ECsafeSEAFOOD

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

Grant agreement no: 311820

Deliverable D5.8

Toxicity of selected contaminants (i.e. biotoxins) using mouse bioassay

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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)	

Decision on mouse bioassays

Mechanisms of action for several regulated marine biotoxins are quite well described, but for emerging toxins these may be largely unknown. Marine toxins present a comparably higher acute life-threatening risk than other chemical pollutants in seafood, and therefore can be of high risk. Therefore, their toxicological assessment is important for the hazard characterization and *in vivo* assays have to be applied when assessing this toxicity. For this reason, the ECsafeSEAFOOD project contemplated that in case of identifying new toxins for which no toxicological data were available during the execution of the project, a possibility of performing *in vivo* assays could be applied. This has not been the case.

For the management of risks associated to marine toxins, mouse bioassays can be used when no equivalent or alternative methods exist, thus providing publically acceptable justification for animal experiments. Mouse bioassay, in spite of the inconsistencies, lack of specificity and questionable ethical justification has been used over the years for risk assessment of biotoxins from harmful algal blooms. Development of methods alternative to the mouse bioassay, including analytical instrumental methods, has been hampered by the complexity of toxin families with numerous congeners that may differ in toxicity, but also by the limited availability of standards and reference materials needed for routine monitoring programmes, as well as by continuously changing patterns of toxins found in seafood products. Functional assays developed for biotoxin detection include cytotoxicity assays with cell lines and various assays for neurotoxic toxins using neuroblastoma. However, in order to describe the toxicity and potency of emerging toxins, which is a key issue for hazard characterization, both functional *in vitro* and *in vivo* assays are requested.

Researchers working in WP5 dealing with toxicology of emerging contaminants have committed to the 3R principle (Replacement, Refinement and Reduction) at the beginning of the project. In the ECsafeSEAFOOD project proposal mouse bioassays were optionally included in the case of new emerging biotoxins that might be found within monitoring activity in WP2 and further used for cytotoxicity and bioavailability assays, could need an *in vivo* approach to better characterize their toxicity. Limited by the contaminated seafood products and the lack of biotoxin standards, bioavailability and cytotoxicity assays have been performed on tetrodotoxin (TTX), Azaspiracid-1 (AZA-1) and ciguatoxins (CTXs), For TTX, toxicity data already exist from *in vitro* and *in vivo* mouse bioassays (Nieto et al., 2008; Ogata et al., 2001; Xu et al., 2003; Yang et al., 2008), likewise for AZA-1 (Aasen et al., 2011, 2010; Aune et al., 2012; Ito et al., 2002; Vale et al., 2007) and CTXs (Au et al., 2016; Bottein Dechraoui et al., 2008; Morey et al., 2008; Ryan et al., 2010, 2007).

Therefore, at M36 project meeting in Porto (February 2016) we discussed potential knowledge gaps that would demand and justify the use of mouse bioassays. Since these tests on TTX, AZA-1 and CTX have already been performed, toxicity mechanisms identified and published, we saw no need to perform additional *in vivo* assays and to cause harm to test laboratory animals. For that reason, it was concluded that mouse bioassay tests were not justified within the ECsafeSEAFOOD project taking into consideration the nature of the toxins identified within the project.

The corresponding budgetary resources were allocated to the oragization of the final project conference in Brussels in January 2016.

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