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Derivation of toxicity equivalency factors for marine biotoxins associated with Bivalve Molluscs

Luis M. Botana [a, *], Philip Hess [b], Rex Munday [c], Arnich Nathalie [d], Stacey L. DeGrasse [e], Mark Feeley [f], Toshiyuki Suzuki [g], Martin van den Berg [h], Vittorio Fattori [i], Esther Garrido Gamarro [j], Angelika Tritscher [k], Rei Nakagawa [k], Iddya Karunasagar [j]

[a] Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago, 27002 Lugo, Spain
[b] Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Laboratoire Phycotoxines (DYNECO/PHYC), Rue de l'Ile d'Yeu, BP 21105, Nantes Cedex 3 44311, France
[c] AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[d] French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Risk Assessment Department, Maisons-Alfort, France
[e] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Drive, College Park, MD 20740, USA
[f] Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, ON, Canada
[g] National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan
[h] Institute for Risk Assessment Sciences, University of Utrecht, Utrecht, The Netherlands
[i] Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy
[j] Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy
[k] Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland

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ABSTRACT

Background: Seafood toxins pose an important risk to human health, and maximum levels were imposed by regulatory authorities throughout the world. Several toxin groups are known, each one with many analogues of the major toxin. Regulatory limits are set to ensure that commercially available seafood is not contaminated with unsafe levels.

Scope and approach: The mouse bioassay was used to measure the toxicity in seafood extracts to determine if a sample exceeded regulatory limits. The advantage of this approach was to provide an estimation of the total toxicity in the sample. As instrumental methods of analysis advance and serve as replacements to the mouse bioassay, the challenge is translating individual toxin concentrations into toxicity to determine whether regulatory limits have been exceeded. Such analyses provide accurate quantitation of the toxin analogues, by they have widely dissimilar potencies. Thus, knowledge of the relative toxicities is required for risk assessment and determining overall toxicity. The ratios between the toxicity of the analogues and that of a reference compound within the same toxin group are termed "Toxicity Equivalency Factors" (TEFs).

Key findings and conclusions: In this document, the requirements for determining TEFs of toxin analogues are described, and recommendations for research to further refine TEFs are identified. The proposed TEFs herein, when applied to toxin analogue concentrations determined using analytical methods, will provide a base to determine overall toxicity, thereby protecting human health.

1. Introduction

Bivalve molluscs may be contaminated with marine biotoxins produced by microalgae and these toxins are an important cause of seafood intoxications, with symptoms that vary from mild diarrhoea to permanent neuropathy or death. Their presence is expanding worldwide, for reasons that are not fully understood, but appear to be linked to climate change, eutrophication and international trade (Hallegraef, 2015).

The limits for marine biotoxins for international trade are set by the CODEX Committee on Fish and Fishery Products (CCFFP), that has developed the Standard for Live and Raw Bivalve Molluscs

* Corresponding author.
E-mail address: luis.botana@usc.es (L.M. Botana).

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(Codex, 2008). This Standard identifies maximum levels in mollusc flesh for 5 toxin groups, saxitoxin (STX), <0.8 mg/STX equivalents (eq.)/kg, okadaic acid (OA), <0.16 mg/OA eq./kg, domoic acid (DA), 20 mg/kg, brevetoxin (BTX), 200 mouse units/or eq./kg, and azaspiracid (AZA), 0.16 mg/kg. Each group of seafood toxins is comprised of many analogues of the major toxin, yet the regulatory levels are represented according to the total toxicity of the analogues. Traditionally regulatory limits were assessed using the mouse bioassay (MBA), which involves the intraperitoneal injection of seafood extracts (AOAC, 2005a; T. Yasumoto, Murata, Oshima, Matsumoto, & Glardy, 1984; T. Yasumoto, Oshima, & Yamaguchi, 1978b). The advantage of the MBA is that it provides an estimate of the total toxicity of the sample. Instrumental analytical approaches are becoming available as alternatives to the MBA; such methods include liquid chromatography with ultraviolet, fluorescence or mass spectrometric detection (AOAC, 2005b; EU, 2011; These, Klemm, Nausch, & Uhlig, 2011). These methods permit the quantitation of toxin analogues when compared to a certified standard of the toxin (Antelo, Alfonso, & Alvarez, 2014).

Quantitation of the toxin analogues is not, however, sufficient for monitoring and regulatory decision making, since the different analogues may have widely dissimilar toxic potencies. For such assessment, it is necessary to know the relative toxicities of the components of the toxin mixture. These are termed "Toxicity Equivalency Factors" (TEFs), which are defined as the *toxicity ratio of a compound from a chemical group that shares the same mode of action of a reference compound in the same group*. The toxicity of the analogue is expressed as a fraction of the toxicity of the reference compound (Botana et al., 2010; Van den Berg et al., 2006).

Accurate TEFs are essential for the monitoring and control of regulatory limits set for groups of related compounds. The 34th Session of CODEX Committee on Methods of Analysis and Sampling (CCMAS) encouraged CCFFP to investigate TEFs for the marine biotoxins listed in the Standard. For this purpose, an Expert Group was created by Food and Agricultural Organization (FAO) and World Health Organization (WHO) to elaborate and propose a list of TEFs for each toxin group for which limits are recommended in the Codex standards for Live and Raw Bivalve Molluscs.

An additional toxin group, tetrodotoxin (TTX), was also considered given its reported presence in shellfish (A. D. Turner, McNabb, Harwood, Selwood, & Boundy, 2015; Vlamis et al., 2015). While TTXs are not specifically mentioned in the CODEX standard, they have the same mode of action as STXs and can be grouped along with the PSTs.

2. Deriving TEFs

The calculation of the amounts of different substances, sharing the same mechanism of action, into the equivalent value for a single compound is a complex process. It requires an understanding of both the mechanism of action of the toxins, and how this mechanism translates into toxicity. In many cases, such an understanding is not available, as with OA and its analogues, the dinophysistoxins (DTXs). This toxin group, referred to as DSTs (diarrhetic shellfish toxins) has been known for many years (T. Yasumoto et al., 1978b). Their toxicity has been suggested to result from inhibition of protein phosphatases, particularly PP2A (Bialojan & Takai, 1988), thereby disrupting duodenal paracellular permeability due to alterations of tight junction integrity (Tripuraneni, Koutsouris, Pestic, De Lanerolle, & Hecht, 1997). However, recent research results call into question both the target (Espina et al., 2010; Wang et al., 2012) and the mechanism of toxicity of this group (Munday, 2013).

The Expert Group agreed on an approach for establishing TEFs which is summarized in Fig. 1. With respect to the relevance of toxicity data in the derivation of TEFs the following order of priority

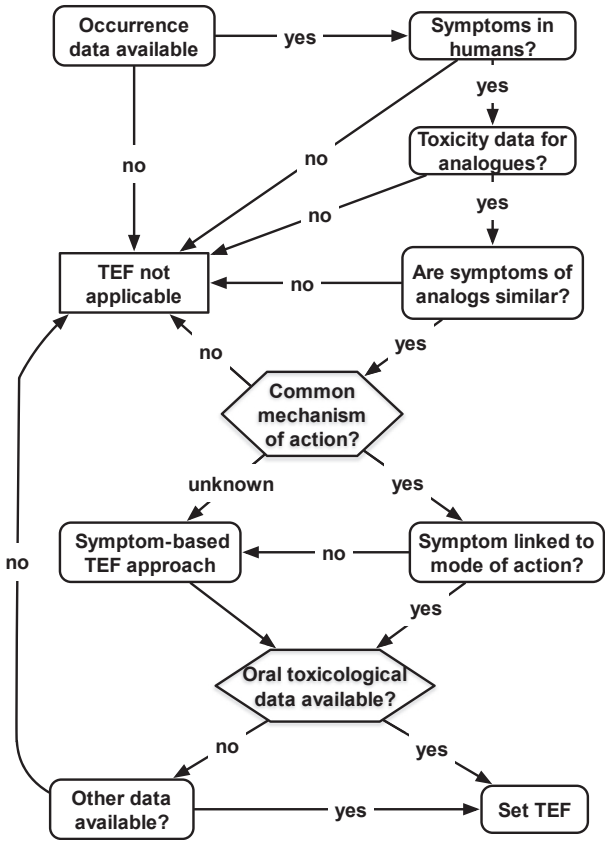

Fig. 1. Scheme of decisions to define and apply a TEF.

was agreed:

1. Data from human intoxications, the most relevant data for the human situation.
   2, Acute toxicity data through oral administration to animals, relevant to the route of human exposure.
3. Acute toxicity data through intraperitoneal (i.p.) administration to animals is less valuable, since this is less relevant for the route of human exposure. It should also be noted that there is no correlation between LD_{50} values obtained by i.p. injection and those by oral administration.
4. *In vitro* data. Such data are particularly useful when the mechanism of action of the toxin is known, and the *in vitro* test system is relevant to this mechanism.

For those toxins with no clearly defined mode of action, with several known targets, such as AZAs (Botana et al., 2014) or with no reported lethal effect in humans, such as DSTs (EFSA, 2008c), the data reported in the literature may be confusing. While values for an LD_{50}, a minimum lethal dose (MLD) or the non-specific term "lethality" have been reported (Munday, 2014). It is of little use to define a TEF for humans based on the dose of AZA that kills a mouse. Therefore, the toxic potency of AZAs in humans is somewhat biased by reference to effects in rodents, although there is presently no other way to quantify them. Another important bias is the lack of information on the chronic effect of toxins that cause death after repeated sub-lethal doses (Ferreiro et al., 2016b), and which may also be toxic through the long-term ingestion of non-lethal amounts, such as described for DA (Truelove, Mueller, Pulido, & Iverson, 1996; Vieira et al., 2015).

The approach applied by the Expert Group to establish TEFs is