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Toxicity equivalence factors for regulated and non-regulated marine toxins

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Marine Toxins are classified into groups such as saxitoxin, okadaic acid, azaspiracid, domoic acid, brevetoxin and palytoxin. They cause various shellfish poisonings after the consumption of seafood naturally contaminated. Many countries worldwide have established maximum permitted concentration of several toxin groups in seafood expressed as the level of the reference compound. The estimation of the total toxicity of seafood samples for risk assessment requires the determination of Toxicity Equivalence Factors (TEF). At present, TEFs are largely based on toxicity by intraperitoneal injection in mouse. Since humans are exposed to marine toxins by oral route, relevant parameters for updating TEFs are data from lethal doses via oral administration in animals. For non-regulated groups, not enough information is available to determine TEFs.

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Introduction

Marine toxins can accumulate in molluscs and cause poisoning or even death in animals and humans. Those toxins are known for the syndromes they cause: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP) and neurotoxic shellfish poisoning (NSP). According to their chemical structure marine toxins are classified into groups namely saxitoxin (STX), okadaic acid (OA), azaspiracid (AZP), domoic acid (DA), brevetoxin (BTX), palytoxin (PLTX) or tetrodotoxin (TTX) [1•].

The primary objective of a toxin monitoring programme is to protect consumers. Therefore the monitoring system employed should guarantee that the toxin levels of shellfish placed on the market are below values established in the legislation. The Standard for Live and Raw Bivalve Molluscs [2] developed by the Codex Committee on Fish and Fishery Products (CCFFP) established the maximum level of each toxin group expressed as the level of the reference toxin [3•] (Table 1). Many countries worldwide have enacted regulatory limits based on those indicated in the Codex Alimentarius Standard 292-2008, such as the European Union [1•,4], the United States, or South Africa [5]. Every group of toxins includes several analogues, therefore to estimate the total toxicity of seafood samples for risk assessment it is necessary to know the relative toxicity of each one. This requires the determination of toxicity equivalency factors (TEFs), 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 [6••].

For the establishment of TEFs, toxicity data are considered in the following order of importance: data from human cases, oral lethal doses in animals, intraperitoneal (i.p.) lethal doses in animals, mouse bioassay (MBA) and *in vitro* assay [6••]. Due to the lack of appropriate human toxicological information, the toxicity studies by oral administration in animals were considered to be most relevant for updating TEFs. However they should be conducted by a uniform validated method, for instance toxicological studies by oral administration described in the guidelines issued by the OECD [7•]. In addition it is important to use pure toxins in the form of certified standards to obtain the TEF values [8].

This review is an up-to-date compilation of the available literature on TEFs related to regulated (STX, OA, AZP, DA) and currently non-regulated (BTX, PLTX, TTX) marine toxins in the European Union.

Regulated marine toxins

Saxitoxin group

In this group of toxins, that has STX as the reference compound, 57 analogues have been reported of which 18 have toxicological relevance [9]. They can be classified in three structural sub-groups: the N-sulfocarbamoyl, the decarbamoyl, and the carbamoyl saxitoxins in increasing order of toxicity in MBA [10]. The MBA is an *in vivo* test to determine the quantity of toxin in a sample based in the relationship between the doses of toxin administered to mice by i.p. injection and the time of death of the

Table 1

Classification of marine toxins by the syndrome they cause and the limit in the Codex standard expressed as level of the reference toxin for each group

Syndrome	Reference toxin	Maximum level of toxin/kg shellfish meat
PSP	STX	≤0.8 mg of saxitoxin equivalent
DSP	OA	<0.16 mg of okadaic acid equivalent
	AZP	<0.16 mg
ASP	DA	<20 mg of domoic acid
NSP	BTX	<200 mouse units or equivalent

animals. The MBA has been used for comparing the toxicity of STX analogues with the assumption that the dose-death time relationship is the same for all of them [10]. However, recent investigations demonstrated that this assumption is not always valid [11].

The toxins of this group bind to the voltage-gated sodium channels (Na_v) and block conduction of action potential in excitable cells entailing the appearance of the main PSP symptoms such as muscular paralysis, respiratory depression and death. This action mechanism has been used by some *in vitro* assays to compare the toxic effects of the STX analogues [12].

The European Food Safety Authority (EFSA) published TEFs for the most prominent STX analogues based largely on the relative potencies by MBA and also on the *in vitro* study of the effect of certified toxins on sodium channels [13•] (Table 2). Considering these TEFs the most potent analogues are neosaxitoxin (NeoSTX), gonyautoxin 1 (GTX1), and decarbamoylsaxitoxin (dcSTX) with the same potency than STX (TEF = 1).

Table 2

Comparison of TEF values for saxitoxin group toxins. TEFs established by EFSA and TEFs based on LD_{50} by gavage or LD_{50} by voluntary oral consumption

Compound	TEF		
	EFSA	Gavage	Voluntary feeding
STX	1.00	1.00	1.00
NeoSTX	1.00	1.70	2.54
GTX1	1.00	–	–
GTX2	0.40	–	–
GTX3	0.60	–	–
GTX4	0.70	–	–
GTX5	0.10	0.063	0.050
GTX6	0.10	0.038	–
C1	–	–	–
C2	0.1	–	–
C3	–	–	–
C4	0.1	–	–
dcSTX	1.00	0.457	0.368
dcNeoSTX	0.4	0.216	0.224
dcGTX2	0.2	–	–
dcGTX3	0.4	–	–

However a relevant parameter for toxicity comparison would be the median lethal dose (LD_{50}) via oral administration, since this is the route through which humans are exposed to STX and analogues. In agreement with this, the relative potencies for some of the toxins were re-evaluated by oral administration (gavage or voluntary feeding) of certified materials [6••,11,14] (Table 2). The symptoms of intoxication were the same as those recorded after i.p. injection, although the time to onset of the changes was greater and relative toxicities of some STX analogues were different. On the basis of the available LD_{50} by oral administration to mice, TEFs for GTX1, gonyautoxin 2 (GTX2), gonyautoxin 3 (GTX3), gonyautoxin 4 (GTX4), C1 and C2 are similar to those proposed by EFSA, while TEFs for gonyautoxin 5 (GTX5), gonyautoxin 6 (GTX6), dcSTX and decarbamoylneosaxitoxin (dcNeoSTX) were lower [11,14]. It needs to be taken into account that the high oral toxicity of NeoSTX indicates a TEF value higher than that proposed by EFSA [11,13•]. On the basis of the available oral toxicity data, TEFs for some of the STX analogues should be revised as was recently recommended in the Expert Panel review [6••].

Okadaic acid group

This group includes OA as the reference compound, dinophysistoxin 1 (DTX1) dinophysistoxin 2 (DTX2) and a number of OA acid derivatives such as diol esters: dinophysistoxin 3 (DTX3) and water soluble sulfated diesters: dinophysistoxin 4 (DTX4), dinophysistoxin 5a (DTX5a), dinophysistoxin 5b (DTX5b), dinophysistoxin 5c (DTX5c) [15]. Those toxins inhibit serine/threonine protein phosphatases, specifically PP2A and as secondary targets PP1 and PP2B [16]. The affinity of PP2A for DTX1 is 1.6-fold higher and for DTX2 is 2-fold lower than for OA [16–18]. Therefore TEFs based on PP2A inhibition are 1.0, 1.6 and 0.5 for OA, DTX1 and DTX2 respectively. For PP2A and PP5 DTX1 is of greater potency than OA (TEFs 1.6 and 1.8 respectively). However for PP1, which is less sensitive to this group of toxins, the relative potency of DTX1 is 0.72. The relative potencies for DTX2 based on the inhibition of three PPs (PP2A, PP5 and PP1) ranged between 0.33 and 0.5 [19].

PPs affinity cannot fully explain the difference in toxicity between compounds of the OA group. Therefore other actions must be involved in their toxicity. In cytotoxicity assays and based on effective concentration that produces 50% of cell mortality (EC_{50}), DTX1 displayed the most toxic effect showing a 2–4 times greater activity than OA while DTX2 showed less toxicity than OA by a factor between 0.3 and 0.7 [20,21]. Since the gut is the major target of the OA group, it is remarkable that the toxic