polluted rivers and along the coast where human activity is high. Choosing seafood species from open seas and oceans, where pressure from human activity is low, and diversifying seafood consumption with a focus on species occupying low trophic levels, will help prevent the uptake of unknown and unmonitored contaminants through human consumption.
EFFECTS OF ORIGIN AND BIOLOGY ON THE LEVELS OF BIOTOXINS

The EU has established regulations for all marine toxins that are considered hazardous for consumers, and information concerning the levels of toxins in seafood are shared by the Member States:

- Across the EU, accredited laboratories implement harmonised methods under high quality standards so equivalent approaches for establishing the levels of toxins in seafood are implemented;
- All production areas for shellfish are regularly monitored in the EU;
- Regardless of origin and species, seafood products that follow EU commercial regulations and are commercialised through the appropriate channels should be safe for consumption.

Exceptions include the biotoxin “ciguatoxin” in fish and “tetrodotoxin” in shellfish and pufferfish, which are emerging issues in the EU.

However:

- Only Canary Islands and Madeira have reported cases of ciguatoxin, and the Netherlands, the UK and Mediterranean area have reported tetrodotoxin.
- Ciguatoxin is found mainly in amberjack (*Seriola sp.*) and tetrodotoxin is found mainly in pufferfish and shellfish.
- Regulatory bodies in these regions have already set up management procedures through local regulations to limit the consumption of seafood that has a high risk of contamination with ciguatoxin and tetrodotoxin.

INDUSTRY RECOMMENDATIONS:
Regulated biotoxins are covered by official monitoring programmes that control biotoxins in shellfish harvesting areas. However, the seafood industry could consider increasing internal controls for marine toxins according to their specific needs for the management of seafood production and commercialisation.

Ciguatoxin has so far only been reported by the Canary Islands and Madeira so the seafood industry should avoid commercialisation of amberjack from these areas.

EFFECT OF INDUSTRIAL PROCESSING ON CONTAMINANTS

The ECsafeSEAFOOD project findings were as follows:

- Cooking and peeling of raw prawns increased the Hg concentration but reduced the concentration of all other elements including inorganic arsenic (iAs), total arsenic (tAs), chromium (Cr), zinc (Zn), selenium (Se), cadmium (Cd), copper (Cu) and iron (Fe);
- Trimming (removal of fatty tissue) and smoking of salmon did not significantly change the lipid, dry matter and contaminant (elements, Perfluorooctanesulfonic acid, α-HBCD (Hexabromocyclododecane)) levels;
- Smoking increased the level of polychlorinated biphenyl (PCB) compounds in halibut per wet weight, but levels were not changed based on lipid or dry matter basis;
- Non-toxic organic arsenic was not transformed to carcinogenic inorganic arsenic by industrial smoking or cooking of seafood;
- In all cases, the levels of all measured contaminants were still lower than the current EU maximum levels for cadmium (Cd), lead (Pb), mercury (Hg) and PCB in seafood.

INDUSTRY RECOMMENDATIONS:
To evaluate contaminant risk of processed seafood, analysis of contaminants must be done on the final processed product rather than on the raw unprocessed seafood, since processing may change the concentration levels. This recommendation is in accordance with the rules set out in the EC regulation 1881/2006.
EFFECT OF COOKING ON HARMFUL CONTAMINANTS IN SEAFOOD
Cooking can affect environmental contaminant levels in seafood such as methylmercury (MeHg), inorganic arsenic (iAs), perfluorinated compounds (PFCs), endocrine disruptors (EDCs), brominated flame retardants (BFRs), musks, polycyclic aromatic hydrocarbons (PAHs) and UV filters. Seafood species with higher water loss during cooking (steaming) show increased levels of contaminants. Tested species were ranked according to the number of contaminants showing increased levels after cooking by steaming, from the lowest (canned mackerel) to the highest (mussels).

Yet, the levels of contaminants after cooking do not present a potential health risk for consumers.

steaming increases the concentration of the specific contaminant level in particular seafood species, however these levels do not represent a potential health risk for consumers.

For a more detailed table outlining the effect of cooking on specific contaminants, see: http://ecsa.seafood.eu/cookingspecificcontaminants
CASE STUDY: PHYCOREMEDIATION AS A TOOL TO REDUCE PRIORITY CONTAMINANTS

Phycoremediation involves the use of algae for the removal or biotransformation of contaminants from aquatic systems. This technology is environmentally friendly and less expensive than most other contaminant reduction methods. ECsafeSEAFOOD carried out phycoremediation research, using the seaweeds *Saccharina latissima* and *Laminaria digitata*, to discover that seaweeds can contribute substantially to the uptake of organic and inorganic contaminants from seawater.

ECsafeSEAFOOD research results showed:

- A strong decrease (more than 85%) in venlafaxine (VEN) from seawater was observed during the first 12 hours of an experiment with the seaweed *S. latissima*.
- Uptake of VEN is faster in *S. latissima* than in *L. digitata* (24 hours instead of 120 hours).
- *L. digitata* proved to be the better candidate for removal of arsenic from seawater (reduction of more than 75%).
- Neither *S. latissima* nor *L. digitata* were able to decrease concentrations of tetrabromobisphenol-A (TBBPA) and bisphenol-A (BPA) in seawater.

Seaweeds can also slightly reduce the bioaccumulation of some contaminants in mussels:

- Best results were obtained for VEN, inorganic arsenic (iAs) and diflubenzuron, with decreases of 25%, 84% and 70%, respectively.
- Levels of lindane, Cu (copper) and Cd (cadmium) did not decrease in mussels with the presence of *L. digitata*.

The presence of contaminants in the medium did not affect the physiological condition of the seaweeds.

INDUSTRY RECOMMENDATIONS:

Phycoremediation by the seaweeds *Saccharina latissima* and *Laminaria digitata* can be a good mitigation technology to reduce the levels of certain contaminants in bivalves and/or in water environment. *S. Latissima* significantly reduces venlafaxine (VEN) in seawater and *L. digitata* decreases arsenic levels in seawater and mussels. For more information, contact Sara Cunha (sara.cunha@ff.up.pt) or see Anacleto et al. (2017).

Figure 1. a) Trial: of *L. digitata*; b) Trial: water glass beakers spiked with venlafaxine (VEN); c) Trial: water plastic beakers spiked with inorganic arsenic (iAs).
CONCLUSIONS

Seafood is a unique, high quality, safe and nutritious food item. A diet rich in seafood contributes to a healthy life. Although contaminants of emerging concern can be found in raw and cooked seafood, the levels are low and represent no potential health risks for consumers following the recommendations of health authorities of eating two servings of seafood per week. To ensure a balanced and healthy diet, consumers need to diversify seafood consumption, prioritising species occupying lower trophic levels (e.g. mackerel, sardines), species from sustainable and responsible fishing and farming practices, and species harvested in areas subjected to efficient environmental regulation mechanisms. The new, fast, sensitive and robust analytical and biosensor tools validated for contaminants in seafood samples have huge potential to be used by the seafood industry as part of the internal quality assurance procedures (HACCP).

To improve consumers’ confidence in the seafood industry, transparent information about product characteristics (e.g. origin, date of capture, safety guarantee) along the trade chain (i.e. producers, processors, distributors and retailers) is needed. The industry should also implement mitigation measures to strengthen the safety of their products, including those related to processing and phycoremediation. Understanding consumer needs and perceptions in relation to seafood safety is crucial to ensuring successful acceptance of seafood products.

ADDITIONAL INFORMATION

- The following table indicates the current EU legislation on regulated contaminants (including regulatory limits).

<table>
<thead>
<tr>
<th>Regulated contaminants in seafood</th>
<th>Legislation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo(a)pyrene (PAHs), PCBs, dioxins, Pb, Cd, Hg, PFAS</td>
<td>EU Reg. 1881/2006; EU Reg. 629/2008; EU Recom. 2010/161 (PFAS); EU Reg. 589/2014 (PCBs) (Chemical contaminants)</td>
</tr>
<tr>
<td>Listeria monocytogenes, Salmonella, Escherichia coli, histamin, Estafilococos coagulase positivos</td>
<td>EU Reg. 2073/2005; EU Reg. 1441/2007 (Microbiological criteria)</td>
</tr>
</tbody>
</table>

- The project website of the EC funded ECsafeSEAFOOD project, contains general project information as well as partner information, project outputs and more. See: www.ecsafeseafood.eu

- The EU REACH Regulation (Registration, Evaluation, Authorisation and Restriction of Chemical substances) was introduced in 2007 to improve the protection of human health and of the environment through better and earlier identification of the intrinsic properties of chemical substances. Under the REACH regime, manufacturers and importers are required to gather information on the properties of their chemical substances and to register the information. By registering and limiting the use of dangerous chemicals in the industrial production chain, REACH will indirectly reduce the contamination of marine waters and marine organisms by toxic substances. This will in turn decrease the risk of contamination of fish and seafood for human consumption. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006R1907.

- The EU’s Rapid Alert System for Food and Feed (RASFF) is a key tool to ensure the cross-border flow of information to swiftly react when risks to public health are detected in the food chain. RASFF enables information to be shared efficiently between its members (EU-28 national food safety authorities, European Commission, European Food Safety Authority, EFTA Surveillance Authority, Norway, Liechtenstein, Iceland and Switzerland) and provides a round-the-clock service to ensure that urgent notifications are sent, received and responded to collectively and efficiently. For more information or to access the RASFF portal, see: http://ec.europa.eu/food/safety/rasff_en.
ECsafeSEAFOOD has developed a number of useful resources that can help seafood industry stakeholders in improving contaminant diagnostics and seafood risk assessments. They can also be used to highlight the deficits in seafood contaminant research. These resources include an online tool for stakeholders that balances the benefits and risks associated with seafood consumption and a unique repository that collates data on contaminants of emerging concern in seafood species.

NOVEL ONLINE DATABASE OF THE RELEVANT PRIORITY ENVIRONMENTAL CONTAMINANTS IN SEAFOOD

The ECsafeSEAFOOD Contaminants Database collates data on contaminants of emerging concern in seafood species. The contaminants in question include brominated flame retardants, endocrine disruptors, toxic element species, pharmaceuticals, personal care products, perfluorinated compounds, microplastics, and marine biotoxins among others.

USEFUL RESOURCES

- FDA Fish and Fishery Products. Hazards and Controls Guidance (Fourth Edition) – April 2011. These guidelines are intended to assist processors of fish and fishery products in the development of their Hazard Analysis Critical Control Point (HACCP) plans. The guidelines will help processors of fish and fishery products to identify hazards that are associated with their products and formulate control strategies. www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf.
- EFSA Chemical Contaminants Database, email johanrobbens@ilvo.vlaanderen.be, then visit: www.ecsafeseafooddatabase.eu.

FISHCHOICE: NEW ONLINE TOOL TO EVALUATE BENEFITS AND RISKS OF SEAFOOD CONSUMPTION

The ECsafeSEAFOOD project partners have developed an online tool for stakeholders that balances the benefits and risks associated with seafood consumption while taking the exposure to contaminants into consideration. The tool is called FISHCHOICE and is available at: www.fishchoice.eu. Professional access can be requested online.
SOURCES


ECsafeSEAFOOD project:
www.ecsafeseafood.eu
@ECsafeSEAFOOD
Contact: Dr António Marques
(amarques@ipma.pt)
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**ECsafeSEAFOOD** has been assessing food safety issues related to priority contaminants present in seafood as a result of environmental contamination (including those originating from harmful algal blooms and those associated with marine litter) and evaluating their impact on public health.

Availability of safe and high-quality food is a growing public concern and research plays a very important role in ensuring consumer confidence in this sector. **ECsafeSEAFOOD** will provide scientific evidence to serve as a basis for further development of common food safety, public health, and environmental policies and measures, by seeking to establish a quantitative link between the contamination of the marine environment and that of seafood.

**ECsafeSEAFOOD** aims to increase consumer confidence through the provision of clear and practical communication and information, working in close collaboration with food safety authorities.

For more information, please visit the project website, [www.ecsafeseafood.eu](http://www.ecsafeseafood.eu), or contact the project coordinator, Dr. António Marques, [amarques@ipma.pt](mailto:amarques@ipma.pt).

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Editing, proofing and design was carried out by AquaTT – Marieke Reuver, Tanja Calis, Eva Greene, Ruth McAvinia, Cliona Ní Cheallacháin, Anne-Marie Williams and Laura Macaulay.

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**HOW TO REFERENCE THIS GUIDE**

Written permission is not required for the distribution or use of this guide. However, the authors request that reference to the guide, or any of the content therein, is cited as follows:


The **ECsafeSEAFOOD** project sincerely thank the below organisations for their contribution to the validation of this document.

This guide is not intended as a comprehensive seafood safety guide. It is designed to provide information to assist decision-making regarding seafood safety. It has been developed based on scientific evidence from the project, as well as the best available evidence at the time of development of this publication.