ECsafeSEAFOOD

Priority environmental contaminants in seafood: safety assessment, impact and public perception

Grant agreement no: 311820

Final report

Month 1 (February 2013) – Month 48 (January 2017)

Period Covered: 01-02-2013 to 31-01-2017

Actual submission date: M50

Start date of the project: 02/2013 **Duration:** 48 months

Organisation name of lead contractor: IPMA

Revision: V1

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	x
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

4.1. Final publishable summary report

Executive summary

There is still limited information about levels of contaminants in seafood, for which no maximum permitted levels (MPLs) in seafood have been set by European authorities in seafood, i.e. contaminants of emerging concern, such as some toxins from harmful algal blooms, marine litter and associated chemicals, endocrine disruptors, pharmaceutical and personal care products, metal species and brominated flame retardants. In this way, the ECsafeSEAFOOD project assessed food safety issues related to those contaminants as a result of environmental contamination and evaluated their impact on public health, contributing to the improvement of seafood risk management and communication. For this purpose, the main achievements of ECsafeSEAFOOD project were: 1) Creating a unique free online database (www.ecsafeseafooddbase.eu) containing information on the levels of contaminants of emerging concern in seafood, allowing users to download or upload relevant information (WP1); 2) Monitoring the presence of marine toxins, microplastics and chemical contaminants of emerging concern (e.g. pharmaceuticals and personal care products, endocrine disruptors, metals speciation, brominated flame retardants, perfluorinated compounds) in commercial seafood species available in different European countries, integrating the effect of origin, season and cooking (WP2); 3) Developing new methods for the assessment of contaminants in seafood (e.g. microplastics, toxins, pharmaceuticals), and in-house reference material was prepared for emerging toxins (e.g. tetrodotoxins) (WP2); 4) Understanding seafood consumption pattern, risk perception and information needs of consumers, and the possible impact of information messages targeted at the general public (WP3); 5) Identifying methylmercury and the brominated flame retardant PBDE99 as the only contaminants requiring further refinement of the exposure assessment through the seafood diet (WP3); 6) Establishing the Lowest Observed Adverse Effect Level (LOAEL) for ciguatoxins (CTX) in an episode of ciguatera fish poisoning (WP3); 7) Establishing the areas at risk for Ciguatera in a regional study evaluating the presence and concentration of CTXs in Lionfish; 8) Developing and validating a free online consumer tool, named FishChoice to balance risks and benefits of seafood consumption and to advice consumers and health professionals on healthier seafood choices (www.fishchoice.eu) to improve consumer education (WP3); 9) Validating macroalgae as a mitigation solution to

reduce contaminant levels in seafood (WP3); 10) Developing three validated guides with useful information on chemical contaminants in seafood that can be used by consumers, seafood industry and policy makers (WP3); 11) Identifying the most appropriate strategies to reduce the levels of contaminants by seafood industrial processing and consumers (WP3); 12) Developing and validating new sensitive and fast detection systems with real food samples for marine toxins (azaspiracids and tetrodotoxins), antibiotics (sulphonamides) and tetrabromobisphenol A, enabling controlling seafood safety and creating a potential business area for SMEs fabricating sensors (WP4); 13) Identifying acute and sub-acute effects of emerging contaminants through bioaccessibility and bioavailability (human cell lines and zebrafish) *in vitro* assessment of contaminants in raw and processed seafood (WP5); 14) Understanding toxicokinetics, accumulation, elimination and carryover of contaminants from the feed to fish, crabs and bivalves, taking into account the impact of microplastics and climate change (WP6); and 15) Ensuring transparent messages for seafood consumers through numerous dissemination materials like press releases, articles, newsletters, posters, videos, scientific and technical publications, conference proceedings stakeholders seminars and a final project event (WP7).

Such achievements will enable to increase consumers' confidence in seafood consumption, and will have a positive economic effect as a result of the promotion of seafood consumption throughout Europe in a conscientious way.

Summary description of project context and objectives

Seafood is widely recognized as a high quality, healthy and safe food, but like other food items, some can accumulate environmental contaminants with potential impact on human health. In Europe, seafood is regularly controlled for some environmental contaminants (e.g. Pb, Hg, Cd, toxins, PCBs, Dioxins and PAHs) through efficient monitoring programs, providing crucial information for management and risk assessment purposes. However, little information is still available for contaminants for which European authorities have not established MPLs, i.e. contaminants of emerging concern, such as toxins from harmful algal blooms, marine litter and associated chemicals, endocrine disruptors, pharmaceutical and personal care products, metal species and brominated flame retardants. In order to contribute to the improvement of seafood risk management and risk communication, to

increase the safety of seafood for European consumers, and to reduce human health risks, ECsafeSEAFOOD assessed safety issues mainly related to non-regulated contaminants of emerging concern present in seafood as a result of environmental contamination and to evaluate their impact on public health. The project also addressed the European Marine Strategy Framework Directive, especially descriptor n°9 on contaminants in fish and seafood, with relevance to determine the Good Environmental Status of EU waters. For this purpose, the ECsafeSEAFOOD project integrated a set of specific objectives to fulfill the main objective:

• Monitor the presence of environmental contaminants of emerging concern in the environment and in seafood and prioritize those contaminants that are real hazards for human health (WP1 and WP2).

• Study the effect of processing / cooking on the behaviour of environmental contaminants of emerging concern in seafood (WP2).

• Optimize methods for the detection and quantification of emerging toxins from harmful algal blooms (WP2).

• Investigate what information is needed and how it should be disseminated, in coordination with risk managers, to the general public and to vulnerable groups of consumers in order to reduce public health risks from seafood consumption, and test the possible impact of dissemination on public opinion (WP3).

• Perform risk assessments in order to measure the potential impact of seafood contaminants on public health using in-depth probabilistic exposure tools (WP3).

• Develop mitigation tools to be implemented by risk managers, like two online tools (FishChoice; http://www.fishchoice.eu/) for different stakeholders (i.e. one simpler tool for consumers and another with more detailed information for other stakeholders like doctors, nutritionists, researchers, policy makers, food safety authorities) and guidelines, as well as test innovative technologies to decrease contaminant levels in seafood, such as phycoremediation and industrial processing (WP3).

• Confirm/refine the European Maximum Reference Levels (MRLs) in seafood for contaminants of emerging concern that are real hazards and for which no legislation exists or information is still insufficient (WP3).

• Develop, validate and provide new tools to make the assessment of the presence of environmental contaminants in seafood products easier and faster (WP4).

• Develop further understanding of public health impacts of these chemical hazards, through the toxicological characterization of selected seafood contaminants in realistic conditions, i.e. assessing synergistic and antagonistic effects between contaminants, and analyzing the bioaccessibility and bioavailability of real seafood product samples before and after undergoing processing and culinary treatment (WP5).

• Quantify the transfer of relevant environmental contaminants of emerging concern between the environment and seafood, taking into account the effects of climate change (WP6).

• Effectively inform, communicate and disseminate project outputs to key stakeholders (policy makers, food producers) and consumers, and the general safety of seafood to reduce the public health risks (WP7).

ECsafeSEAFOOD activities addressed these objectives and were structured in eight work packages (WPs) targeting environmental contaminants of emerging concern, including toxins from harmful algal blooms and marine litter. WP1 elaborated a database with relevant information required for risk assessment gathered from literature and national monitoring programs. WP2 monitored contaminants in seafood using an ambitious sampling strategy following the recommendations of the Marine Strategy Framework Directive (Descriptor 9) and assessed the effect of seafood processing/cooking on contaminants. In WP3, risk assessment (with data from WP1-2) and mitigation strategies were implemented to reduce the impact of risky contaminants on human health. WP4 developed fast screening/detection methods for relevant contaminants tailored to suit stakeholders needs to promote consumers' confidence in seafood. WP5 carried out the toxicological characterization of contaminated seafood in realistic conditions and used alternative toxicological methods to provide tools for the risk assessment (WP3). WP6 assessed the links between the level of contaminants in the environment and that in seafood through controlled trials and case-study species, taking into account the effect of climate changes. WP7 implemented a strategy for education, training with clear and practical dissemination of results. Finally, WP8 ensured efficient project management.

Description of main S & T results/foregrounds

WP 1: Database and selection of priority contaminants

In WP1 the ECsafeSEAFOOD database web application was developed using the blog software WordPress. The database is available at http://www.ecsafeseafooddbase.eu and a link is also available at the project website. The purpose of this database is to gather information available about levels of contaminants in seafood, as well as their toxicity. Relevant criteria for contaminant selection were defined and incorporated in the database, including details for each study about the contaminant, seafood species, origin, season, levels of contaminant, toxicity, methodology used and reference (see details in D1.1). The database was created in April 2013 and was kept up to date during the whole project duration. A comprehensive literature review and collection of data concerning contaminants data was performed by all partners involved in WP1. Relevant sources of information included scientific literature, national and international monitoring programs, and reports. Contaminants were ranked based on 1) concentration levels and 2) toxicity effects. Based on this ranking lists, the outcome of the hotspot sampling (WP2) and expert judgment, a final selection of contaminants from the database was made (D1.3) leading to 40 chemical contaminants divided in 6 classes (see Table 1). Additionally, microplastics were also considered as relevant together with the following toxins: Azaspiracids, Ciguatoxins, Palytoxins, Tetrodotoxins and Cyclic Imines (Spirolides, Pinnatoxins, Gymnodimines).

Table 1 – List with the final selection of contaminants for further activities in the project

PerFluorinated Compounds (PFCs)	Toxic elements	Endicrine Disruptores	Brominated Flame Retardants (BFRs)	Pharmaceuticals	Musks	Others		
PFOS PFOA PFNA PFHxS PFDcA PFUnA PFDoA PFBS PFPeA PFHxA	Inorg AS MeHg	Bisphenol A Triclosan Methylparaben Nonylphenol TBEP	HBCD PBDEs HBB DEC602 Metho PBDE TBBPA 2,4,6-tribromphenol	Diclofenac .Sulfamethaxole Sotalol Diazepam Carbamazepine Venlafaxine Citalopram Oxytetracycline	Galaxolide Tonalide Cashmeran	UV-filters	PAH oxyPAH MethylPAH	Microplastics

The database will remain available also after the end of the project. The ECsafeSEAFOOD database contains 4938 studies out of 437 articles corresponding to 375 contaminants. Results of analyses of contaminants in commercial species (WP2) were also incorporated in the database, as well as information about climate change. This valuable source of data is open for all interested stakeholders. Presently, the ECsafeSEAFOOD database received 185

login requests (e.g.: EFSA, FAO, Seafish, Cefas, Ifremer, Visfederatie, Universities). In the last year, the database had 12.160 visitors counting for 68,770 visits (table 2), indicating a high interest. The publication, "Environmental contaminants of emerging concern in seafood – European database on contaminant levels", was published at Environmental Research Journal. It summarizes the project's description and the database relevance. In this paper the current status on the knowledge of human exposure, toxicity and legislation is presented and the outcomes from scientific publications reporting the levels of these compounds in seafood is presented and discussed.

Table 2 – ECsafeSEAFOOD database visitors by date and country.

Visitor	Visit
Today:	257
Yesterday:	320
Last 7 Days (Week):	3,998
Last 30 Days (Month):	19,781
Last 365 Days (Year):	68,770
Total:	124,544

Rank	Flag	Country	Visitor Count
1		United States	1,561
2		Unknown	1,253
3		India	1,158
4		Romania	775
5		France	678
6		Italy	664
7		Brazil	553
8		Philippines	545
9		United Kingdom	533
10		Serbia	503

WP2: Monitoring priority contaminants in seafood

To properly assess the levels of contaminants in seafood an elaborated sampling protocol and plan for the collecting, processing and distribution of samples in the project was prepared (details in D2.1). This plan describes how and what samples, both from hot spots as well as commercial species from open seas, should be collected, treated and shipped to the partners for quantification of chemical contaminants, microplastics and toxins from harmful algal blooms. A two-step approach was used; first were potential hotspots for high

environmental pollution (e.g. estuaries of large rivers in idustrialised areas) monitored for contaminants that could be quantified in the marine environment. Based on these findings the commercial seafood samples from open seas were analysed for this set of contaminants. The sampling protocol for chemical contaminants is differentiated from the sampling plan established for toxins and microplastics. Logistically, the implementation of this sampling protocol was a challenge as it involved the harmonized collection of specific seafood samples in several European countries, processing for transport according to the specificities required for each contaminant and shipped to the partners involved in contaminant quantification. Despite minor delays the implementation of hot spot and commercial sampling was a success for two reasons. The hotspot sampling enabled a better focus on contaminants to be analysed in the commercial samples of open seas andthe list of samples (species, size and location) was prepared to address all tasks and objectives of WP2 with the least amount of samples. The strategy to investigate the effect of cooking on contaminant levels, according to activities/tasks foreseen in the DoW is also included in the document. As samples have to be collected all over Europe and beyond and shipped to partners, freeze drying methods were successfully tested to minimize the deterioration of most chemical contaminants in seafood samples during transport. Therefore, freeze dried samples were shipped without refrigeration and unforeseen delays in shipment (such as custom control) did not cause deterioration of samples. Only for volatile compounds, such as low-chlorinated PCBs and PAHs freeze-drying the risk of loss of contaminants exists and freeze-drying was therefore used for these samples. For toxins, the effect of freeze drying was evaluated in ciguatoxins (CTXs) in fish. For most samples, a higher matrix effect was observed in freeze-dried samples in comparison with fresh samples. Regarding the concentration of CTXs, the trend was that CTX concentrations were lower in freeze dried samples than in fresh samples.

Hot spot and commercial samples, collected in two rounds (round I and II) were analyzed for a wide range of contaminants and microplastics. Final results of the selected priority contaminants analyses were compiled and reported in Deliverable 2.4.

The use of micro-CT techniques to identify the transport and distribution of ingested microplastics was investigated. The results from UGent indicate that the use of micro-CT imaging is a promising technique for microplastic research. However, since X-rays interact with electrons in the sample, electron-dense structures can be visualized in a CT-image as a

contrast pattern against the background. Since biological soft tissue, as well as plastic, is composed of low-atomic-number elements (such as carbon, oxygen and hydrogen), it produces very little contrast. Therefore, both soft tissue as well as microplastics have to be labeled/treated to ensure an effective visualization of the soft tissues and microplastics, by using extra-dense microplastics (here, microplastics embedded with barium sulphate). These extra-dense microplastics act as a radiocontrast agent, resulting in denser particles and hence better visible on the scan. It was decided to limit the sample for micro-CT imaging to the digestive gland (instead of the entire mussel) since both the stomach and intestine are located in this gland and a higher resolution of the micro-CT images could be obtained by reducing the size of the sample. Using micro-CT we were able to identify microplastics ingested by the exposed mussels. These results indicate that the use of micro-CT imaging is a promising technique for microplastic research. Nonetheless, this technique is not applicable for the identification of microplastic in natural samples (i.e. organisms collected in the field). However, this technique is useful for laboratory experiments on ingestion and translocation of microplastics. Therefore, traditional extraction techniques, i.e. tissue destruction need to be used for the assessment of microplastic burden in mussels. Microplastic extraction was based on the principle of wet digestion of tissues using acid. Two acid digestion methods were performed on mussel samples from hotspots: (a) Acid mix Method used a combination of nitric acid and perchloric acid; and (b) Nitric acid Method. The results of this exercise are reported in deliverable D2.3. Microplastic abundance data obtained for sediment and mussel originating from four European hot spot locations: Po estuary (Italy), Tagus estuary (Portugal), Ebro estuary (Spain) and Western Scheldt (Netherlands) (reported in D2.3) were used to model the impact of microplastic ingestion on bioaccumulation of hydrophobic organic chemicals (HOC) by the mussel. The mechanistic model from Bart Koelmans (Wageningen Marine Research) was used and described in D2.11. At environmentally relevant microplastic concentrations, microplastic ingestion is an irrelevant factor for environmental risks of HOC to mussels and other seafood.

There is still a lack of monitoring programmes targeting the assessment of these contaminants of emerging concern in seafood, resulting in little data about the levels of these contaminants in seafood. Next to this, the effect of culinary processing on levels of these contaminants in seafood is mostly unknown. In order to assess the contaminant level after seafood processing/cooking, selected seafood species from the second round of

commercial sampling were cooked using the normal household practices and results are reported in D2.7. Seafood samples were selected taking into account species showing the highest levels of contaminants in round I. The selected seafood samples were analysed as raw and cooked, according to the procedures defined in the sampling plan (D2.1). Overall, an increase in contaminant levels was registered for most contaminants regardless of seafood species.

The effect of parameters like size and fat content of various individuals and species on the level of contamination were assessed in commercial samples from round I and II. From the commercial seafood species collected in round 1, two sizes were sampled (small and large) for sole, plaice, octopus, monkfish, Pacific hake, Atlantic hake and imported tuna. Due to the unavailability of imported tuna, this species was replaced by Atlantic tuna. Overall, biological parameters, such as size and species, affected the contamination levels of several commercial available seafood species studied, such as:

- As expected from the literature, the contaminant levels of Hg and MeHg were higher in larger species of Pacific tuna and Mediterranean octopus. However, larger specimens of Mediterranean sole and North Sea plaice contained lower Hg and MeHg levels. Next to this, Channel plaice showed higher contaminant levels in larger specimens in round II, whereas the opposite occurred in round I. This apparent different result could be explained by the biology of these species. Especially for plaice it has been shown that younger, smaller fish lives and feeds in shallow coastal waters, which are in general more polluted that deeper waters in the open seas. This can result in higher mercury pollution. As it increases in size it goes to deeper waters, were by growth the mercury levels decrease
- Concerning the old BFRS, PBDEs, only BDE 28, BDE 47 and BDE 99 were detected in Portuguese monkfish and Pacific Tuna, with contamination levels increasing with size;
- As far as new BFRs are concerned, HBB levels increased with size for Portuguese monkfish, but decreased with size in Pacific tuna. As for MBDE congeners (2-MBDE-68 and 6-MBDE-47), the contamination level increased with tuna size;
- In terms of PFAs, contaminant levels were higher in larger species of Pacific tuna and Mediterranean octopus;

- Contaminant levels of UV-filters were higher in larger specimens of Portuguese monkfish, whereas contaminant levels were higher in larger Pacific tuna from round I, but lower in round II;
- Within Musks, contaminant levels were higher in larger specimens of Atlantic hake and Mediterranean sole, but such levels were lower in larger specimens of Portuguese monkfish, Pacific hake, Channel plaice, Mediterranean octopus and pacific tuna compared to small specimens;
- Regarding pharmaceuticals, contaminant levels of sotalol and diazepam were lower in larger species of Pacific tuna;
- As for endocrine disruptors (EDCs), contaminant levels were higher in larger species of North Sea and Channel plaice, whereas larger species of Pacific tuna, Mediterranean sole and Portuguese monkfish presented lower contamination levels.

From all these data it could be concluded that several parameters have a large influence on the levels of contaminants of emerging concern in seafood.

The effects of origin on the levels of priority contaminants were evaluated in several species. Large differences were observed, depending on species origin. Overall, the effect of the origin on contaminant levels in seafood species widely varied, and some locations were clearly associated to higher levels of contamination. This is described in more detail in D2.10.

The effects of species on the levels of priority contaminants were also assessed. The habitat choices and feeding behaviour also had effects on the observed levels of contaminants. In general, carnivorous fish such as tuna tend to accumulate more pollutants than seafood species low in the food chain at the same location. For more details see D2.9.

The period of the year that the seafood was sampled also lead to large differences for some species and some contaminants between the two sampling rounds.

Next to these parameters, it should not be forgotten that the type of contaminant itself can affect the levels also. Some contaminants tend to bioaccumulate in the food chain, such as BDEs and mercury, while more water soluble contaminants like PFOS or some pharmaceuticals do not. The distribution of these pollutants in the marine environment can also be different. It is therefore important to evaluate all these factors and parameters when one wants to predict the levels of contaminants in seafood.

WP3: Risk assessment/modeling of consumers' exposure and mitigation strategies to reduce health risks

Consumer survey: Because of the gap between European consumer perception versus scientific facts with respect to priority contaminants in seafood and the marine environment, we explored the relevant determinants of this gap (i.e. behavioral, attitudinal and socio-demographic factors) through a consumer survey in Italy, Spain, Ireland, Portugal and Belgium. Primarily, results of the survey pointed out that the highest seafood consumption was found among Portuguese participants who consumed about 2.78 times per week 150-200 g of seafood. Portugal was followed by Spain (2.64) and Italy (2.11). The lowest seafood consumption was measured for Ireland and Belgium with a mean consumption frequency of 1.61 and 1.09 per week, respectively. Hence, Irish and Belgian consumers did not meet the recommendation of eating two portions of fish a week. Considering the risk and benefit perception towards seafood, it was concluded that the perceived benefits outweigh the perceived risks for most participants. However, 39% of European participants were concerned about the amount of environmental contaminants and 42% were concerned about the safety of seafood. Heavy metals emerged as the contaminants that worried the participants the most, followed by plastic residues. The results of the pan-European survey also indicated that participants tend to trust the organizations that perform controls on the safety of seafood. In general, a higher degree of confidence was noticed in the national food safety authority, whereas retailers scored lowest. Within the ECsafeSEAFOOD project, we also conducted an information experiment with the aim to study the effect of a message containing information related to seafood on several variables. The information in the message focused on the health risks and health benefits of seafood, the sustainability aspect of seafood consumption, and advice about the optimal seafood consumption frequency and recommendations to buy seafood in a sustainable way. Consumers' attitude towards the message was in general positive and no significant differences were noticed between the information conditions used in the study. There were also no significant differences in the difference score (measurements after exposure to the message (ex post), minus baseline (ex ante) measurements) for behaviour, attitude, intention and Perceived Consumer Effectiveness (PCE) regarding seafood consumption and in relation to the marine environment when comparing between the different information conditions. PCE (predictor of pro-environmental behavior) significantly

increased after reading the message. However, the resulting PCE score was still below the neutral point of the measurement scale and a significantly higher intention to act more sustainably when buying seafood was only seen among participants in Belgium. Barriers related to price, satisfaction with conventional seafood and low perceived availability of more sustainable seafood alternatives also influenced sustainable choice behaviour.

Human health risk assessment: A probabilistic exposure assessment was performed for priority contaminants, based on concentration data obtained in WP1 and WP2 and detailed consumption data from different European countries (Belgium, Ireland, Italy, Portugal and Spain) obtained in Task 3.1. The assessed exposures were compared with toxicological reference values in order to determine whether a potential health concern existed for these priority contaminants through seafood consumption. For all the contaminants considered, the average contaminant exposure and the 95th percentile were the highest for Spain and Portugal, compared to Italy, Ireland and Belgium. This was explained by the higher total seafood consumption frequency in these two countries compared to the seafood consumption frequency in the other three countries. The obtained results indicate however that it is unlikely that a potential health risk exists through the seafood diet for most priority contaminants in the five European countries involved in the study. However, further refinement of the exposure assessment through the seafood diet and risk reduction measurements are certainly needed for MeHg and are recommended for PBDE99.

Mitigation strategies involving communication - Development of a European consumer tool to balance risks and benefits of seafood consumption: A new online tool, called FishChoice, was designed, developed and validated by WP3 partners. The online tool is freely accessible at www.fishchoice.eu, which is made available in two versions and five languages (English, Dutch, Norwegian, Portuguese and Spanish). The tool provides information about the nutrient and contaminant content of a personalised seafood diet as entered by the tool user, which can be consumers or any other type of stakeholder or policy maker. FishChoice was registered as a European brand. An exhaustive test was undertaken to FishChoice tool functionalities within and outside the consortium, by the members of the Scientific Advisory Committee and by consumers from five European countries (Belgium, Spain, Portugal, Ireland and Norway). A high acceptance of the online tool was registered among European consumers. The majority of consumers indicated that they would use the tool in the future

and would adopt the advice given by the tool. Potential pitfalls were identified, including strategies to addressing these in the future.

Mitigation strategies involving communication – Guidelines: Three sets of guidelines were developed: a guide for consumers, a guide for the seafood industry, and a guide for policy makers. The contents were defined and adjusted taking into account the target stakeholder, and based on project results and its specific research domain, i.e. emerging chemical contaminants. These guides were validated by external institutions.

Mitigation strategies involving industrial processes – Phycoremediation: Experiments were performed in three separate phases (A, B, and C). In phase A, the suitability of two different kelp species (*Saccharina latissima* and *Laminaria digitata*) were tested for the uptake of bisphenol A, tetrabromobisphenol A, venlafaxine and inorganic arsenic. Once it was demonstrated that the macroalgae were capable of accumulating/degrading the emergent contaminants, phase B was started, in which trials were performed combining both blue mussels (*Mytilus galloprovincialis*) and oarweed (*Laminaria digitata*). The dispersal and uptake of the tested contaminants venlafaxine, inorganic arsenic, lindane, diflubenzuron, cadmium and copper in mussels, seaweed and seawater was tested over time. In phase C, a technical and economical evaluation of phycoremediation was performed for the removal of specific contaminants, looking at effective reduction of these contaminants in target seafood in the presence of seaweed. It was concluded that the application of phycoremediation is technically possible using the current technology. However, this study also concluded that phycoremediation is economically unfeasible at the moment with the state-of-the-art production systems employed, since the production and maintenance of *L. digitata* biomass in an integrated closed aquaculture system is very expensive at the present moment.

Mitigation strategies involving industrial processes – Seafood processing: Mitigation of contaminants in industrial processing was studied for Greenland halibut (cold smoked), Atlantic salmon (cold smoked and trimmed) and prawns (cooked and peeled). Several essential elements and regulated and emerging contaminants were determined. Elements (inorganic arsenic, arsenic, chromium, zinc, selenium cadmium, copper and iron) were determined in all seafood types, as well as persistent organic contaminants (perfluorinated compounds, polychlorinated biphenyls and the brominated flame retardants α,β,γ-HBCDs, TBBPA, 2,4,6, tribromophenol and PBDEs) in the wild caught and long lived Greenland

halibut. Comparison of individually raw versus processed fillets showed that cold smoking significantly increased PCB congeners (10–22%) in Greenland halibut per wet weight, but levels were not changed based on lipid or dry matter. For the Atlantic salmon cold smoking and trimming did not significantly change any levels (elements and the persistent organic contaminant; α-HBCD) when calculated both per wet weight, dry weight or lipid content. Results of processing prawns showed that peeling prawns increased mercury, but reduced other elements including inorganic arsenic, arsenic, chromium, zinc, selenium and especially cadmium, copper and iron. The seasonal variation in prawns showed that raw prawns had significantly higher cadmium, chromium, iron, selenium and zinc levels in autumn than in spring, while summer levels were typically intermediate. Finally, for all processed seafood non-toxic organic arsenic that occurred in relatively high concentrations in seafood was not transformed in carcinogenic inorganic arsenic by industrial smoking or cooking in salmon, Greenland halibut and prawn (Fig. 1).

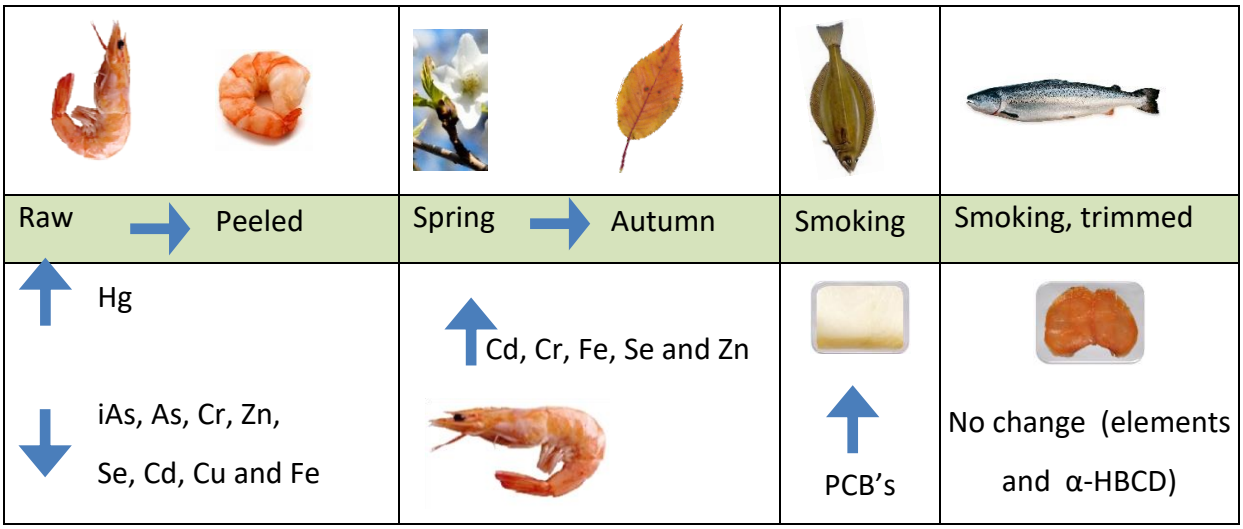


Figure 1 – Main results obtained with seafood processing activities.

WP 4: Development of fast screening methods to detect contaminants in seafood

The use of different recognition elements to the development of sensors was studied. Antibodies, aptamers and molecularly imprinted polymers (MIPs) provided the best results for different applications with seafood.

Antibodies: During the project an antiserum for the detection of cyclic imines was developed. Initial characterization of the antibodies indicated increasing titres of pinnatoxin antibodies in the serum, although further immunization may be needed with a new lot of

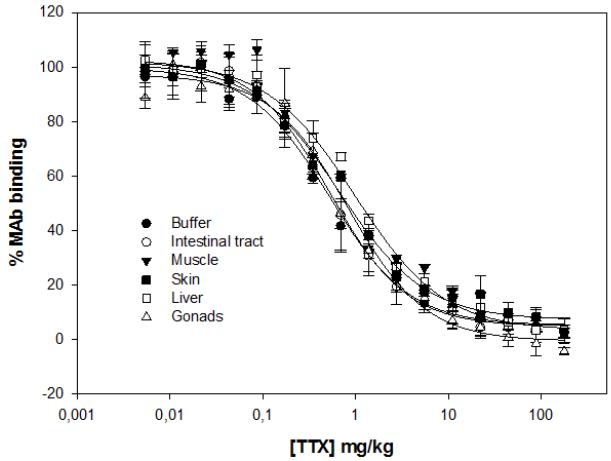
pinnatoxin A conjugated to cBSA. A plate coater prepared by conjugation of pinnatoxin A to OVA was prepared and tested in the assay, but did not improve assay performance. Due to time constraints (the development of antibodies takes a lot of time and many difficulties regarding the availability of sufficient pinnatoxin A for the immunization arose during the project) it was not possible to get a final suitable antibody for sensor development. As an alternative already developed antibodies were used for the detection of azaspiracids (AZAs), tetrodotoxins (TTX), sulfonamides and tetrabromobisphenol A (TBBPA).

Aptamers: The development of aptamers for the detection of brominated flame retardants (TBBPA, HBCD, BDEs), perfluorinated compounds (PFOS, PFOA), toxic elements (arsenic) and polycyclic aromatic hydrocarbons was performed. Selected environmental contaminants are quite hydrophobic substances that caused many problems during the selection of aptamers. In the end, suitable results were obtained only for the more polar compounds: TBBPA and arsenic.

MIPs: MIPs were developed and characterized to evaluate their suitability for the selective and sensitive detection of different target contaminants: BFRs (TBBPA, HBCD and DBDE), PFCs (PFOA and PFOS), nonylphenol and toxic elements (arsenic and methylmercury). The MIPs fabricated with PFCs showed the strongest affinity that correlated well with the data from the *in silico* study (best binding energy per monomer was achieved also for these MIPs). Moreover these MIPs had the highest number of active sites. Therefore, they are suitable for further development of new methods. Indeed they were used for the development of a suitable sample treatment protocol and recoveries around 80-90 % were achieved with different seafood. Finally, WP4 developed biosensors for different targets such as marine toxins (TTX, AZAs), and antibiotics (sulphonamides) and TBBPA. These new tools (ELISAs, electrochemical sensors and SPR sensors) were validated with real seafood samples and results compared with reference methods.

TTX: Three different sensing technologies were studied for the immunodetection of the marine toxin tetrodotoxin (TTX) present in puffer fish and recently identified in shellfish: a new colorimetric enzyme-linked immunosorbent assay (mELISA), an optical immunosensor, an electrochemical immunosensor. The antibody used in all cases was obtained from collaboration with Dr. Katrina Campbell from Queen's University of Belfast (Northern Irleand). The mELISA is based on the immobilization of TTX through dithiol-carboxylate monolayers self-assembled on maleimide-activated plates. Very good sensitivity was

achieved (LOD of 2.28 µg/L) and the working range was between 2.28 and 95.19 µg/L. When applied to real seafood samples very good results were also obtained after the application of suitable correction factors depending on the tissue being analysed (see Figure below). The optical immunosensor is based on SPR technology and it provided very similar results in comparison to mELISA: LOD of 4.27 µg/L and a working range between 4.27 and 23.88 µg/L). In the analysis of puffer fish a LOD of 0.11 mg/kg was estimated (Fig. 2). Thanks to the similar affinity of maleimide and gold for thiol groups, the SAM-based strategy used for the development of the mELISA was implemented in the development of an electrochemical immunosensor and similar results were achieved.



Limit of detection: 0.03 mg/kg

Figure 2 - MAb binding percentage of TTX in buffer and in liver, muscle, gonads, intestinal tract and skin puffer fish matrices obtained by mELISA. MAb binding percentage is expressed as percentage of the control (without toxin); x values refer to initial toxin concentrations in 10 mg/mL matrix (mg of TTX/kg of matrix).

AZAs: Two different colorimetric immunoassays using an antibody developed by NVI were optimized for quantification of the marine toxin Azaspiracid (AZA): one using microtiter plates and another using magnetic beads as antibody supports. In both cases the obtained results demonstrate the applicability of these immunoassays to the analysis of mussel samples and their use as both screening tool at the regulatory level and quantification analysis technique in a simple, fast and reliable way (Fig. 3). The methodology with magnetic beads was also adapted to an electrochemical detection with an LOD of 1.0 ± 0.1 µg/L and a working range between 1.9 ± 0.1 and 46 ± 4 µg/L. The high accuracy obtained in the determination of AZAs content in mussel samples, with no matrix effect being observed, clearly makes the use of magnetic immunocomplexes a valuable strategy for the development of immunoassays for AZAs detection in shellfish.

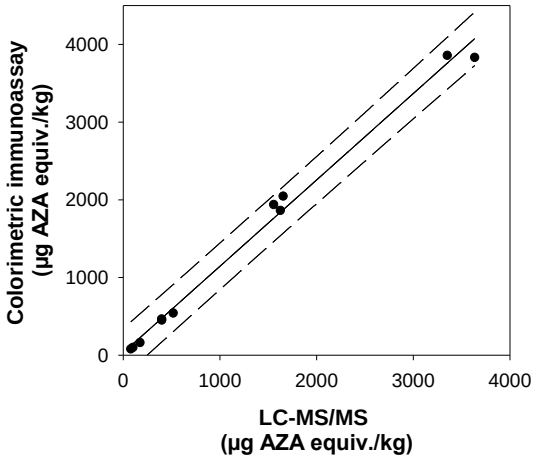


Figure 3 - Linear regressions for the correlations between sample quantifications by the magnetic bead-based colorimetric immunoassay and sample quantifications by LC-MS/MS analysis. Dashed lines represent the prediction intervals of 95%.

Sulphonamides: An indirect competitive ELISA for sulphonamides was developed and characterized. LODs were between 0.075 and 0.132 ng/mL and the optimal assay presented an IC_{50} in the range between 1.60 and 1.90 ng/mL for sulfathiazole and between 7.0-7.5 ng/mL for the sulfapyridine. A good specificity of the assay for sulphonamide antibiotics versus other structurally related compounds and other antibiotics was also observed. This immunoassay was then transferred to the SPR platform. After optimisation of the assay the IC_{50} value was IC_{50}: 3.051 ± 0.384 ng/L and it showed a good performance when applied to seawater and seafood extracts.

TBBPA: A nanobody (anti-TBBPA VHH T3-15 from Dr. Bruce D. Hammock group at UC Davis (USA)) was used for this purpose. It consists of a variable domain of a heavy chain antibody (VHH). During the optimization of the protocol kinetics variables, such as flow rate and time of contact, were studied as well as the concentration of nanobody and the presence of different amount of organic solvent. With the optimum conditions the IC_{50} was 4.84 ng/mL and the limit of detection was 1.78 ng/mL.

WP 5: Toxicological impact of chemical contaminants in public health

Activities in WP5 focused on determining toxicity and bioavailability of contaminants of emerging concern (CEC), present in seafood.

For determination of contaminant bioavailability from foods, bioaccessibility and intestinal cross epithelial transport were separately evaluated. Bioaccessibility of CEC was assessed in

several seafood matrixes (including fish, molluscs, crustaceans and seaweed) using an *in vitro* digestion method. The effect of culinary treatment (steaming) in CEC bioaccessibility was also evaluated. Table 3 describes the different CEC investigated in the project and resumes the main bioaccessibility results in raw seafood. MeHg bioaccessibility ranged between 10% (octopus) and 61% (hake). As bioaccessibility was high in all analysed species. Cd bioaccessibility was high for mussel (100%) and shrimp (75%), and low for tuna (41%). Mackerel (71.5%, PFUnA) and flounder (61.9%, PFOS) revealed high perfluorinated compounds bioaccessibility. In relation to brominated flame retardants (BFRs), low PBDEs (BDE47, BDE100) bioaccessibility (18-45%) was observed in mussel and mackerel. α-HBCD bioaccessibility in mackerel (90%) and plaice (63%) was higher than in mussel (14%). Pharmaceuticals and personal care products (PPCPs) bioaccessibility was above 75% in all studied species. Lowest (75%) and highest (95%) bioaccessibility was observed for octocrylene (tuna canned) and venlafaxine (mullet), respectively.

Table 3 - Resume of bioaccessibility results of CEC obtained for several seafood species.

High bioaccessibility (≥ 50 %)	**Low bioaccessibility (< 50)**
MeHg (hake*, monkfish*)	MeHg (tuna*, mackerel*, octopus*)
As (fish, molluscs, crustaceans, seaweed*)	Cd (tuna)
Cd (mussel*, shrimp*)	BDE47 and BDE100 (mackerel, mussel*)
PFCs (PFOS - flounder, PFUnA - mackerel)	α-HBCD (bivalves)
α-HBCD (fish)	AZA-toxins (mussels)
PPCPs (venlafaxine – mullet*, mussel, methylparaben – mussel, octocrylene – seabream, tuna canned)	TTX toxins (puffer fish gonads)
OA-toxins (mussels)	

Another objective of the project was to evaluate the bioaccessibility of marine toxins. High bioaccessibility was observed for okadaic acid (OA-toxins) in steamed mussels (60%). Nevertheless, for azaspiracids the bioaccessibility ranged between 43 and 48 %. Tetradotoxin (TTX) bioaccessibility was evaluated in puffer fish gonads and only 15% of this toxin is available to absorption after digestion.

The effect of culinary treatment (steaming) in CEC bioaccessibility was also evaluated. MeHg bioaccessibility decreased in all species after steaming. For As, bioaccessibility decreased only in seaweed. In the case of Cd, bioaccessibility decreased in mussel and shrimp. PBDEs

bioaccessibility decreased at least 50% in mussel after steaming. Venlafaxine showed a significant bioaccessibility decrease in steamed mullet, while no differences were found in mussel.

Transport across epithelia was evaluated using H4 epithelial cells growing on microporous membranes to estimate bioavailability. Large differences in MeHg bioavailability were found between seafood matrices. Regardless the iAs species (iAs(III) or iAs(V)), 15-20% of inorganic arsenic crossed epithelia. Of the tested toxins, AZA-1 showed low bioavailability, while organic contaminants had a diverse bioavailability from low (0.3%, EHMC) to very high (62%, BP3). In particular, no specific correlation was observed between bioavailability and lipophilicity of compounds. Plant derived polyphenols were found to significantly decrease bioavailability of certain contaminants. Our study suggests that both contaminant as well as food matrix may have a profound influence on bioavailability and, consequently, human exposure to contaminants.

For cytotoxicity and genotoxicity studies, cell lines of intestinal epithelia (H4), macrophages (TLT) and hepatocytes (HepaRG) were used. Although there were differences in the results between cell lines and end-point assays, MeHg and iAs were the most toxic compounds. PFCs were less toxic, but PFNA was substantially more toxic than PFOA and PFOS. α-HBCD toxicity could not be properly determined, as only low concentrations could be obtained in cell culture media. TBBPA was toxic with a potency in the range of PFNA, while HHCB-Lactone was a compound of low toxicity even at the highest tested concentrations. In genotoxicity experiments, iAs(V), PFCs and HHCB-lactone showed a potential genotoxic activity in HepaRG cells, while TBBPA and HHCB-Lactone were also potent in TLT cells. It is important to note that stability of hydrophobic substances in cell culture based aqueous media was thoroughly checked in order to properly conduct toxicity tests. Carboxymethylcellulose was successfully applied for solubilization and stabilization of hydrophobic contaminants. To evaluate estrogenic effects of typical contaminant mixtures identified in seafood, no significant synergistic effects have been found between them.

In parallel to cell lines, zebrafish as an invertebrate model was applied for toxicity studies. Arsenite showed a different toxic effect in zebrafish in comparison to arsenate. These results were confirmed by evaluating gene expression as well as lipid peroxidation and stress the fact that inorganic arsenic should be monitored and evaluated at species level. Exposure of zebrafish to PFCs showed that the most affected genes were *hsp70* and *gstp1*

indicating that oxidative stress and general stress were also the biological processes mostly involved by the exposure to these chemicals. In all cases the effect on those two genes seemed increasing with the increasing of the concentration of exposure. Oxidative stress was investigated further for this compound by detecting their capacity to produce lipid peroxidation. All these three compounds, with the exception of PFOA at 1 mg/L, produced lipid peroxidation. At 10 mg/L a significantly higher increase in TBARS was detected for PFNA. The exposure to PFNA also produced statistically different levels of TBARS at the two concentrations tested (1 and 10 mg/L). For PFOA and PFOS no significant differences were detected between exposure performed at 1 and 10 mg/L. In conclusions PFNA seems to produce a greater lipid peroxidation with respect to the other two PFCs.

In addition to single compounds, several typical mixtures of contaminants found in seafoods were tested. Each type of mixture, even though containing the same compound, produced different effects depending on the other compounds present. Interestingly, the mixture of PFCs at double the concentration that started affecting gene expression of some selected genes caused a lethal effect on zebrafish embryos showing a far greater degree of toxicity with respect to the individual compounds. All in all these results show that it is important to study mixture and chemical compounds in a realistic way. When testing the effects of PFOS + MeHg, the latter had a major influence on the effects, in the case of MeOH-BDE47 + MeHg, both compounds contributed equally to the effect produced by the mixture. In the case of PFOS + 6-MeOH-BDE47 the effect was mainly of the latter. Oxidative stress was the main effect detected by those compounds.

Toxicity of contaminants in zebrafish has also been tested at organ level. Chemical contaminants such as MeHg are taken up by adult zebrafish directly from the water and accumulate in various organs. Adverse effects were detected at organ level by gene expression analysis. Contaminated feed was also tested with adult zebrafish and adverse effects were detected at organ level with a variety of end-points. Microplastics produced a great increase in the toxic and biological effects of chemical contaminants. This was confirmed by the results obtained in fish fed with a feed containing both microplastics and chemical contaminants. The effects at physiological level could be detected especially in the liver at, as well as by the evaluation of gene expression and in the brain at gene expression level.

WP6 Transfer of relevant priority contaminants from the marine environment to seafood and effect on climate changes

The transfer of toxicologically relevant priority environmental contaminants between water, sediment, feed, microplastics and seafood, considering the effect of climate change was investigated.

Feeding trials to study the carryover of relevant priority contaminants trials using salmon, and seabream as case studies: Marine plastic debris has got increased awareness, with scientists and regulators trying to uncover the effects of this physical contaminant on marine organisms and humans. When the ECsafeSEAFOOD project started, there was no experimental evidence of the extent to which the presence of microplastic associated with feed may alter the bioaccessibility of persistent organic pollutants (POPs) in fish. Hence controlled trials to access bioavailability and associated toxicological impacts of microplastic with associated POPs were included in the project. The transfer of persistent contaminants from feed to Atlantic salmon (*Salmo salar*) and sea bass (*Dicentrarchus labrax*) were studied in feeding trials through modelling the elimination coefficients and assimilation efficiencies from elimination and accumulation regression lines (Fig. 4 and 5).

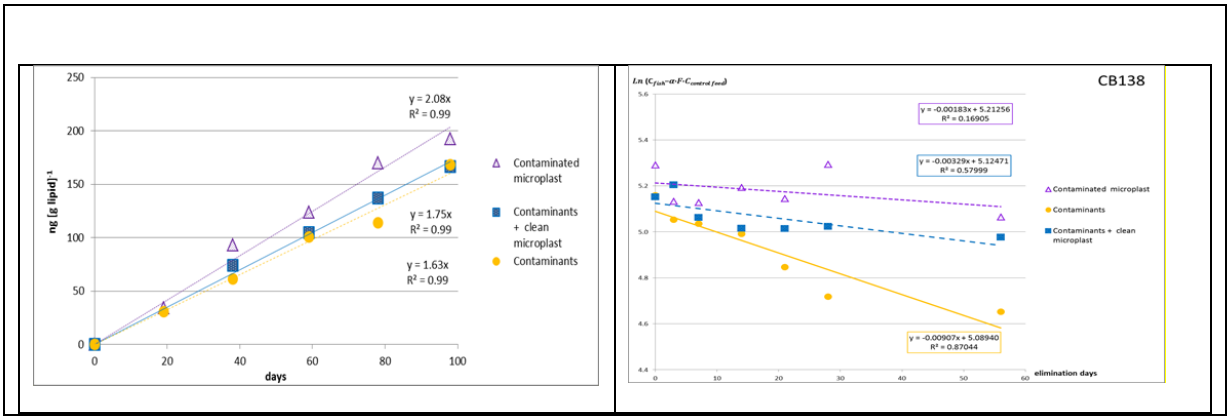
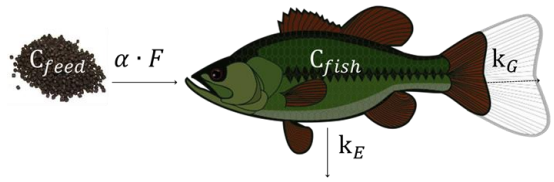


Figure 4 - Example of accumulation regressions of measured Cfish (here PCB138) of three contaminated diets modelled for obtaining the assimilationefficiency (α) and elimination regressions of ln Cfish of same diets modelled for obtaining the elimination coefficients (kE).

The toxicokinetic modelling parameters are illustrated in the following figure. The influence of inclusion of 2% 125-250 µm Low Density-Polyethylene (LD-PE) microplastic particles either as clean particles added to contaminated feed, or as contaminants sorbed to microplastic before being added to the feed, were compared to feed with similar contaminant levels. The measured and modelled carryover from feed to fish (assimilation efficiencies) of individual PCBs, PBDEs, HBCDs, PFCs and methyl mercury are also tools to

access food safety of aquaculture products like farmed salmon and sea bass, produced and consumed throughout Europe.



α: assimilation efficiency (carryover)

F: feeding rate, g feed/g fish/day

$$C_{fish\ growt\ corrected}(t) = C_{fish} \cdot (1 + k_G \cdot t)$$

$$ln\ (C_{fish} - C_{from\ control\ feed}) = constant - k_E \cdot t$$

$$\frac{dC_{fish}}{dt} = \alpha \cdot F \cdot C_{feed} - k_E \cdot C_{fish}$$

$$C_{fish}(t) = \frac{\alpha \cdot F \cdot C_{feed}}{k_E} \cdot (1 - \exp(k_E \cdot t))$$

Figure 5 - The toxicokinetic parameters for modelling elimination coefficients(k_E) and assimilation efficiencies(α) (k_G: growth dilution coefficient, C_{fish}: concentration in fish, C_{feed}: concentration in feed).

For fat soluble contaminants, the results revealed lower elimination coefficients for trials with lipophilic contaminants sorbed to the microplastic before including in the feed compared to those with feed contaminated through the oil with or without clean microplastic. The same tendencies were seen for ~11 PCBs, PBDEs, HBCDs, as well as for the separate salmon trial performed in 2015 and sea bass trial 2016 in different facilities. The assimilation efficiencies were similar or higher for the diet with contaminated microplastic likely due to increased supply of bile surfactants thus improving the uptake from the GI (gastrointestinal tract) i.e. presence of contaminated or clean microplastic did not reduce the bioaccessibility. The reason for the slower contaminant elimination for feed with microplastic in equilibrium with contaminants is not clear. However, an explanation may be that contaminated microplastic has a prolonged passage through the intestine thereby prolonging and delaying the time for contaminants uptake. For methyl mercury, the background concentration was high and the elimination was low, so no significant differences were observed between diets. The mean of individual elimination coefficients and assimilation efficiencies are listed in table 4.

Feeding trials to study the carryover of relevant priority contaminants trials using edible crab as case studies: The levels of azaspiracid-1 (AZA1, Trial 1) and pinnatoxin G (PnTxG, Trial 2) and their metabolites were followed over time after oral dosing of crabs with artificially contaminated mussel homogenate. No significant uptake of PnTxG, nor any of its metabolites, was detected in crabs dosed with 31 µg/kg PnTxG on a body-weight basis.

Table 4 - Mean of individual elimination coefficients (kE ·102) and assimilation efficiencies (α) of the trials.

	Fish	***salmon***			***sea bass***		
	Feed	**Contaminated plast**	**Contaminated + clean plast**	**Contaminated**	**Contaminated plast**	**Contaminated + clean plast**	**Contaminated**
$k_E \cdot 10^2$	HBCD	0.21	0.27	0.71	0.62	1.06	1.17
	PCBs	0.14±0.12	0.29±0.08	0.72±0.17	0.40±0.12	1.01±0.08	0.75±0.16
	PBDEs	0.23±0.09	0.44±0.09	0.50±0.09	0.80±0.22	1.45±0.31	1.22±0.33
	MeHg	-0.4	0.1	-0.1	0.1	-0.04	0.02
α	HBCD	0.56	0.42	0.53	0.54	0.54	0.63
	PCBs	0.65±0.10	0.55±0.07	0.63±0.08	0.45±0.06	0.40±0.06	0.46±0.06
	PBDEs	0.71±0.12	0.61±0.08	0.61±0.09	0.23±0.09	0.44±0.09	0.50±0.09
	MeHg	0.69	0.98	0.84	1.04	0.96	1.08

Results from dosing crabs with AZA1 at 20 µg/kg (on a body-weight basis) were not able to be interpreted due to the heavy natural contamination found with high-but-variable levels of AZAs. Analysis of crabs naturally contaminated with AZAs in Trial 2 allowed the half-life for AZA1 to be estimated at approximately 1 month. This, together with the measured natural variation in AZA concentration in crabs, and the presence of AZAs almost exclusively in the hepatopancreas (brown meat) and their absence from the white meat, are important information that can be used to design future studies and to develop more effective monitoring and regulatory frameworks. The results, although only preliminary due to the short length of the experiment relative to the estimated half-life, and the high variability of AZA1 in naturally contaminated crabs, indicate that AZA1 is slowly depurated from crabs. The comparison of the ELISA and LCMS results give an indication in the degree at which this reduction in AZA1 is due to metabolisation into other AZA-analogues (as the antibodies used in the ELISA recognise a wide range of AZA analogues, while the LCMS analysis is specific for the AZA analytes under analysis). The high levels of AZAs detected in crabs from Frøya in two successive autumns confirm this region as a hot spot for AZAs in crabs. Furthermore, the high levels of AZAs naturally present in crabs, together with the high variability (Fig. 6) and the long depuration time, indicate that it would be futile to conduct studies on the uptake, distribution, metabolism and excretion of AZAs in crabs collected in this region. The Norwegian Food Safety Authority has been informed about the outcome of these experiments.

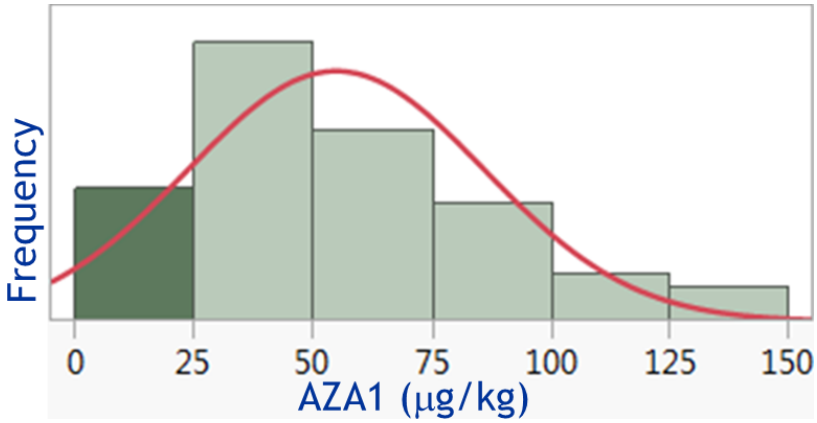


Figure 6 - Distribution of azaspiracid-1 in hepatopancreas of crabs.

Study the influence of simulated climate change on the behavior and transfer of relevant priority contaminants in marine clams: The effect of climate change (seawater warming and acidification) on the bioaccumulation and elimination of emerging chemical contaminants in bivalves (*Ruditapes philippinarum*), i.e. Task 6.2, was investigated by exposing organisms to environmentally relevant concentrations of contaminants from different groups and distinct chemical characteristics. First, two trials were conducted separately, as described in Deliverable D6.2: 1st trial – bivalves were exposed via feed to diclofenac, sulfamethoxazole, sotalol, venlafaxine, citalopram, carbamazepine, triclosan, methylparaben, dechloranes 602, 603 and 604, hexabromobenzene, and tris 2-butoxyethyl phosphate; 2nd trial - bivalves were exposed via feed to inorganic As, bisphenol A, tetrabromobisphenol A, and perfluorinated sulfonic acid precursors. Results obtained in both trials were inconclusive for most contaminants as they were not successfully stabilized in bivalve feeds, raising the need to use a different experimental approach, i.e. bivalves (in this case the Mediterranean mussel *Mytilus galloprovincialis*) exposed to contaminant mixes via seawater (instead of via feed) as biological models. In trial III all groups of contaminants were included, except for dechloranes, TBEP and HBB, as the results obtained in the first trial were robust. The results showed that contaminants bioaccumulation and elimination mechanisms are largely dependent on the behaviour and chemical properties of each compound and, thus, variations were observed within contaminant groups. Furthermore, data clearly showed that bioaccumulation and elimination mechanisms are strongly affected by both seawater temperature and acidification. For instance, increased temperatures promoted higher bioconcentration of some contaminants (e.g. sotalol, carbamazepine, triclosan and TBBPA), but also lower bioconcentration in some cases (e.g. PFOA and PFOS). On the other hand,

acidification increased the bioconcentration of methylparaben and PFOS, but impaired accumulation of venlafaxine, citalopram, triclosan, PFOA and iAs. Furthermore, when both environmental stressors where combined, even higher bioconcentration of sulfamethoxazole, TBBPA and PFOA occurred in mussels, whereas even lower levels of venlafaxine, citalopram, methylparaben and iAs were observed. Finally, data also indicated that a 20 day period of clearance is not sufficient for a complete elimination of most contaminants.

Working Group for Marine Toxins:

Development of an ELISA for the Detection of Azaspiracids: An indirect competitive immunoassay was developed for the detection of azaspiracids using 96 well plates. Immunizing antigens were prepared from synthetic fragments of the constant region of AZAs, while plate-coating antigen was prepared from AZA-1. The first promising plate-coating antigen, OVA–BrAZA-1, gave an optimized assay (average of 13 assays) with a working range (I20–I80) of 0.45–8.6 ng/mL and the I50 at 1.9 ng/mL using the AZA-1 certified reference material (CRM). This change to OVA–BrAZA-1 (and AgR367-11b) made the assay 8-fold more sensitive compared to previous plate-coaters. The ELISA provides a sensitive and rapid analytical method for screening large numbers of samples. It has a limit of quantitation for total AZAs in whole shellfish at 57 µg/kg, well below the maximum permitted level set by the European Commission at 160 µg/kg. The ELISA has good cross-reactivity to AZA-1–10, -33, and -34 and 37-epi-AZA-1. Naturally contaminated Irish mussels gave similar results whether they were cooked or uncooked, indicating that the ELISA also detects 22-carboxy-AZA metabolites (e.g., AZA-17 and AZA-19). ELISA results showed excellent correlation with LC-MS/MS analysis, both for mussel extract spiked with AZA-1 and for naturally contaminated Irish mussels. The assay is therefore well suited to screening for AZAs in shellfish samples intended for human consumption, as well as for studies on AZA metabolism.

Immunorecognition magnetic supports for the development of an electrochemical immunoassay for azaspiracid detection in mussels: A magnetic bead (MB)-based direct immunoassay for the detection of azaspiracids (AZAs), using protein G-coated magnetic beads (MBs) as supports for antibody immobilisation and peroxidase-labelled AZA as a tracer was developed. A colorimetric approach was first developed to optimise the

experimental parameters and establish the cross-reactivity factors for AZA-1–10. The subsequent combination of the immunorecognition MBs with 8-electrode arrays enabled the multiplexed electrochemical detection of AZAs (Fig. 7). Naturally-contaminated mussel samples were analysed and the results obtained showed an excellent correlation with LC-MS/MS analysis. The MB-based immunoassay had an LOD of (3 µg AZA-1 equiv./kg, below the European regulatory threshold, using a protocol that requires very few steps and a short analysis time (~15 min). The simplicity, cost-effectiveness, rapidity, robustness, selectivity and precision of the assay provide a valuable tool for the detection of all regulated AZAs and other toxic AZA analogues, which can be used by end users.

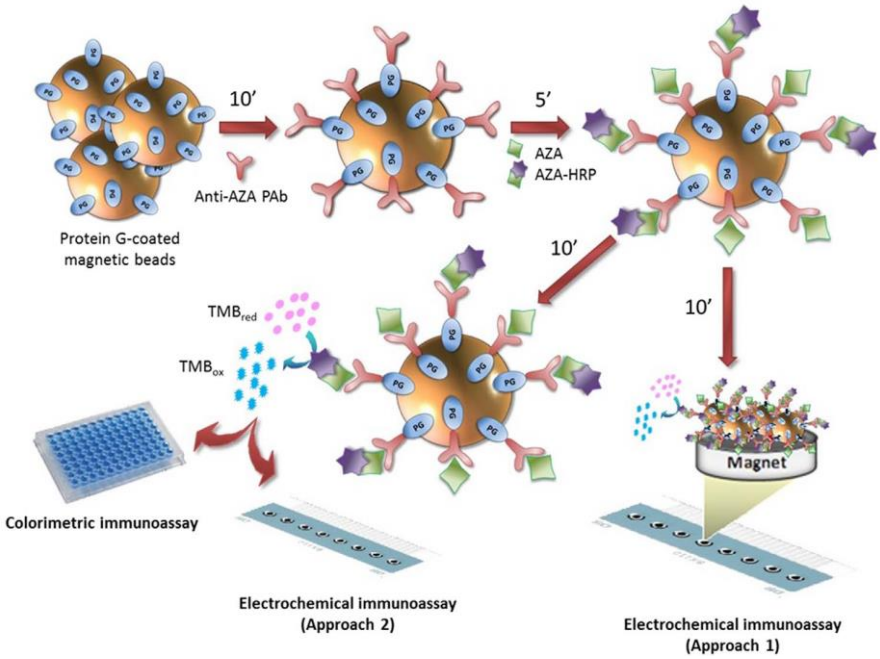


Figure 7 - Schematic representation of the MB-based immunoassay and the different approaches developed for AZA detection by colorimetric and electrochemical detection methods.

Effects of cooking on azaspiracid profiles in mussels: A study on the effects of heating on the azaspiracid profile in contaminated mussels revealed that while some azaspiracids are stable to heat (e.g. AZA1 and AZA2), some carboxylated azaspiracid metabolites (including the known AZA17, AZA19, AZA21, AZA23, and the new azaspiracids AZA44–47 discovered during this study) are converted via decarboxylation into other azaspiracids. The regulatory analysis is based only on AZA1–3 in uncooked shellfish, however AZA3 is only produced in significant amounts when the shellfish are cooked (via decarboxylation of AZA17) (Fig. 8). This study showed that cooking of mussels can increase the concentration of regulated AZAs by as much as 4-fold via heat-induced decarboxylation. However, since most AZAs tested so far

are toxic in *in vitro* tests, the current regulatory regime may significantly underestimate the total toxin content of azaspiracid-contaminated shellfish. Similar heat-induced changes of the azaspiracid profile were also observed in the hepatopancreas (brown meat) of contaminated crabs during the ECsafeSEAFOOD project.

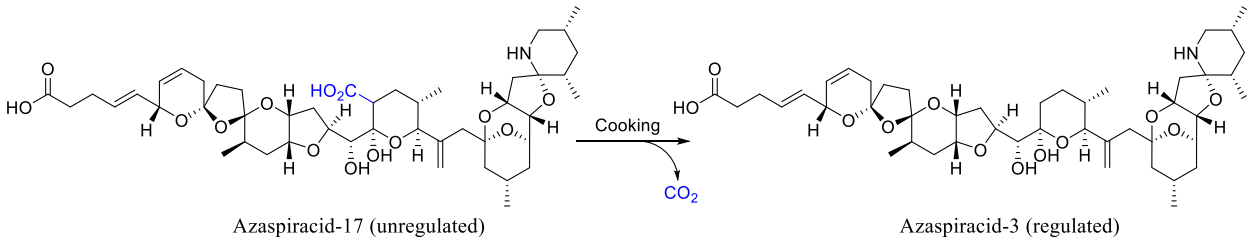


Figure 8 - Heat-induced transformation of carboxylated AZA metabolites during cooking.

Identification of Gymnodimine D: A novel cyclic imine toxin, Gymnodimine D, was isolated from a culture of *Alexandrium ostenfeldii* and its structure was determined by mass spectrometry and nuclear magnetic resonance spectrometry. The structure of gymnodimine D is the first gymnodimine to contain two tetrahydropyranyl rings within the macrocylic ring system. A range of other gymnodimine analogues were detected by LC–MS in the same culture but the amounts of these were insufficient for isolation and structure determination. This work revealed an unexpected degree of structural diversity in the gymnodimines. The isolated compounds can be used as reference compounds of cyclic imine toxins.

Detection of tetrodotoxins in puffer fish by a self-assembled monolayer-based immunoassay and comparison with surface Plasmon resonance, LC-MS/MS, and mouse bioassay: A new enzyme linked immunosorbent assay (mELISA) was developed for the detection of tetrodotoxins (TTXs). This assay was based on the immobilization of TTX through dithiol monolayers self-assembled on maleimide plates, which provides an ordered and oriented antigen immobilization and favours the antigen−antibody affinity interaction. The mELISA had a limit of detection (LOD) of TTX of 0.23 mg/kg of puffer fish matrix. The mELISA and a surface plasmon resonance (SPR) immunosensor were used to establish the cross-reactivity factors (CRFs) of several TTX analogues, as well as to determine TTX equivalent contents in puffer fish samples. Results obtained by both immunochemical tools showed good correlation, as well as with those obtained by liquid chromatography tandem mass spectrometry (LC-MS/MS). The mELISA has demonstrated to be fit for the purpose for screening samples in monitoring programs and in research activities.

Confirmation of Pinnatoxins and Spirolides in Shellfish and Passive Samplers from Catalonia (Spain) by Liquid Chromatography Coupled with Triple Quadrupole and High-Resolution Hybrid Tandem Mass Spectrometry: The first detection of pinnatoxin G (PnTX-G) occurred in Spain and 13-desmethyl spirolide C (SPX-1) in shellfish from Catalonia (Spain, NW Mediterranean Sea). Cyclic imines were found at low concentrations (2 to 60 µg/kg) in 13 samples of mussels and oysters (22 samples analyzed). Pinnatoxin G has been also detected in 17 seawater samples (out of 34) using solid phase adsorption toxin tracking devices (0.3 to 0.9 µg/kg-resin). Pinnatoxin G and SPX-1 were confirmed with both low and high resolution (<2 ppm) mass spectrometry by comparison of the response with that from reference standards.

The novel ovatoxin-g and isobaric palytoxin (so far referred to as putative palytoxin) from Ostreopsis cf. ovata (NW Mediterranean Sea) - structural insights by LC-high resolution MSn: Two analogs of palytoxin were found in cultures of six strains of *O. cf. ovata* isolated from the south of Catalonia (NW Mediterranean Sea). In addition to already known ovatoxins, these strains produced two minor compounds, ovatoxin-g and a putative palytoxin, whose structure was analysed in crude algal extracts through liquid chromatography– electrospray ionization high-resolution mass spectrometryn (LC-ESI-HRMSn) in positive ion mode.

Contribution to the risk characterization of ciguatoxins: LOAEL estimated from eight ciguatera fish poisoning events in Guadeloupe (French West Indies): From 2010 to 2012, 35 ciguatera fish poisoning (CFP) events involving 87 individuals who consumed locally-caught fish reported in Guadeloupe (French West Indies). For 12 of these events, the presence of ciguatoxins (CTXs) was indicated in meal remnants and in uncooked fish by the mouse bioassay (MBA). Caribbean ciguatoxins (C-CTXs) were confirmed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis. Using a cell-based assay (CBA), and the only available standard Pacific ciguatoxin-1 (P-CTX-1), the lowest toxins level detected in fish samples causing CFP was 0.022 mg P-CTX-1 equivalent(eq.)·kg-1 fish. Epidemiological and consumption data were compiled for most individuals afflicted, and complete data was obtained to establish the lowest observable adverse effects level (LOAEL) from 8 CFP events involving 21 individuals. Based on toxin intakes, the LOAEL was estimated at 4.2ngP-CTX-1eq./individual, corresponding to 48.4 pg P-CTX-1eq.kg^{-1} body weight (bw). The calculated LOAEL is consistent with EFSA and the U.S. Food and Drug Administration guidance levels for CTXs (0.1 mg C-CTX-1eq.kg-1 and 0.01 mg P-CTX-1eq.kg-1 fish).

Prevalence of ciguatoxins in lionfish (Pterois spp.) from Guadeloupe, Saint Martin, and Saint Barthélemy Islands (Caribbean): The prevalence of ciguatoxins (CTXs) was assessed in lionfish from the French Antilles, a ciguatera-endemic region. The neuroblastoma-2a (N2a) cell assay was used to assess composite cytotoxicity in 120 fish samples collected from the surrounding waters of Guadeloupe (n = 60), Saint Barthélemy Islands (n = 55) and Saint Martin (n = 5). Twenty-seven of these samples exhibited CTX-like activity by the N2a assay. Ciguatoxin (CTX) was confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple samples that presented highest composite toxicity levels by N2a. Those fish containing CTXs were all from Saint Barthélemy. Lionfish from Guadeloupe and Saint Martin did not exhibit toxin activity, although the sample size from Saint Martin was insufficient to draw any conclusions as to the incidence of CTXs. It was demonstrated the utility of the cell-based assay combined with LC-MS/MS to assess activity and to provide structural confirmation of CTXs respectively.

Ostreopsis cf. ovata from western Mediterranean Sea: Physiological responses under different temperature and salinity conditions: The physiological effects of changes in water temperature and salinity were analyzed, and their interaction through a multi-factorial experiment using two strains of *O.* cf. *ovata* in culture that had been isolated from the western Mediterranean Sea. Results of the physiological study showed that growth was inhibited at 19 °C for all salinities. The highest growth rates were registered at 24 °C for both strains (0.48 ± 0.05 div day^{-1}), and a significant variability in growth rate was found among salinities at 24 °C and 28 °C. The concentration of palytoxin-like compounds in the cultures increased with time and significantly higher amounts of toxin were found at 28 °C in comparison to 24 °C. The results suggest that climate change may not affect intensity of blooms, but their toxicity may be enhanced.

Ostreopsis cf. ovata dynamics in the NW Mediterranean Sea in relation to biotic and abiotic factors: In order to identify the most influential environmental factors that trigger proliferations of O. cf. ovata in the area of the adjacent shallow rocky coast of the Ebro Delta (NW Mediterranean Sea) a three-year survey was performed. Seawater temperature was the primary driver defining the ecological niche of *O.* cf. *ovata*. Negative correlations between the abundance of *O.* cf. *ovata* and nitrate concentrations and significant positive correlation with salinity were observed. The temporal pattern of O. cf. ovata dynamics from mid-July to early-November is probably due to the fact that this species is observed only

above a certain threshold temperature of seawater. Toxicity in planktonic cells was negatively correlated with cell abundance in the water column, achieving maximum concentrations of 25pg PLTX eq $cell^{-1}$.

Contribution to the genus Ostreopsis in Reunion Island (Indian Ocean): molecular, morphologic and toxicity characterization: Information on morphology, 5.8S and ITS data and toxin content was collected from thirty three strains isolated along the west coast of Reunion Island, in the Indian Ocean. Two morphotypes, non-overlapping in size, were distinguishable: the small morphotype (DV = 53.5 ± 6.9 µm; W = 37.7 ± 5.6 µm) with a typical tear-drop shape and the large morphotype (DV = 103.9 ± 5.1 µm;W = 85.3 ± 6.9 µm) with a rounded shape (Fig. 9). Phylogenetic analysis revealed the presence of three genotypes. Haemolytic analysis resulted in no palytoxin-like activity in any species.

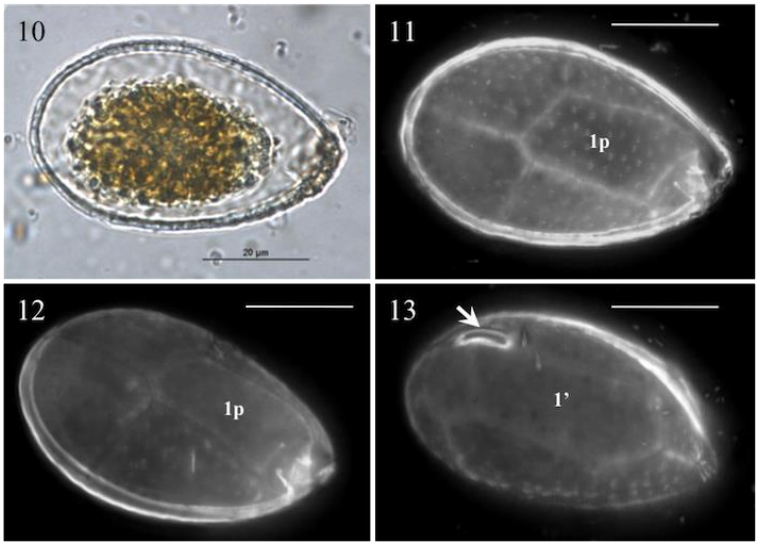


Figure 9 - Morphology of the "Small" morphotype identified in field samples (lugol-stained cells). 10-12. Shape and plate arrangement of the hypotheca. 13. Shape and plate arrangement of the epitheca. The apical pore position is indicated by a white arrow. 10. Bright Field microscopy images. 11-13. Epifluorescence microscopy images with Calcofluor staining. Bar scale = 20 µm.

Potential impact and main dissemination activities and exploitation results

Potential ECsafeSEAFOOD impact

Seafood plays an important role in a balanced diet and has many health benefits. However, like any other food type, seafood can also be a source of harmful contaminants with the potential to impact human health negatively. Although for most people the overall benefits of seafood consumption (following official recommendations) outweigh potential food safety risks, seafood information available to consumers may sometimes be confusing at best or even conflicting. The ECsafeSEAFOOD project aimed to boost consumer confidence in seafood safety, an area which is of growing public concern. Research and new knowledge play a very important role in addressing consumers' confidence in seafood safety and in recent years research funding has been committed to understanding newly emerging risks in relation to seafood consumption. ECsafeSEAFOOD was one such initiative, dedicated to assessing potential risks of seafood consumption and ways of minimising them. ECsafeSEAFOOD has gained further insight into the presence of contaminants of emerging concern in the most consumed seafood items in the EU. The project also contributed to establishing quantitative links between environmental pollution and seafood safety, which will enable dietary advice on seafood consumption for a reduced contaminant exposure. The information obtained has been extensively disseminated to a broad range of consumer interest groups, food producers as well as food authorities, and hence contributes to an improved common understanding on seafood safety between different sectors.

ECsafeSEAFOOD will have long-term tangible impacts in terms of (i) European competitiveness and innovation particularly of food-producing SMEs and local communities; (ii) supporting the offering of safe and high quality seafood to consumers; (iii) positive economic effects as a result of increasing seafood consumption due to higher awareness and confidence in these products in Europe; (iv) scientific breakthroughs in different areas, including priority contaminants monitoring, risk assessment, toxicity, mitigation measures, as well as by establishing a quantitative link between the contamination of the marine environment and that of seafood involving climate changes aspects; (v) societal impacts: supporting seafood education improvement, supporting increased employment in biotech companies, improving nutrition and increasing the sustainability of an important food

sector; and (vi) contributing to descriptors 9 (contaminants) and 10 (marine litter) of the Marine Strategy Framework Directive: (9) Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards and (10) Properties and quantities of marine litter do not cause harm to the coastal and marine environment.

Priority chemical contaminants: Despite the fact that a few of the contaminants that have been studied in ECsafeSEAFOOD (e.g. some brominated and perfluorinated compounds, cadmium and some marine toxins) are already regulated, essential knowledge is still lacking, thus requiring a revision of the maximum levels which are currently set. The vast majority of harmful compounds that were targeted by ECsafeSEAFOOD are not yet regulated and there is scarce information about their presence in the marine environment (e.g. marine litter, emergent toxins, pharmaceutical and personal care products, toxic metal species). ECsafeSEAFOOD generated important information about the presence of priority contaminants in marine organisms and helped assess the potential impact of these pollutants in the environment and the risk of their presence in seafood. Ultimately, the information gathered will help to protect the environment and further educate our society, helping to raise awareness of environmental problems and about food quality. The Working Group on Marine Toxins (WGMT) contributed significantly to identify and quantify emerging toxins in seafood and in the environment, an issue that EFSA is currently requesting information on to the Scientific Community in order to undertake accurate risk assessment for European consumers. Hence, results of the ECsafeSEAFOOD regarding marine toxins, have immediate application to evaluate food safety risks and identify geographical areas at risk. The project has also identified species of seafood at risk of contamination.

Health authorities, international and national agencies, SMEs and consumers: The results generated in the ECsafeSEAFOOD project are of great interest to the scientific community, international organisations, agencies, authorities and bodies responsible for monitoring seafood safety and the status of marine environment. Scientific evidence from ECsafeSEAFOOD can serve as a basis for further development of common food safety, public health and environmental policies and mitigation measures. This definitively reduces risks and optimises the management of food from harvesting, thus reducing the risks and costs.

This research also enabled an increase in the capacities of EU and national policy makers to communicate risk of environmental contaminants in seafood and their impact on public health across the EU. The findings from ECsafeSEAFOOD will help both policy initiatives and food alerts to be communicated across Europe more efficiently to targeted groups, e.g. to vulnerable groups and to the general public. This will be possible through the ECsafeSEAFOOD's user-friendly online tool that allows people to balance the risks and benefits of eating seafood. Two different versions were developed, one of which will target health professionals and health authorities. This tool will be useful for them to gain access to straightforward and up-to-date information about the risks and benefits related to seafood consumption. The main language of the tools will be English but it will also be available in several other languages. The second version of the online tool will be specifically developed for the European seafood consumer to help them to decide which species to buy and consume, in order to maximise the health benefits of seafood consumption and minimize the risks. Insight into citizens and consumer concerns will contribute to the development of seafood safety communication strategies (by e.g. health authorities, SMEs, NGOs, industry, consumer organizations) improving their effectiveness in improving seafood choices and public health. ECsafeSEAFOOD had an important impact on the national and European regulatory organizations. In this framework, the development of guides with a scientific basis is generally taken seriously by people, they were produced with collaborations from highly reputable and credible scientific organisations. In summary, the ECsafeSEAFOOD results are expected to have a significant impact on society, with a notable interest of health authorities, the general population and food safety agencies.

Marine strategy: Throughout its work, the ECsafeSEAFOOD project has addressed the Marine Strategy Framework Directive (MSFD) - European legislation that aims to achieve Good Environmental Status of EU marine waters by 2020. The Directive defines Good Environmental Status (GES) as: The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive. To help Member States interpret what GES means in practice, the Directive sets out 11 qualitative descriptors that describe what the environment will look like when GES has been achieved. In particular, the ECsafeSEAFOOD project has addressed Descriptor 9 (contaminants in seafood are below safe levels) through assessments of the levels of

emerging chemical contaminants in seafood and the compilation of these data into a consultable database, and Descriptor 10 (marine litter does not cause harm) through the assessment of microplastics in seafood. Additionally, a quantitative link was established between the contamination of the marine environment and that of seafood, taking into account the effect of climate changes, fully complying with the needs of the MSFD.

Seafood toxicology: Toxicological analysis within the ECsafeSEAFOOD project were carried out using real seafood samples and mixtures of contaminants to account for synergistic and antagonistic effects, contrary to standard toxicological tests that use standard chemical compounds and undertake testing in non-realistic conditions. The specific approach will impact health authorities, by enabling risk assessment to be more accurate and relevant for identification of specific groups of high-risk consumers. Bioaccessibility, bioavailability, synergistic/antagonistic effects and toxicity data will help policy makers set permissible levels of contaminants in seafood products based on differences between animal species and culinary treatments. Consumers will benefit by setting clear and understandable rules about healthy seafood consumption and preparation to minimize risk of exposure. The use of alternative models (e.g. mammalian cell cultures and zebrafish) will also help to reduce animal experimentation in toxicology. SMEs will use the knowledge obtained in the project to design detection tools for contaminants, and to prepare seafood with minimized exposure to contaminants.

Fast screening methods: The new, cost-effective, fast screening analytical techniques developed for target chemical contaminants will be used or adapted to analyse other seafood species or aquatic organisms to monitor pollution and food safety not only in marine species, but in freshwater organisms and in other food matrices. The development of rapid methods for the detection of contaminants in seafood will help the food industry to get cost-effective instruments that will provide a better characterisation and control of the safety of raw materials, ingredients and seafood products. The implementation of the proposed detection systems would ensure the reduction of food safety related costs without losing the quality of the data obtained. Therefore, these solutions may strengthen the confidence of consumers in seafood products and hence, the competitiveness of European seafood producers. The generation of new devices and technologies for

contaminants screening will allow for SMEs devoted to the development of sensors to commercialise these products and look at the food and environmental application market as a potential business opportunityWithin the WGMT, the project also developed analytical methods applicable to the identification of marine toxins. These methods include: 1) instrumental analytical methods (LC-MS/MS and LC-HRMS) for the unequivocal identification of marine toxins; and 2) Immunoassays and biosensors for marine toxins which simplify in the shortest time possible the identification of toxins.

Dissemination and communication: Through effective dissemination and communication of tailored messages to specific end-users, ECsafeSEAFOOD results will help secure global awareness of seafood safety for consumers. Improved public awareness and understanding of the acute risks of contaminants and the pro-active risk management strategies to protect human health that are operated by European and national authorities will improve consumers' safety, increase their confidence in the seafood industry and ultimately strengthen the seafood industry as a result. Details on the specific dissemination activities carried out during the project are outlined below..

Links between RTDs and SMEs: The ECsafeSEAFOOD project provided strong interactions between universities, organisations and SMEs. Through ECsafeSEAFOOD, the relevant SMEs will be able to strengthen their economic and IPR positions, show a steady growth which will eventually create more job opportunities in the EU.

In summary,
Direct impacts:

• ECsafeSEAFOOD will have a direct impact on the simultaneous monitoring of various environmental contaminants in seafood and in the environment by means of multi residue analysis developed as part of the project. This will lead to more effective monitoring of the environmental contaminants both in seafood and in the environment.

• The development of fast screening methods will be useful to quickly measure undesirable compounds in seafood "in loco" by non-trained technicians.

• An improved understanding of the risk of contamination in seafood that will help authorities and industry to understand the effects of contaminants on the quality of seafood.

• Data obtained will help the industry to optimise their processes in order to prevent or reduce the risk of contamination.

• Improved monitoring methods to further improve the chances of detecting any contaminants before they can cause any risk for consumers.

Medium-term impacts:

• Protection of marine resources, by understanding levels of contaminants of emerging concern in seafood that can trigger the implementation of mitigation strategies to reduce the pressure on marine resources.

• More evidence for policy making regarding chemical and biological hazards in seafood.

• Improve the relationship with National Food Safety Agencies, European Food Safety Authority, agencies responsible for analytical methods, including the European Committee for Standardisation (CEN), seafood industry and consumer organisations, by improving the channels of communication for efficient knowledge exchange about seafood safety issues. Additionally, the project partners have also improved their relationships with these organisations.

• Improvement of consumer protection, through the implementation of innovative and cost effective analysis.

• Reduction in unnecessary precautionary aquaculture-closures/product-recalls through early detection from screening and utilization of fast screening methods.

• Reduced need to analyse biotoxin-free samples with expensive instrumental methods.

• Improved consumer confidence in European seafood through the development of robust screening/detection methodologies and the development of consumer guidelines.

• Reduction of costs to producers and consumers due to the use of less expensive screening methods.

• Increased likelihood of detecting analogues of known toxins, if present, through the optimization of detection methods.

• Reduced likelihood of trade barriers based on toxin contamination due to earlier and easier detection.

Dissemination activities and exploitation of results

Information regarding human health risks and food safety is sensitive and has to be handled with extreme care. Hence, the project results were carefully evaluated before being communicated. Beside the work package devoted to communication, a Communication Committee (CC) was created being responsible of screening the project outcomes from a risk communication perspective before dissemination, creating a link with European and national authorities to ensure an optimum incorporation of project outputs and their dissemination. The CC worked closely with the IPRC (Intellectual Property Right Committee) to ensure that they received all information gathered in WP7. Therefore, a strong collaboration and exchange of information occurred between the CC, IPRC and WP7. The IPRC ensured that any Intellectual Property Rights (IPR) issues relevant to the project were identified, advising the Foreground owners. In total the IPRC and CC examined and proposed recommendations for 108 different activities (i.e. papers, oral presentations, posters).

Effective communication and dissemination was carried out through numerous different channels, including the dedicated project website, project Twitter account and related Twitter accounts, scientific peer reviewed articles, press releases, partner interviews, newsletters, videos, radio, a project feature on Euronews, presentations and discussions at events, popular media, a contaminants database, an online tool to balance the benefits and risks of seafood consumption, three stakeholder guides (consumer, industry and policy) and stakeholder consultations (workshops and online surveys).

The ECsafeSEAFOOD contaminants database collates data on emerging chemical contaminants in seafood species. In the last year of the ECsafeSEAFOOD project, 68,770 visitors used the database with a spread of different nationalities from all over the globe (Table 5). In addition 185 external users requested an account. The database contains 437 literature records and 4938 contaminants records.

The online benefit/risk tool, FishChoice (www.fishchoice.eu), had major impact in terms of dissemination and communication across different communication media (press, TV, internet) and was received with great interest by consumers. Press releases were published by the involved partners. In addition, a scientific peer reviewed article on the online benefit/risk tool was published in the second Special Issue of the project.

Table 5 - ECsafeSEAFOOD database visitors by date and country.

Visitor	Visit
Today:	257
Yesterday:	320
Last 7 Days (Week):	3,998
Last 30 Days (Month):	19,781
Last 365 Days (Year):	68,770
Total:	124,544

Rank	Flag	Country	Visitor Count
1		United States	1,561
2		Unknown	1,253
3		India	1,158
4		Romania	775
5		France	678
6		Italy	664
7		Brazil	553
8		Philippines	545
9		United Kingdom	533
10		Serbia	503

The three stakeholder guides are expected to have a major impact for the seafood production and processing industries, policy and decision makers, and consumers. By communicating project specific results in a practical way, the different stakeholders will be able to assess health risks from their activities and reduce the effective risk of contamination through seafood diet. They will also enable the promotion of seafood consumption throughout Europe in a conscientious and informed way.

Other dissemination activities carried out in ECsafeSEAFOOD include:

- Development and maintenance of an ECsafeSEAFOOD project website (www.ecsafeseafood.eu). The ECsafeSEAFOOD project website was regularly updated throughout the project with results, public reports, dissemination activities, links to the consumer tool and to the database, and events. The main objective of the website was to facilitate communication and dissemination of the ECsafeSEAFOOD project both within and outside project consortium, ensuring widespread project awareness to all stakeholders and possible end users. The website presents general information about the project and is easily accessible to end users, including the general public. It was designed and created for an easy transfer of knowledge between beneficiaries and to disseminate and exploit project results during the project lifetime. The project website has an open space for public information, including general information about the project, its objectives, project methodology, partnership, activities/events, news and a media centre. Additionally, a closed area for project participants is also available, where exchange of sensitive

information and fast communication between partners takes place. The website's Google analytics script has been utilised to keep track of website statistics. The accumulative number of visits from 10 June 2013 (M5) to 19 January 2017 (M48) is 11,967, with 9,632 of these (80.5%) being new visitors. The total number of page views is 24,437 (Pages/Visit = 2.04). The website has received visitors from 145 different countries since the website was launched. United States, Portugal and Spain are the top four countries in terms of the accumulated number of visits.

- Numerous dissemination materials, such as press releases, newsletters (3), posters/oral presentations in 34 events, videos (2), scientific and technical papers (including three special issues in Environmental Research and Food Chemical Toxicology, of which one is still ongoing). In total, 204 dissemination activities, 1 Paper in Proceedings of a Conference and 2 chapters in an edited book were prepared since the beginning of the project.
- AquaTT launched a Twitter account for the ECsafeSEAFOOD project on 11 November 2015 (https://twitter.com/ECsafeSEAFOOD). Since its establishment, the ECsafeSEAFOOD Twitter account has Tweeted 119 times and has 178 followers. AquaTT also used its own Twitter (1,426 followers) and LinkedIn accounts to disseminate ECsafeSEAFOOD press releases and news. Additionally, the project also created a Facebook account for the final event (https://www.facebook.com/ECsafeSEAFOODConference/).
- External media promotion of the project including numerous articles in the popular press and websites, a project feature on the FUTURIS television programme broadcasted at Euronews, radio interviews and written interviews.
- Peer reviewed publications and conference proceedings to inform scientists about ECsafeSEAFOOD's work and findings. 64 scientific publications were published or in in press format, 14 of which in a special project issue of the Environmental Research journal. A second special issue is due to be published in 2017, where 11 manuscripts were accepted from the project. A third Special Issue is currently ongoing with the outcomes of the final project event.
- Stakeholder consultations (3 workshops and online surveys) to identify relevant stakeholders, determine stakeholder needs, gather regulatory frameworks for risk

assessments and to collaborate with end-users to develop effective strategies for dissemination and knowledge transfer (For more, see D7.7).

- Three safe seafood guides for consumers, industry and policy makers (available since March 2017 at www.ecsafeseafood.eu).
- The ECsafeSEAFOOD final conference and stakeholder event was organised in Brussels in January 2017 with 94 participants, including the EC and EFSA, which allowed ECsafeSEAFOOD to present relevant outcomes of the project to key stakeholders and to establish closer links between among stakeholders.
- The ECsafeSEAFOOD project was also closely linked with other European projects, including the EFSA EUROCIGUA and Sea-on-a-Chip FP7.

Exploitation was an integral part of the ECsafeSEAFOOD project design, which was undertaken through knowledge management activities. Knowledge outputs (KO)s were identified, mainly related to methodologies, protocols and experimental approaches used in the project, and were collected and analysed. By monitoring, collecting and managing project outputs, it was possible to fast-track such knowledge to impact. The impact of ECsafeSEAFOOD's results was achieved by their adoption by scientists working in the field and the wider research community, but also through the application of the knowledge by national and international policy makers in the fields of food safety, environment and public health, including food safety authorities (FSAs), the seafood industry and consumer organisations. For example, the ECsafeSEAFOOD partner NVI shared its results with the Norwegian FSA, as they revealed high levels of azaspiracids in brown meat from crabs in autumn 2013. Since then, a permanent contact mechanism has been established with the Norwegian FSA and other FSAs all over Europe. In total, 81 knowledge outputs were collected from all project partners. Eight items (single KOs or clustered KOs) were prioritised for transfer to the industry and policy stakeholders at the ECsafeSEAFOOD International Stakeholder Event, which took place on 25th January 2017 in Brussels, Belgium. More information can be found in D7.9 Final report on dissemination and knowledge transfer generated within the project.

Project public website address:

The EcsafeSEAFOO publica website is http://www.ecsafeseafood.eu.

The FishChoice toll is located at http://www.fishchoice.eu/.

The EcsafeSEAFOOD WP1 database is located at http://www.ecsafeseafooddbase.eu

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