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

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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.

Highlights

► Marine toxins TEF are revised according to recent toxicology studies. ► TEF for each toxin group are proposed. ► The proposed TEF were agreed by a joint FAO-WHO working group.

Keywords : Marine toxins, Toxicity Equivalency Factors, FAO, WHO, Bivalve, Mollusc

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 (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, Y. Oshima, & M. 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.

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 Figure 1. With respect to the relevance of toxicity data in the derivation of TEFs the following order of priority 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 summarized in Figure 1. Table 1 lists the uncertainties associated to TEF definitions for each toxin group.

Saxitoxin group

This group of toxin analogues has saxitoxin (STX) as the reference compound, and they share a common structure of tetrahydropurine. These toxins are mainly produced by dinoflagellates of the genus *Alexandrium*, but *Pyrodinium* and *Gymnodinium* are also potential sources (Wiese, D'Agostino, Mihali, Moffitt, & Neilan, 2010). More than 50 compounds have been reported (Wiese, et al., 2010)

and at least 18 have been demonstrated to have toxicological relevance. They are soluble in water and thermostable at acidic pH; at alkaline pH they are quickly degraded (Kodama & Sato, 2008).

STX and analogues exert their toxic effects in animals by binding to the voltage-gated sodium channel (Na_v) (Payandeh, Scheuer, Zheng, & Catterall, 2011). This channel contains one alpha subunit and one to three small beta subunits. There are 9 alpha subunits of the Na_v channel (Na_v 1 to 9) (Wingerd, Vetter, & Lewis, 2012), and originally they were divided into tetrodotoxin (TTX)-sensitive (Na_v 1, 2, 3 and 7) and TTX insensitive. The alpha subunits contain 4 homologous domains, each with 6 hydrophobic transmembrane segments. There are 6 binding sites that are the targets for many toxins, including several phycotoxin groups. Site 1 is the receptor for TTX and STXs and site 5 is the receptor for ciguatoxins (CTXs) and BTXs (Hartshorne & Catterall, 1981). The major molecular mechanism of toxicity of both TTX and STX is to block the channel pore, thereby inhibiting the conductance of the channel and the transmission of electrical action potentials generated by the influx of sodium ions into the cell. This mechanism is responsible for muscle paralysis, potentially leading to paralysis of the diaphragm and death.

Sommer and Mayer reported a quantitative MBA for STX (Sommer & Meyer, 1937), which is based on the dose-death time relationship in mice dosed intraperitoneally with this toxin. This MBA, which is now an approved AOAC method (Hungerford, 1995), has been widely used for comparing the toxicity of STX analogues (Oshima, 1995). The assumption is that the dose-death time relationship is the same for all analogues, yet that is not case, (Munday, Thomas,

194 Gibbs, Murphy, & Quilliam, 2013), which calls into question the validity of this
195 assay for the calculation of TEFs.

196 Table 2 shows the relative potencies determined by MBA as presented in the
197 scientific literature. There is a correlation between the relative specific activity
198 and relative toxicity by ip. injection with some STX derivatives; however, with
199 NeoSTX, GTX 1&4 and dcGTX 2&3, there is no such correlation. This is
200 attributable to differences in the dose-death time relationship (Munday, et al.,
201 2013). The differences among the values shown in Table 2 in many cases most
202 likely reflect the use of impure compounds, although the estimates for NeoSTX
203 reported (Munday, et al., 2013) and (Vale, Alfonso, et al., 2008), using certified
204 toxins, are significantly different. As certified STX analogues became available, a
205 list of relative potency was proposed by the European Food Safety Authrority
206 (EFSA) based on the effect of certified toxins on neuronal cultures and on MBA
207 (EFSA, 2009). These values were reevaluated using oral administration (gavage
208 or feeding) (Munday, et al., 2013). In some cases, the results were similar to
209 those obtained through i.p. administration, but differences were observed for
210 other congeners. The TEF for dcSTX was 0.64 in the MBA and 0.785 by the i.p.
211 route compared to 0.37 by feeding and 0.46 by gavage. The TEF for dcNeoSTX
212 was 0.4 in the MBA and 0.058 by i.p. injection compared to 0.22 by both gavage
213 and feeding. Importantly, the TEF for the oral toxicity of NeoSTX was higher (1.7
214 by gavage, 2.5 by feeding) compared to 1.16 by i.p. injection. The TEF for the oral
215 toxicity of NeoSTX was (1.7 by gavage, 2.5 by feeding) compared to 1.16 in the
216 MBA and 3.12 by i.p. injection.

217 There are *in vitro* methods that compare the effects of STX with its congeners, as
218 shown in Table 3. The EFSA TEFs for GTX-1&4, GTX-2&3 and C1,2 are consistent

with those determined by oral administration. In contrast, the TEF for NeoSTX was significantly higher than that proposed by EFSA, while the proposed TEFs for GTX5, GTX6, dcSTX, dcNeoSTX were lower. There are two toxins that require further clarification: dcSTX was recently reported by some authors to be less toxic than STX, with TEF of 0.8 (Vale, Alfonso, et al., 2008), 0.64 (Munday, et al., 2013), 0.478 (Suzuki & Machii, 2014) and 0.37 (Suzuki & Machii, 2014), and NeoSTX from 1 (Alonso, Alfonso, Vieytes, & Botana, 2016; EFSA, 2009) to 2.54 (Munday, et al., 2013). It is interesting to note that there is a better match between the results obtained with Na_v subtype 1.2 channel blockage (Alonso, et al., 2016) and with oral administration to mice (Munday, et al., 2013).

Okadaic acid group

This group of toxins has OA as the reference compound. OA was originally isolated from the sponge *Halichondria okadaii* (Tachibana, et al., 1981) and linked to diarrhetic shellfish poisoning (DSP) (T. Yasumoto, Y. Oshima, & M. Yamaguchi, 1978a) through dinophysistoxin-1 (DTX1), produced by *Dinophysis fortii.* Dinophysistoxin-2 (DTX2) was discovered as a third main analogue (Hu, et al., 1992) in Irish mussels associated with diarrhetic episodes. OA and DTXs are produced by *Dinophysis* and *Prorocentrum* species.

OA is a polyether characterised by a carboxylic acid group and three spiro-keto ring assemblies, one of which connects a five with a six-membered ring. OA, DTX1 and DTX2 withstand a wide pH and temperature range in methanolic NaOH solution. Strong mineral acids cause their rapid degradation in 20 min at 76 °C even with shellfish matrix in the extract (T. Yasumoto, Murata, Oshima, &

Sano, 1985), but food itself can buffer the acid and the toxins may be stable in the stomach after a meal (Alfonso, et al., 2008).

There are different types of esters of OA and DTXs. They are all fatty acid esters (palmitic being the most common) of OA, DTX1 and DTX2, of variable chain length and referred to as DTX3. The multitude of compounds potentially present in shellfish (free toxins, diol esters and their derivatives, fatty acids and mixtures of diol- and fatty acid esters) complicates the determination of overall toxic potential of shellfish samples. All of the esters are quantitatively cleaved through treatment with strong base, e.g. 0.3 molar methanolic NaOH at 76 °C for 10 to 40 min (Marr, Hu, Pleasance, Quilliam, & Wright, 1992).

The target of OA and analogues is suggested to be protein phosphatases (PP), especially PP2A (ID_{50} 1,2 nM) and, as secondary targets, PP1 (ID_{50} 315 nM) and PP2B (ID_{50} 4530 nM)(Bialojan & Takai, 1988; Takai, Bialojan, Troschka, & Ruegg, 1987). Table 4 shows the intraperitoneal (i.p.) and in vitro (i.v.) toxicities of this group of compounds. There is remarkable consistency among the cell lines tested, with DTX-1 showing a 2-4 fold higher activity than OA, and DTX-2 showing less toxicity than OA, by a factor of between 0.35 and 0.73.

DTX-1 shows similar toxicity in mice when administered either intraperitoneally or by oral administration, with fluid accumulation in the gastrointestinal tract of mice dosed with DTX1 at 0.4 and 0.32 µg/mouse for OA and DTX1, respectively (Tubaro, Sosa, Bornacin, & Jungerford, 2008). The lethal dose of DTX1 by oral administration has been reported as below 300 µg/kg b.w. (all animals dead) in fasted animals (Munday, 2014; Ogino, Kumagai, & Yasumoto, 1997), while other studies reported no deaths in mice or rats given DTX-1 orally at 750 mg/kg b.w. (Ito & Terao, 1994; Terao, Ito, Ohkusu, &

Yasumoto, 1993). No published reports regarding the oral toxicity of DTX2 are available, although a work not yet published (Louzao*, pers comm.*) has concluded that the oral LD_{50} is 2,150 µg/kg b.w. (death at 24 h, mice fasted for 12 h, administration by gavage) and that the LD_{100} is 3,000 µg/kg b.w., all animals dying in less than 5 h. No damage to the GI tract mucosa was observed in this study. Although the toxicity of DTX-1 by gavage appears to be higher than that of OA, the variability among published values precludes an estimate of TEFs based on oral toxicity. A recent study on the cardiotoxic effects of OA (20 µg/kg b.w.) and DTX1 (16 µg/kg) in rats showed no cardiotoxic effects of these compounds in acute experiments as assessed either by the electrocardiogram or by biomarkers (Ferreiro, et al., 2015). The TEFs recommended by the Expert group for OA group are indicated in Table 6.

Azaspiracid group

This group of toxins has AZA1 as the reference compound. The first intoxication by AZAs was recognised in 1996 (McMahon, 1996). These compounds are produced by the genera *Azadinium* and *Amphidoma* (Krock, et al., 2012; Tillmann, Salas, Jauffrais, Hess, & Silke, 2014). Their structure is unusual in that they have a unique spiro ring assembly and a cyclic amine instead of a cyclic imine group; a carbocyclic or lactone ring is absent (Satake, Ofuji, Naoki, et al., 1998); their mechanism of toxicity is presently unknown (Botana, et al., 2014). Long term effects are inconclusive (EFSA, 2008b; Ito, et al., 2002), although damage to multiple organs was reported following oral administration to mice, with injury

293 to the intestinal epithelium, lamina propria and villi, and a lethal oral dose of 700
294 µg/Kg b.w. (Ito, et al., 2002).
295 AZAs are readily absorbed after oral administration to mice (Aune, et al., 2012).
296 They were first detected 30 minutes after administration to pigs, with peak
297 levels achieved after 4 h (Twiner, Hess, & Doucette, 2014). In humans, they cause
298 vomiting, nausea, diarrhoea and stomach cramps within a few hours after
299 ingestion (Klontz, Abraham, Plakas, & Dickey, 2009). No deaths from AZA
300 ingestion have been reported. The EFSA working group established an acute
301 reference dose (ARfD) of 0.2 µg AZA equivalents/kg body weight (b.w.) (EFSA,
302 2008b). The Joint FAO/IOC/WHO *ad hoc* Expert Consultation established a
303 provisional ARfD of 0.04 µg/kg b.w. body weight but were unable to establish a
304 Tolerable Daily Intake (A. CODEX, 2006).
305 AZAs target several apoptotic modulators (Botana, et al., 2014; Roman, et al.,
306 2002; Twiner, et al., 2005), such as caspase, cytoskeleton (Vilarino, Nicolaou,
307 Frederick, Vieytes, & Botana, 2007), cytochrome release (Twiner, Hanagriff,
308 Butler, Madhkoor, & Doucette, 2012), c-jun-N-terminal protein kinase (JNK),
309 calcium levels (Cao, LePage, Frederick, Nicolaou, & Murray, 2010; Vale,
310 Wandscheer, et al., 2008), fatty acid biosynthesis (Twiner, et al., 2008). AZAs
311 decrease cell volume mediated by potassium and chloride efflux (Vale, Nicolaou,
312 Frederick, Vieytes, & Botana, 2010), deplete ATP (Kellmann, et al., 2009), inhibit
313 endocytosis (Bellocci, Sala, Callegari, & Rossini, 2010) and decrease procathepsin
314 pools in endocytosis (Sala, Bellocci, Callegari, & Rossini, 2013). The observation
315 that AZAs are present only in mussel samples with high levels of glutaric acid is
316 intriguing, and a combination of AZA and glutaric acid blocks voltage-dependent

317 sodium channels (Chevallier, et al., 2015), which could explain the neurotoxicity
318 linked to AZA (Twiner, et al., 2014).
319 AZAs block open hERG potassium channels (Twiner, Doucette, et al., 2012), and
320 this translates into the *in vivo* acute (11 μg/kg) and subacute (four doses of 10
321 μg/Kg in 15 days) cardiotoxicity of AZAs through hERG channels in rats
322 (Ferreiro, et al., 2016b; Ferreiro, et al., 2014). Ultrastructural changes in the
323 hearts of rats were observed at a dose of 1 μg/kg b.w. i.p. The possible
324 cardiotoxic effect of this group requires further investigation.
325 Acute toxicity data on AZAs are shown in Table 5. The TEFs recommended by the
326 Expert group for AZAs are indicated in Table 6.
327
328 **Domoic acid**
329
330 Domoic acid is a globally distributed excitotoxin produced by the red macroalga
331 *Chondria armata* (Takemoto & Daigo, 1958), and by diatoms of the genera
332 *Nitzschia*, *Pseudo-nitzschia* (Bates, et al., 1989) and *Amphora* (Dhar, et al., 2015).
333 The worldwide distribution of the toxin producing organisms makes the
334 presence of DA rather ubiquitous in the world oceans. A TEF of one is applicable,
335 as only DA and its diastereoisomer, epi-DA, have been shown to be of
336 toxicological relevance (sum of DA and epi-DA expressed as DA).
337
338 **Brevetoxins**
339 Brevetoxins target the neurotoxin receptor site five voltage gated sodium
340 channels, resulting in membrane depolarization, prolongation of open time,
341 prevention of channel inactivation, induction of the channel activation at more

342 negative potentials, thereby causing repetitive firing and increases in sodium
343 currents (Atchison, Luke, Narahashi, & Vogel, 1986; Trainer, Moreau, Guedin,
344 Baden, & Catterall, 1993). These effects lead to rapid reductions in respiratory
345 rate, cardiac rhythm alterations, and hypothermia (Templeton, Poli, & LeClaire,
346 1989).
347 Brevetoxins have a polyether backbone and can be grouped into two types.
348 BTX1 (also referred to in the literature as PbTx1) represents the A-type toxins
349 and has been reported to be the most toxic of the BTX analogues (Shimizu, Chou,
350 Bando, Van Duyne, & Clardy, 1986). BTX2 (also referred to as PbTx2),
351 representing the B-type toxins, is the most abundantly produced by the source
352 dinoflagellate *Karenia brevis* (Shimizu, et al., 1986). Following a neurotoxic
353 shellfish poisoning outbreak in New Zealand in 1992-1993, it was found that
354 BTXs are extensively metabolized in shellfish (Ishida, et al., 1995). Of the
355 metabolites identified in shellfish from New Zealand BTX-B1 was found to be
356 most toxic (Ishida, et al., 1995). BTX-B4 was threefold more toxic than BTX-B2
357 and comparable to the toxicity of BTX3 (also referred to as PbTx3) (Baden &
358 Mende, 1982; Morohashi, et al., 1999; Poli, Mende, & Baden, 1986). There is
359 limited human oral data available for establishing TEFs; currently, the CODEX
360 Standard method for BTXs is the MBA and the regulatory limit is expressed as
361 mouse units. For this reason, TEFs for BTXs are not currently proposed.
362
363
364 **The special case of tetrodotoxin and emerging toxins**
365 TTX is a marine toxin of bacterial origin and is produced, amongst others, by
366 *Pseudomonas* and *Vibrio* spp. (Bane, Lehane, Dikshit, O'Riordan, & Furey, 2014).

It is becoming a concern in Europe given its presence in gastropods (Rodriguez, et al., 2008; Silva, et al., 2012) and in shellfish (A. Turner, Powell, Schofield, Lees, & Baker-Austin, 2015; Vlamis, et al., 2015). Because TTX in shellfish is a newly-discovered phenomenon, there is presently no surveillance programme for TTX in place. The mode of action is similar to that of STX, with main difference between the toxin groups being the subtypes of Na_v for which they preferentially bind. In the case of TTX, the Na_v 1.7 is the main target (Alonso, et al., 2016; Walker, et al., 2012), although TTX can bind with lower affinity to other Na_v subtypes. TTX binds to human Na_v 1.7 with 38 fold more potency than STX (Walker, et al., 2012).

The human lethal dose of TTX is 2 mg (Noguchi, Onuki, & Arakawa, 2011). Based on the intraperitoneal toxicity to mice, relative toxicities of TTX analogues have been reported in the literature (Bane, et al., 2014).

The lethality of TTX decreases with the route of administration, from 10 µg/kg b.w. i.p., to 16 µg/kg b.w. subcutaneous, and 332 µg/kg b.w. oral (Kao, 1966; E. G. Moczydlowski, 2013).

The Expert Group suggested an emerging need to establish TEFs for TTX analogues that may be found in bivalves, indicating a requirement for oral toxicity data on the analogues.

It was also suggested that other emerging toxins, such as palytoxin, ovatoxins, ostreocins and cyclic imines should be further investigated to determine the actual risk to consumers (Munday, 2014).

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393 Meeting on "Toxicity Equivalency Factors for marine biotoxins associated with
394 bivalve molluscs" held in Rome on February 22-24, 2016. The full report of the
395 meeting will be available on FAO/WHO websites.
396

Table 1. Uncertainties in the definition of TEFs, from high (+++) to low (-) or no relevance if complete information is not available.

Toxin group	Mode of action (to explain toxicity)	Animal data relevant to human effect	Known potency of each analogue	Chronic toxicity information available
STX *	-	-	++	-
OA **	++	++	-	++
DA #	-	+	-	+++
BTX @	+	+	+	+
AZA &	+++	++	+	++

* Most of the required information is available for common toxins, but new toxins, such as benzoate derivatives, and dcSTX or NeoSTX require further research about their potency. Benzoate derivatives toxicity and an enhanced understanding of the pharmacokinetics of the group are also needed.

** Analogues that lack phosphatase inhibition have potent cellular effects (Espina, et al., 2010), therefore the mechanism for diarrhoea needs to be understood (Louzao, et al., 2015; Munday, 2013). Other phosphatase inhibitors do not show a diarrhetic effect. This suggests other factors are involved in the mechanism of toxicity, i.e. neuropeptide Y inhibition (Louzao, et al., 2015). No damage to the mucosa was observed while severe diarrhoea was induced (Vieira, et al., 2013). Oral studies with the same methodology are also required.

Target is well known (Hogberg & Bal-Price, 2011), but long term effects are unclear with regard to endocrine (Crespo, et al., 2015), cardiotoxic (Vieira, et al., 2016; Vranyac-Tramoundanas, Harrison, Sawant, Kerr, & Sammut, 2011), or prenatal toxicity (Levin, Pizarro, Pang, Harrison, & Ramsdell, 2005).

@ Several aspects of toxicity needs further investigation, such as effects on smooth muscle mediated by the autonomic nervous system, cardiotoxicity, or body temperature (Abraham, et al., 2005; Berman & Murray, 2000; Gordon, Kimm-Brinson, Padnos, & Ramsdell, 2001).

& Several target candidates, but no identified mode of action (Botana, et al., 2014; Twiner, Doucette, et al., 2012; Vilarino, et al., 2007). Many compounds without mechanistic studies (Marine-Institute, 2014). Unclear long term toxicity (EFSA, 2008a; Ferreiro, et al., 2016b).

Table 2. Relative toxicity of STX derivatives as indicated by the MBA.

Compound	Relative specific activity in the MBA	Relative LD_{50} by i.p. injection[1]
Saxitoxin	1.0	1.00
NeoSTX	0.50, 0.75[2], 0.90, 0.90, 1.0, 1.16[1], 1.2	3.12
GTX-1	0.80, 1.0	
GTX-4	0.30, 0.70	
GTX-1&4	0.70, 1.02[1], 0.65[2]	1.90
GTX-2	0.40, 0.40	
GTX-3	0.60, 1.1	
GTX-2&3	0.60, 0.60[1], 0.52[2]	0.757
GTX-5	0.10, 0.10, 0.20, 0.1[5]	0.222
GTX-6	0.10, <0.1[5]	0.122
C-1	0.02, 0.00	
C-2	0.10, 0.17	
C-3	0.0, 0.01	
C-4	0.0, 0.10	
dcSTX	0.40,0.48[3], 0.50, 0.50, 0.60, 0.64[1],	0.785

	1.0, 1.02[2]	
dcNeoSTX	0.40, 0.020[4]	0.058
dc-GTX-1	0.5	
dc-GTX-2	0.20, 0.20, 0.30	
dc-GTX-3	0.20, 0.40, 0.50	
dc-GTX-4	0.50	
dc-GTX-2&3	0.20, 0.19[2]	0.695
11α-Hydroxy-STX	0.60	
11β-hydroxy-STX	0.70	
TTX[5]		1.1 (compared to STX)
11-deoxy-TTX		7.7
6,11-dideoxy TTX		46
11-oxo-TTX		1.7
4-epi-TTX		7
6-epi-TTX		6.6
4,9-Anhydro-TTX		53.6
11-nor-TTX-6(S)-ol		5.9
11-nor-TTX-6(R)-ol		7.6

Data are taken from Table 13 of the 2009 ESFA report on saxitoxin group toxins (EFSA, 2009) and modified as indicated by superscript numerals.

1. (Munday, et al., 2013). 2. (Vale, Alfonso, et al., 2008). 3. (Suzuki & Machii, 2014). 4. Munday, unpublished results. 5 (Watanabe, Suzuki, & Oshima, 2010).

A mouse unit for STX is 0.183 μg (9.15 μg/kg b.w.) (AOAC, 2005a; Schantz,

McFarren, Schafer, & Lewis, 1958), and the potency of STX is 10 percent higher than TTX. 5. A basic TEF list for TTX, compared to STX (T. Yasumoto, Yotsu-Yamashita, Murata, & Naoki, 1988). Further information is needed for each TTX derivative.

Table 3. Relative toxicities of STX and derivatives in mice through oral administration (gavage/voluntary feeding) and relative activities toward sodium channels *in vitro*.

Compound	Relative toxicity by voluntary feeding/ gavage *	Relative activity toward sodium channels *in vitro* by type of assay method[1]								
		1 squid axon	2 rat cortex	3 frog sciatic nerve	4 frog muscle fibre	5 rat muscle	6 cultured neurons	7 cerebellar neurons	8, $Na_v1,6$	8, $Na_v1,2$
STX	1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
neoSTX	2.54/ 1.7	-	0.69	4.5	1.0	3.6 / 3.7	0.82	1.02	1.2	2.0
GTX 1		-	-	-	-	0.28	-	-		
GTX 1&4	0.936/ 0.739	-	0.98	-	-	-	0.53	0.50	1.4	0.54
GTX 2		0.2		0.22		0.15/ 0.16				

GTX 2&3	0.572/ 0.533	-	0.32	-	-	-	0.38	0.28	0.15	0.4
GTX 3		0.42	-	1.4	-	0.96				
GTX 4		-	-	-	-					
GTX 5	0.064/ 0.05	-	0.031 0.039	-	-	0.024	0.09	0.09	0.11	0.01
GTX 6	< 0.017/ 0.038	-	-	-	-	-				
dcSTX	0.368/ 0.457	-	0.097 0.29	-	0.2	0.44	0.84	1.00	0.96	0.25
dcNeoSTX	0.224/ 0.216	-	-	-	0.004	-	0.48	0.44	0.25	0.1
dcGTX2,3	0.108/ 0.167								0.02	0.05
C1	-	-	-	-	-	0.0017/ 0.0028				
C2		-	-	-	-	0.029				
C1,2	0.043/ 0.034								0.09	0.01

C3	-	-	-	-	-	0.002				

Note 1 - Assay methods:

* Relative toxicity by voluntary feeding/ gavage (Munday, et al., 2013).

1: Relative blockade of sodium channels in the squid giant axon (Kao, et al., 1985).

2: Relative binding to sodium receptors of the rat cerebral cortex (Llewellyn, 2006; Usup, Leaw, Cheah, Ahmad, & Ng, 2004).

3: Relative blockade of impulses in frog sciatic nerve (Strichartz, 1984).

4: Relative blockade of sodium current in frog skeletal muscle fibre (Kao & Walker, 1982; Yang, Kao, & Oshima, 1992).

5: Relative blockade of sodium channels from rat muscle plasma membrane (Guo, et al., 1987; E. Moczydlowski, Hall, Garber, Strichartz, & Miller, 1984).

6: Blockade of veratridine-induced changes in membrane potential in cultured neurons (Vale, Alfonso, et al., 2008).

7: Sodium currents voltage-dependent inhibition in primary cultures of cerebellar neurons (Perez, et al., 2011).

8. High-throughput electrophysiology system, in cells stably transfected with specific subunits of sodium channels (Alonso, et al., 2016).

Table 4. Toxicities of OA and its analogues by i.p. injection (Munday, 2014). Large discrepancies are most likely due to the use of non-certified calibrants.

Compound	**Parameter**	**Acute toxicity (µg/kg b.w.)**
OA	LD_{50}	192 (Tachibana, et al., 1981)
OA	LD_{50}	200 (pers. comm. T. Yasumoto, 1991)
OA	No death	200 (Ito & Terao, 1994)
OA	LD_{50}	204 (Aune, et al., 2012)
OA	LD_{50}	210 (Dickey, Bobzin, Faulkner, Bencsath, & Andrzejewski, 1990)
OA	LD_{50}	225 (Tubaro, et al., 2003)
OA	$LD_{40\ to}\ LD_{100}$	mean 227, range 216-242, (Suzuki, 2012)
OA	LD_{100}	375 (Ito & Terao, 1994)
DTX1	MLD	160 (Murata, Shimatani, Sugitani, Oshima, & Yasumoto, 1982; T Yasumoto & Murata, 1990)
DTX1	LD_{50}	160 (pers. comm. T. Yasumoto, 1991)(Dominguez, et al., 2010)
DTX1	LD_{100}	375 (Ito & Terao, 1994)
DTX2	LD_{50}	352 (Aune, et al., 2007)
DTX3	LD_{100}	375 (Ito & Terao, 1994)
DTX3	MLD	500 (T. Yasumoto, et al., 1985)

DTX4	LD_{50}	610 (Hu, Curtis, Walter, & Wright, 1995)

***In vitro* cell toxicities of OA, DTX-1 and DTX-2**

Compound	Relative toxicity in the specified cell line					
	SH-SY5Y (Louzao, et al., 2015)	Neuro-2a (Solino, Sureda, & Diogene, 2015)	NG108-15 (Solino, et al., 2015)	MCF-7 (Solino, et al., 2015)	Caco-2 (Ferron, Hogeveen, Fessard, & Le Hegarat, 2014)	HT29-MTX (Ferron, et al., 2014)
OA	1.0	1.0	1.0	1.0	1.0	1.0
DTX1	4.4	2.1	2.4	3.8	2.2	3.4
DTX2		0.52	0.52	0.73	0.47	0.35

Table 5. Toxicities of AZAs

Intraperitoneal injection

Compound	Parameter	Acute toxicity (µg/kg b.w. b.w) (reference)
AZA1	Lethality	200 (Munday, 2014)
AZA1	MLD	150 (Satake, Ofuji, James, Furey, & Yasumoto, 1998)
AZA1	LD_{50}	74 (Marine-Institute, 2014)
AZA1	LD_{50}	>10 and <55 in rats (Ferreiro, et al., 2016a)
AZA2	Lethality	Approximately 110 (Munday, 2014)
AZA2	LD_{50}	117 (Marine-Institute, 2014)

AZA2	LD_{50} (i.v.)	11 in rats (Ferreiro, et al., 2014)
AZA3	Lethality	Approximately 140 (Munday, 2014)
AZA3	LD_{50}	164 (Marine-Institute, 2014)
AZA4	Lethality	Approximately 470 (Munday, 2014)
AZA5	Lethality	<1 000 (Munday, 2014)
AZA6	LD_{50}	100 (Marine-Institute, 2014)

Oral administration

Compound	Parameter	Acute toxicity (µg/kg b.w.)	Reference
AZA1	Lethality	> 700	(Ito, 2008)
AZA1	LD_{50}	775	(Aune, et al., 2012)
AZA1	LD_{50}	443	(Marine-Institute, 2014)
AZA2	LD_{50}	626	(Marine-Institute, 2014)
AZA3	LD_{50}	875	(Marine-Institute, 2014)

***In vitro* toxicity**

	Cell type				
Compound	Jurkat T (cytotoxicity)	HEK 293 (hERK current)	2-3 Days in vitro mice neurons (cytotoxicity)	Neocortical neurons (LDH release)	Neocortical neurons (calcium oscillations)
AZA1	1	1	1	1	1

AZA2	8.3	1.2	1.89	0.89	1.36
AZA3	4.5	1		4.32	3.22
AZA4	0.6				
AZA5	0.4				
AZA6	7				
AZA8	4.5				
AZA9	0.4				
AZA10	0.2				
AZA33	0.22				
AZA34	5.5				
37-epi-AZA1	5.1				

Table 6. TEFs recommended by the Expert Group for each biotoxin group

Saxitoxin group

Compound	Oshima Relative Toxicity values (MU/μmole)	Mouse LD_{50} (i.p.)	TEF based on LD_{50} by gavage	TEF based on LD_{50} by voluntary consumption	EFSA proposed TEF	**Recommended TEF**	Rationale
Saxitoxin	1	1.00	1.00	1.00	1.0	**1.0**	
NeoSTX	0.92	3.12	1.70	2.54	1.0	**2.0**	Oral studies show more potency than STX. A value of 2.0 is recommended, and supported by Na channel *in vitro* results.
GTX1	0.99				1.0	**1.0**	No new data

GTX2	0.36				0.4	**0.4**	No new data
GTX3	0.64				0.6	**0.6**	No new data
GTX4	0.73				0.7	**0.7**	No new data
GTX5	0.064	0.222	0.063	0.050	0.1	**0.1**	Oral LD_{50} data suggest a lower TEF than i.p. LD_{50}. As for NeoSTX, *in vitro* Na channel assays also support a TEF of 0.1.
GTX6		0.122	0.038		0.1	**0.05**	New oral data show lower than 0.1.
C1	0.006					**0.01**	No new data (rounded up: increments of 0.05 for TEF<0.1)
C2	0.096				0.1	**0.1**	No new data
C3	0.013					**0.01**	No new data
C4	0.058				0.1	**0.1**	No new data

dcSTX	0.51	0.785	0.457	0.368	1.0	**0.5**	New oral data (and supported by i.p. toxicity data)
dcNeoSTX		0.058	0.216	0.224	0.4	**0.2**	New oral data (and supported by *in vitro* data)
dcGTX2	0.15				0.2	**0.2**	No new data
dcGTX3	0.38				0.4	**0.4**	No new data

In case of saxitoxin analogues, for which no oral toxicity data were available, TEFs recommended are based on i.p.data

Okadaic acid group

	TEF based on cytotoxicity	TEF based on PP2A inhibition	TEF based on membrane Paracellular permeability	EFSA proposed TEF	**Recommended TEF**	Rationale
OA	1.0	1.0	1.0	1.0	**1.0**	

DTX1	3.1	1.6	2-15	1.0	**1.0**	There are several analogue specific reports from human intoxications. Human intoxications in Japan suggest a LOAEL of 48 µg DTX1 per person, equivalent to events of 50 µg OA per person in Sweden, Norway, UK and Portugal (EFSA, 2008c). Current used TEF of 1.0 is protective for public health. However *in vitro* studies suggest potency of DTX1 is higher than OA. The uncertainties of these studies (5-fold difference between cell lines) suggest a TEF of 1.0 for DTX1 should be assumed until further data is available.
DTX2	0.52	0.5	0.6	0.6	**0.5**	Consistent among the different assays; based on acute oral and i.p. toxicity in mice, DTX-2 is on average 0.5 times as toxic as DTX1). This value is also supported by the various *in vitro* data

DTX3						The TEF of the hydrolysis product of AO, DTX1 or DTX2 would apply.

Domoic Acid

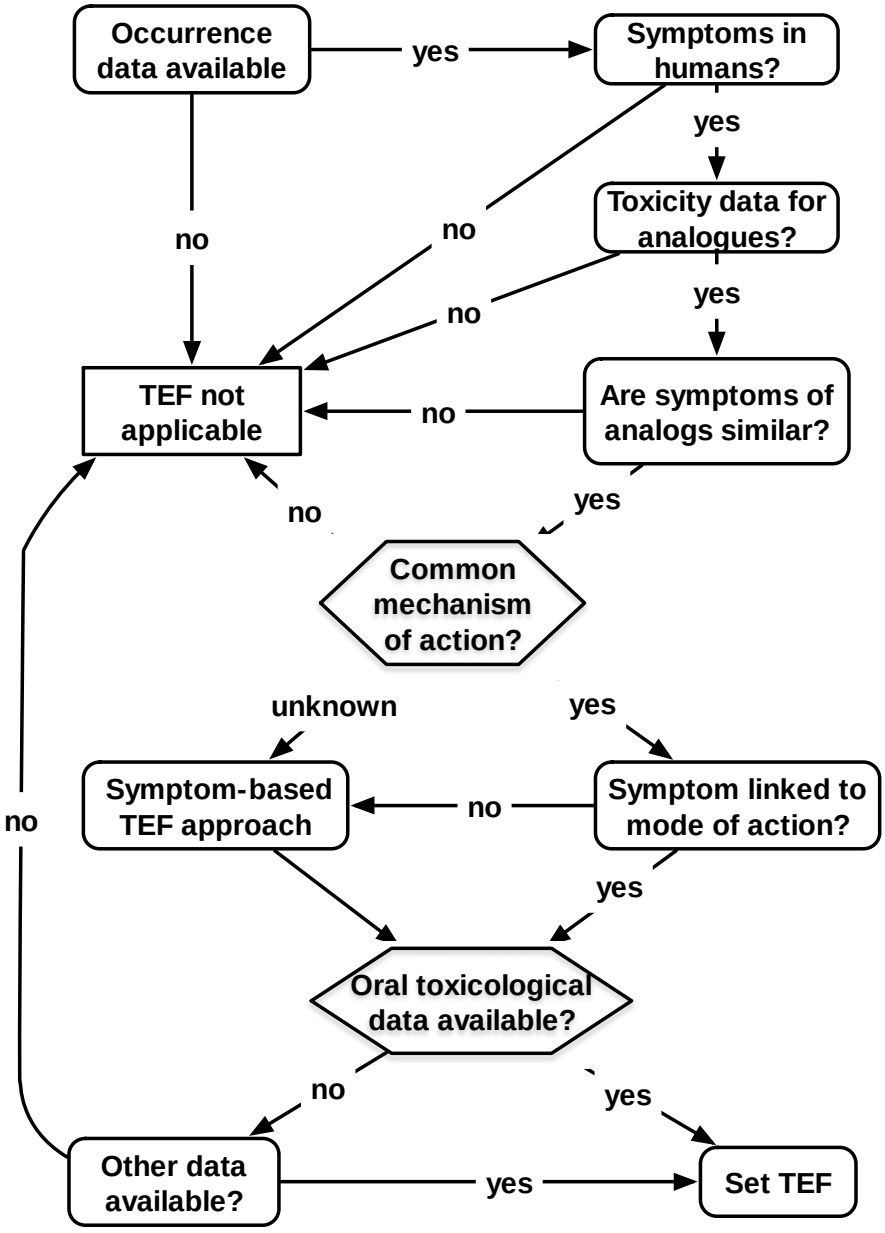
	EFSA proposed TEF	**Recommended TEF**
Domoic Acid (two epimers)	-	1.0

Azaspiracids

	TEF based on i.p. toxicity	TEF based on oral toxicity	EFSA proposed TEF	**Recommended TEF**	Rational
AZA1	1.0	1.0	1	**1.0**	
AZA2	0.6	0.7	1.8	**0.7**	Based on recent oral data. (also consistent with recent i.p. data)
AZA3	0.45	0.51	1.4	**0.5**	Based on recent oral data. (also consistent with recent i.p. data)
AZA4			-		No data

AZA5			-		No data
AZA6	0.7		-	**0.7**	No oral, only i.p. data.

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



References

Abraham, W. M., Bourdelais, A. J., Sabater, J. R., Ahmed, A., Lee, T. A., Serebriakov, I., & Baden, D. G. (2005). Airway responses to aerosolized brevetoxins in an animal model of asthma. *Am J Respir Crit Care Med, 171*, 26-34.

Alfonso, C., Rehmann, N., Hess, P., Alfonso, A., Wandscheer, C. B., Abuin, M., Vale, C., Otero, P., Vieytes, M. R., & Botana, L. M. (2008). Evaluation of various pH and temperature conditions on the stability of azaspiracids and their importance in preparative isolation and toxicological studies. *Anal Chem, 80*, 9672-9680.

Alonso, E., Alfonso, A., Vieytes, M. R., & Botana, L. M. (2016). Evaluation of toxicity equivalent factors of paralytic shellfish poisoning toxins in seven human sodium channels types by an automated high throughput electrophysiology system. *Arch Toxicol, 90*, 479-488.

Antelo, A., Alfonso, C., & Alvarez, M. (2014). Standards for marine and freshwater toxins. In L. M. Botana (Ed.), *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection* (3rd ed., pp. 429-470). Boca Ratón: CRC Press.

AOAC. (2005a). AOAC Official Method 959.08 Paralytic Shellfish Poison. Biological Method. First Action 1959. Final Action. *Official Methods of Analysis of the AOAC.*

AOAC. (2005b). Domoic acid in mussels, liquid chromatographic method. Method 991.26. *Official Methods of Analysis of the AOAC (Association of Official Analytical Chemists).*

Atchison, W. D., Luke, V. S., Narahashi, T., & Vogel, S. M. (1986). Nerve membrane sodium channels as the target site of brevetoxins at neuromuscular junctions. *Br J Pharmacol, 89*, 731-738.

Aune, T., Espenes, A., Aasen, J. A., Quilliam, M. A., Hess, P., & Larsen, S. (2012). Study of possible combined toxic effects of azaspiracid-1 and okadaic acid in mice via the oral route. *Toxicon, 60*, 895-906.

Aune, T., Larsen, S., Aasen, J. A., Rehmann, N., Satake, M., & Hess, P. (2007). Relative toxicity of dinophysistoxin-2 (DTX-2) compared with okadaic acid, based on acute intraperitoneal toxicity in mice. *Toxicon, 49*, 1-7.

Baden, D. G., & Mende, T. J. (1982). Toxicity of two toxins from the Florida red tide marine dinoflagellate, Ptychodiscus brevis. *Toxicon, 20*, 457-461.

Bane, V., Lehane, M., Dikshit, M., O'Riordan, A., & Furey, A. (2014). Tetrodotoxin: chemistry, toxicity, source, distribution and detection. *Toxins, 6*, 693-755.

Bates, S. S., Bird, C. J., De Freitas, A. S. W., Foxall, R. A., Gilgan, M., Hanic, L. A., Johnson, G. R., McCulloch, A. W., Odense, P., Pocklington, R., Quilliam, M. A., Sim, P. G., Smith, J. C., Subba Rao, D. V., Todd, E. C. D., Walter, J. A., & Wright, J. L. C. (1989). Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from Eastern Prince Edward Island. *Canada. Can. J. Fish. Aquat. Sci., 46*, 1203-1215.

Bellocci, M., Sala, G. L., Callegari, F., & Rossini, G. P. (2010). Azaspiracid-1 inhibits endocytosis of plasma membrane proteins in epithelial cells. *Toxicological sciences, 117*, 109-121.

Berman, F. W., & Murray, T. F. (2000). Brevetoxin-induced autocrine excitotoxicity is associated with manifold routes of Ca2+ influx. *J Neurochem, 74*, 1443-1451.

Bialojan, C., & Takai, A. (1988). Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. *Biochem J, 256*, 283-290.

Botana, L. M., Alfonso, A., Vale, C., Vilariño, N., Rubiolo, J., Alonso, E., & Cagide, E. (2014). The mechanistic complexities of phycotoxins: toxicology of azaspiracids and yessotoxins. In J. C. Fishbein & J. M. Heiman (Eds.), *Advances in Molecular Toxicology* (First edition ed., Vol. 8, pp. 1-33). Oxford: Elsevier.

Botana, L. M., Vilariño, N., Elliott, C. T., Campbell, K., Alfonso, A., Vale, C., Louzao, M. C., & Botana, A. M. (2010). The problem of toxicity equivalent factors in developping alternative methods to animal bioassays for marine toxin detection. *Trends Anal. Chem., 29*, 1316-1325.

Cao, Z., LePage, K. T., Frederick, M. O., Nicolaou, K. C., & Murray, T. F. (2010). Involvement of caspase activation in azaspiracid-induced neurotoxicity in neocortical neurons. *Toxicol Sci, 114*, 323-334.

Chevallier, O. P., Graham, S. F., Alonso, E., Duffy, C., Silke, J., Campbell, K., Botana, L. M., & Elliott, C. T. (2015). New insights into the causes of human illness due to consumption of azaspiracid contaminated shellfish. *Sci Rep, 5*, 9818.

Codex. (2008). Standard for live and raw bivalve molluscs. 292. In (pp. 1-7).

CODEX, A. (2006). Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs; FAO/IOC/WHO 2004. ftp://ftp.fao.org/es/esn/food/biotoxin_report_en.pdf. *accessed July 11, 2016.*

Crespo, A., Cifuentes, J. M., Bermúdez, R., Antelo, A., Alemañ, N., & Botana, L. M. (2015). Dose-response and histopathological study, with special attention to the hypophysis, of the differential effects of domoic acid on rats and mice. *Microsc Res Tech, 78*, 396-403.

Dhar, B. C., Cimarelli, L., Singh, K. S., Brandi, L., Brandi, A., Puccinelli, C., Marcheggiani, S., & Spurio, R. (2015). Molecular detection of a potentially toxic diatom species. *Int J Environ Res Public Health, 12*, 4921-4941.

Dickey, R. W., Bobzin, S. C., Faulkner, D. J., Bencsath, F. A., & Andrzejewski, D. (1990). Identification of okadaic acid from a Caribbean dinoflagellate, Prorocentrum concavum. *Toxicon, 28*, 371-377.

Dominguez, H. J., Paz, B., Daranas, A. H., Norte, M., Franco, J. M., & Fernandez, J. J. (2010). Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. *Toxicon, 56*, 191-217.

EFSA. (2008a). Marine biotoxin in shellfish-Azaspiracid group

Scientific Opinion of the Panel on Contaminants in the Food chain. In *The EFSA Journal* (Vol. 723, pp. 1-52).

EFSA. (2008b). Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – azaspiracids. *The EFSA Journal, 723*, 1-52.

EFSA. (2008c). Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – okadaic acid and analogues. *The EFSA Journal, 589*, 1-62.

EFSA. (2009). Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – Saxitoxin group. *The EFSA Journal, 1019*, 1-76.

Espina, B., Louzao, M. C., Cagide, E., Alfonso, A., Vieytes, M. R., Yasumoto, T., & Botana, L. M. (2010). The methyl ester of okadaic acid is more potent than okadaic acid in disrupting the actin cytoskeleton and metabolism of primary cultured hepatocytes. *Br J Pharmacol, 159*, 337-344.

EU. (2011). Commission regulation (EU) No 15/2011 of 10 January 2011 amending Regulation (EC) No 2074/2005 as regards recognised testing methods for detecting marine biotoxins in live bivalve molluscs. *Official Journal of the European Communities, L6*, 3-4.

Ferreiro, S. F., Carrera, C., Vilarino, N., Louzao, M. C., Santamarina, G., Cantalapiedra, A. G., & Botana, L. M. (2015). Acute cardiotoxicity evaluation of the marine biotoxins OA, DTX-1 and YTX. *Toxins, 7*, 1030-1047.

Ferreiro, S. F., Vilarino, N., Carrera, C., Louzao, M. C., Cantalapiedra, A. G., Santamarina, G., Cifuentes, J. M., Vieira, A. C., & Botana, L. M. (2016a). Subacute Cardiotoxicity of Yessotoxin: In Vitro and in Vivo Studies. *Chem Res Toxicol, 29*, 981-990.

Ferreiro, S. F., Vilarino, N., Carrera, C., Louzao, M. C., Cantalapiedra, A. G., Santamarina, G., Cifuentes, J. M., Vieira, A. C., & Botana, L. M. (2016b). Subacute Cardiovascular Toxicity of the Marine Phycotoxin Azaspiracid-1 in Rats. *Toxicol Sci, 151*, 104-114.

Ferreiro, S. F., Vilarino, N., Carrera, C., Louzao, M. C., Santamarina, G., Cantalapiedra, A. G., Rodriguez, L. P., Cifuentes, J. M., Vieira, A. C., Nicolaou, K. C., Frederick, M. O., & Botana, L. M. (2014). In vivo arrhythmogenicity of the marine biotoxin azaspiracid-2 in rats. *Arch Toxicol, 88*, 425-434.

Ferron, P. J., Hogeveen, K., Fessard, V., & Le Hegarat, L. (2014). Comparative analysis of the cytotoxic effects of okadaic acid-group toxins on human intestinal cell lines. *Mar Drugs, 12*, 4616-4634.

Gordon, C. J., Kimm-Brinson, K. L., Padnos, B., & Ramsdell, J. S. (2001). Acute and delayed thermoregulatory response of mice exposed to brevetoxin. *Toxicon, 39*, 1367-1374.

Guo, X. T., Uehara, A., Ravindran, A., Bryant, S. H., Hall, S., & Moczydlowski, E. (1987). Kinetic basis for insensitivity to tetrodotoxin and saxitoxin in sodium channels of canine heart and denervated rat skeletal muscle. *Biochemistry, 26*, 7546-7556.

Hallegraef, G. M. (2015). Harmful marine algal blooms and climate change: progress on a formidable predictive challenge. In L. M. Botana, M. C. Louzao & N. Vilariño (Eds.), *Climate change and marine and freshwater toxins* (pp. 181-193). Berlin: De Gruyter.

Hartshorne, R. P., & Catterall, W. A. (1981). Purification of the saxitoxin receptor of the sodium channel from rat brain. *Proc Natl Acad Sci U S A, 78*, 4620-4624.

Hogberg, H. T., & Bal-Price, A. K. (2011). Domoic Acid-Induced Neurotoxicity Is Mainly Mediated by the AMPA/KA Receptor: Comparison between Immature and Mature Primary Cultures of Neurons and Glial Cells from Rat Cerebellum. *J Toxicol, 2011*, 543512.

Hu, T., Curtis, J. M., Walter, J. A., & Wright, J. L. C. (1995). Identification of DTX-4, a new water-soluble DSP toxin derivatives from the dinoflagellate *Prorocentrum lima*. *J. Chem. Soc. Chem. Comm., 1995*, 597-599.

Hu, T., Doyle, J., Jackson, D., Marr, J., Nixon, E., Pleasance, S., Quilliam, M. A., Walter, J. A., & Wright, J. L. C. (1992). Isolation of a new diarrhetic shellfish poison from Irish mussels. *J. Chem. Soc. Chem. Comm.*, 39-41.

Hungerford, J. M. (1995). 35. Fish and other marine products. AOAC official method 959.08 . Paralytic shellfish poison. Biological method. Final action. In P. Cunniff (Ed.), *Official methods of analysis of AOAC International.* (16th edition ed., Vol. 2, pp. 21-22). Arlington, VA: AOAC.

Ishida, H., Nozawa, A., Totoribe, K., Muramatsu, N., Nukaya, H., Tsuji, K., Yamaguchi, K., & Yasumoto, T. (1995). Brevetoxin B1, a new polyether marine toxin from the New Zealand Shellfish Austrovenus stuchburyi. *Tetrahedron Lett, 36*, 725-728.

Ito, E. (2008). Toxicology of azaspiracid-1: acute and chronic poisoning, tumorigenicity, and chemical structure relationship to toxicity in a mouse model. In L. M. Botana (Ed.), *Seafood and Freshwater toxins: Pharmacology, Physiology and Detection* (2nd edition ed., pp. 775-784). Boca Raton, FL: CRC Press (Taylor and Francis Group).

Ito, E., Satake, M., Ofuji, K., Higashi, M., Harigaya, K., McMahon, T., & Yasumoto, T. (2002). Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussels. *Toxicon, 40*, 193-203.

Ito, E., & Terao, K. (1994). Injury and recovery process of intestine caused by okadaic acid and related compounds. *Nat. Toxins, 2*, 371-377.

Kao, C. Y. (1966). Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmacol Rev, 18*, 997-1049.

Kao, C. Y., Kao, P. N., James, K. M. R., Koehn, F. E., Wichmann, C. F., & Schnoes, H. K. (1985). Actions of epimers of 12-(OH)-reduced saxitoxin and of 11-(OSO3)-saxitoxin on squid axon. *Toxicon, 23*, 647-655.

Kao, C. Y., & Walker, S. E. (1982). Active groups of saxitoxin and tetrodotoxin as deduced from actions of saxitoxin analogues on frog muscle and squid axon. *J Physiol (Lond), 323*, 619-637.

Kellmann, R., Schaffner, C. A., Gronset, T. A., Satake, M., Ziegler, M., & Fladmark, K. E. (2009). Proteomic response of human neuroblastoma cells to azaspiracid-1. *J Proteomics, 72*, 695-707.

Klontz, K. C., Abraham, A., Plakas, S. M., & Dickey, R. W. (2009). Mussel-associated azaspiracid intoxication in the United States. *Ann Intern Med, 150*, 361.

Kodama, M., & Sato, S. (2008). Metabolism of paralytic shellfish toxins incorporated into bivalves. In L. M. Botana (Ed.), *Seafood and Freshwater toxins: Pharmacology, Physiology and Detection* (2nd edition ed., pp. 165-175). Boca Raton, FL: CRC Press (Taylor and Francis Group).

Krock, B., Tillmann, U., Voss, D., Koch, B. P., Salas, R., Witt, M., Potvin, E., & Jeong, H. J. (2012). New azaspiracids in Amphidomataceae (Dinophyceae). *Toxicon, 60*, 830-839.

Levin, E. D., Pizarro, K., Pang, W. G., Harrison, J., & Ramsdell, J. S. (2005). Persisting behavioral consequences of prenatal domoic acid exposure in rats. *Neurotoxicol Teratol, 27*, 719-725.

Llewellyn, L. E. (2006). The behavior of mixtures of paralytic shellfish toxins in competitive binding assays. *Chem Res Toxicol, 19*, 661-667.

Louzao, M. C., Fernandez, D. A., Abal, P., Fraga, M., Vilarino, N., Vieytes, M. R., & Botana, L. M. (2015). Diarrhetic effect of okadaic acid could be related with its neuronal action: Changes in neuropeptide Y. *Toxicol Lett, 237*, 151-160.

Marine-Institute. (2014). Azaspiracids. Toxicological evaluation, test methods and identification of the source organisms (ASTOX II). *Marine Research Sub-programme, (NDP 2007-13)*.

Marr, J. C., Hu, T., Pleasance, S., Quilliam, M. A., & Wright, J. L. (1992). Detection of new 7-O-acyl derivatives of diarrhetic shellfish poisoning toxins by liquid chromatography-mass spectrometry. *Toxicon, 30*, 1621-1630.

McMahon, T. S., J. (1996). Winter toxicity of unknown aetiology in mussels. *Harmful algae, 14*, 2.

Moczydlowski, E., Hall, S., Garber, S. S., Strichartz, G. S., & Miller, C. (1984). Voltage-dependent blockade of muscle Na+ channels by guanidinium toxins. *J Gen Physiol, 84*, 687-704.

Moczydlowski, E. G. (2013). The molecular mystique of tetrodotoxin. *Toxicon, 63*, 165-183.

Morohashi, A., Satake, M., Naoki, H., Kaspar, H. F., Oshima, Y., & Yasumoto, T. (1999). Brevetoxin B4 isolated from greenshell mussels Perna canaliculus, the major toxin involved in neurotoxic shellfish poisoning in New Zealand. *Nat Toxins, 7*, 45-48.

Munday, R. (2013). Is protein phosphatase inhibition responsible for the toxic effects of okadaic Acid in animals? *Toxins, 5*, 267-285.

Munday, R. (2014). Toxicology of seafood toxins: a critical review. In L. M. Botana (Ed.), *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection* (3rd ed., pp. 197-290). Boca Ratón: CRC Press.

Munday, R., Thomas, K., Gibbs, R., Murphy, C., & Quilliam, M. A. (2013). Acute toxicities of saxitoxin, neosaxitoxin, decarbamoyl saxitoxin and gonyautoxins 1&4 and 2&3 to mice by various routes of administration. *Toxicon, 76*, 77-83.

Murata, M., Shimatani, M., Sugitani, H., Oshima, Y., & Yasumoto, T. (1982). Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. *Bull. Jpn. Soc. Sci. Fish., 48*, 549-552.

Noguchi, T., Onuki, K., & Arakawa, O. (2011). Tetrodotoxin poisoning due to pufferfish and gastropods, and their intoxication mechanism. *ISRN Toxicol, 2011*, 276939.

Ogino, H., Kumagai, M., & Yasumoto, T. (1997). Toxicologic evaluation of yessotoxin. *Nat. Toxins, 5*, 255-259.

Oshima, Y. (1995). Postcolumn derivatization liquid chromatographic method for paralytic shellfish toxins. *J. AOAC Int., 78*, 528-532.

Payandeh, J., Scheuer, T., Zheng, N., & Catterall, W. A. (2011). The crystal structure of a voltage-gated sodium channel. *Nature, 475*, 353-358.

Perez, S., Vale, C., Botana, A. M., Alonso, E., Vieytes, M. R., & Botana, L. M. (2011). Determination of toxicity equivalent factors for paralytic shellfish toxins by electrophysiological measurements in cultured neurons. *Chem Res Toxicol, 24*, 1153-1157.

Poli, M. A., Mende, T. J., & Baden, D. G. (1986). Brevetoxins, unique activators of voltage-sensitive sodium channels, bind to specific sites in rat brain synaptosomes. *Mol Pharmacol, 30*, 129-135.

Rodriguez, P., Alfonso, A., Vale, C., Alfonso, C., Vale, P., Tellez, A., & Botana, L. M. (2008). First toxicity report of tetrodotoxin and 5,6,11-trideoxyTTX in the trumpet shell Charonia lampas lampas in Europe. *Anal Chem, 80*, 5622-5629.

Roman, Y., Alfonso, A., Louzao, M. C., de la Rosa, L. A., Leira, F., Vieites, J. M., Vieytes, M. R., Ofuji, K., Satake, M., Yasumoto, T., & Botana, L. M. (2002). Azaspiracid-1, a potent, nonapoptotic new phycotoxin with several cell targets. *Cell Signal, 14*, 703-716.

Sala, G. L., Bellocci, M., Callegari, F., & Rossini, G. P. (2013). Azaspiracid-1 inhibits the maturation of cathepsin D in mammalian cells. *Chem Res Toxicol, 26*, 444-455.

Satake, M., Ofuji, K., James, K. J., Furey, A., & Yasumoto, T. (1998). New toxic event caused by Irish mussels. In B. Reguera, J. Blanco & M. L. Fernandez (Eds.), *Harmful Algae, Proceedings of the VIII International Conference on Harmful Algae, June 1999, Vigo, Spain.* (pp. 468-469). Santiago de Compostela: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.

Satake, M., Ofuji, K., Naoki, H., James, K. J., Furey, A., McMahon, T., Silke, J., & Yasumoto, T. (1998). Azaspiracid, a New Marine Toxin Having Unique Spiro Ring Assemblies, Isolated from Irish Mussels, Mytilus edulis. *J. Am. Chem. Soc., 120*, 9967-9968.

Schantz, E. J., McFarren, E. F., Schafer, M. L., & Lewis, K. H. (1958). Purified shellfish poison for bioassay standardization. *J Assoc Off Anal Chem, 41*.

Shimizu, Y., Chou, H. N., Bando, H., Van Duyne, G., & Clardy, J. (1986). Structure of brevetoxin A (GB-1 toxin), the most potent toxin in the Florida red tide organism Gymnodinium breve (Ptychodiscus brevis). *J Am Chem Soc, 108*, 514-515.

Silva, M., Azevedo, J., Rodriguez, P., Alfonso, A., Botana, L. M., & Vasconcelos, V. (2012). New gastropod vectors and tetrodotoxin potential expansion in temperate waters of the Atlantic Ocean. *Mar Drugs, 10*, 712-726.

Solino, L., Sureda, F. X., & Diogene, J. (2015). Evaluation of okadaic acid, dinophysistoxin-1 and dinophysistoxin-2 toxicity on Neuro-2a, NG108-15 and MCF-7 cell lines. *Toxicol In Vitro, 29*, 59-62.

Sommer, H., & Meyer, K. F. (1937). Paralytic shellfish poisoning. *Arch. Path., 24*, 560-598.

Strichartz, G. (1984). Structural determinants of the affinity of saxitoxin for neuronal sodium channels. Electrophysiological studies on frog peripheral nerve. *J Gen Physiol, 84*, 281-305.

Suzuki, H. (2012). Susceptibility of different mice strains to okadaic acid, a diarrhetic shellfish poisoning toxin. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 29*, 1307-1310.

Suzuki, H., & Machii, K. (2014). Comparison of toxicity between saxitoxin and decarbamoyl saxitoxin in the mouse bioassay for paralytic shellfish poisoning toxins. *J Vet Med Sci, 76*, 1523-1525.

Tachibana, K., Scheuer, P. J., Tsukitani, H., Van Engen, D., Clardy, J., Gopichand, Y., & Schmitz, F. J. (1981). Okadaic acid, a cytotoxic polyether from two

marine sponges of the genus a *Halichondria*. *J. Am. Chem. Soc., 103*, 2469-2471.

Takai, A., Bialojan, C., Troschka, M., & Ruegg, J. C. (1987). Smooth muscle myosin phosphatase inhibition and force enhancement by black sponge toxin. *FEBS Lett, 217*, 81-84.

Takemoto, T., & Daigo, K. (1958). Constituents of Chondria armata. *Chem Pharmaceutical Bull., 6*, 578-580.

Templeton, C. B., Poli, M. A., & LeClaire, R. D. (1989). Cardiorespiratory effects of brevetoxin (PbTx-2) in conscious, tethered rats. *Toxicon, 27*, 1043-1049.

Terao, K., Ito, E., Ohkusu, M., & Yasumoto, T. (1993). A comparative study of the effects of DSP-toxins on mice and rats. In T. J. Smayda & Y. Shimizu (Eds.), *Toxic phytoplankton blooms in the sea.* (pp. 581-586). New York: Elsevier Sci. Publishers B.V.

These, A., Klemm, C., Nausch, I., & Uhlig, S. (2011). Results of a European interlaboratory method validation study for the quantitative determination of lipophilic marine biotoxins in raw and cooked shellfish based on high-performance liquid chromatography-tandem mass spectrometry. Part I: collaborative study. *Anal Bioanal Chem, 399*, 1245-1256.

Tillmann, U., Salas, R., Jauffrais, T., Hess, P., & Silke, J. (2014). AZA: The producing organisms-Biology and trophic transfer. In L. M. Botana (Ed.), *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection* (3rd ed., pp. 773-795). Boca Ratón: CRC Press.

Trainer, V. L., Moreau, E., Guedin, D., Baden, D. G., & Catterall, W. A. (1993). Neurotoxin binding and allosteric modulation at receptor sites 2 and 5 on purified and reconstituted rat brain sodium channels. *J Biol Chem, 268*, 17114-17119.

Tripuraneni, J., Koutsouris, A., Pestic, L., De Lanerolle, P., & Hecht, G. (1997). The toxin of diarrheic shellfish poisoning, okadaic acid, increases intestinal epithelial paracellular permeability. *Gastroenterology, 112*, 100-108.

Truelove, J., Mueller, R., Pulido, O., & Iverson, F. (1996). Subchronic toxicity study of domoic acid in the rat. *Food Chem Toxicol, 34*, 525-529.

Tubaro, A., Sosa, S., Bornacin, A., & Jungerford, J. (2008). Pharmacology and toxicology of diarrheic shellifsh toxins. In L. M. Botana (Ed.), *Seafood and Freshwater toxins: Pharmacology, Physiology and Detection* (2nd edition ed., pp. 229-253). Boca Raton, FL: CRC Press (Taylor and Francys Group).

Tubaro, A., Sosa, S., Carbonatto, M., Altinier, G., Vita, F., Melato, M., Satake, M., & Yasumoto, T. (2003). Oral and intraperitoneal acute toxicity studies of yessotoxin and homoyessotoxins in mice. *Toxicon, 41*, 783-792.

Turner, A., Powell, A., Schofield, A., Lees, D., & Baker-Austin, C. (2015). Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014. *Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin, 20*.

Turner, A. D., McNabb, P. S., Harwood, D. T., Selwood, A. I., & Boundy, M. J. (2015). Single-Laboratory Validation of a Multitoxin Ultra-Performance LC-Hydrophilic Interaction LC-MS/MS Method for Quantitation of Paralytic Shellfish Toxins in Bivalve Shellfish. *J AOAC Int, 98*, 609-621.

Twiner, M., Doucette, G. J., Rasky, A., Huang, X. P., Roth, B. L., & Sanguinetti, M. C. (2012). Marine algal toxin azaspiracid is an open-state blocker of hERG potassium channels. *Chem Res Toxicol, 25*, 1975-1984.

Twiner, M., Hanagriff, J. C., Butler, S., Madhkoor, A. K., & Doucette, G. J. (2012). Induction of apoptosis pathways in several cell lines following exposure to the marine algal toxin azaspiracid. *Chem Res Toxicol, 25*, 1493-1501.

Twiner, M., Hess, P., Bottein, M. Y., McMahon, T., Samons, M. S., Satake, M., Yasumoto, T., Ramsdell, J. S., & Doucette, G. J. (2005). Cytotoxic and cytoskeletal effects of azaspiracid-1 on mammalian cell lines. *Toxicon, 45*, 891-900.

Twiner, M., Hess, P., & Doucette, G. J. (2014). Azaspiracids: toxicology, pharmacology and risk assessment. In L. M. Botana (Ed.), *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection* (3rd ed., pp. 824-855). Boca Ratón: CRC Press.

Twiner, M., Ryan, J., Morey, J., Smith, K., Hammad, S., Van Dolah, F., Hess, P., McMahon, T., Satake, M., Yasumoto, T., & Doucette, G. (2008). Transcriptional profiling and inhibition of cholesterol biosynthesis in human T lymphocyte cells by the marine toxin azaspiracid. *Genomics, 91*, 289-300.

Usup, G., Leaw, C. P., Cheah, M. Y., Ahmad, A., & Ng, B. K. (2004). Analysis of paralytic shellfish poisoning toxin congeners by a sodium channel receptor binding assay. *Toxicon, 44*, 37-43.

Vale, C., Alfonso, A., Vieytes, M. R., Romaris, X. M., Arevalo, F., Botana, A. M., & Botana, L. M. (2008). In vitro and in vivo evaluation of paralytic shellfish poisoning toxin potency and the influence of the pH of extraction. *Anal Chem, 80*, 1770-1776.

Vale, C., Nicolaou, K. C., Frederick, M. O., Vieytes, M. R., & Botana, L. M. (2010). Cell volume decrease as a link between azaspiracid-induced cytotoxicity and c-Jun-N-terminal kinase activation in cultured neurons. *Toxicol Sci, 113*, 158-168.

Vale, C., Wandscheer, C., Nicolaou, K. C., Frederick, M. O., Alfonso, C., Vieytes, M. R., & Botana, L. M. (2008). Cytotoxic effect of azaspiracid-2 and azaspiracid-2-methyl ester in cultured neurons: involvement of the c-Jun N-terminal kinase. *J Neurosci Res, 86*, 2952-2962.

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., & Peterson, R. E. (2006). The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci, 93*, 223-241.

Vieira, A. C., Aleman, N., Cifuentes, J. M., Bermudez, R., Pena, M. L., & Botana, L. M. (2015). Brain Pathology in Adult Rats Treated With Domoic Acid. *Vet Pathol, 52*, 1077-1086.

Vieira, A. C., Cifuentes, J. M., Bermudez, R., Ferreiro, S. F., Castro, A. R., & Botana, L. M. (2016). Heart Alterations after Domoic Acid Administration in Rats. *Toxins, 8*, 68; doi:10.3390/toxins8030068.

Vieira, A. C., Rubiolo, J. A., Lopez-Alonso, H., Cifuentes, J. M., Alfonso, A., Bermudez, R., Otero, P., Vieytes, M. R., Vega, F. V., & Botana, L. M. (2013). Oral toxicity of okadaic acid in mice: study of lethality, organ damage,

distribution and effects on detoxifying gene expression. *Toxins, 5*, 2093-2108.

Vilarino, N., Nicolaou, K. C., Frederick, M. O., Vieytes, M. R., & Botana, L. M. (2007). Irreversible cytoskeletal disarrangement is independent of caspase activation during in vitro azaspiracid toxicity in human neuroblastoma cells. *Biochem Pharmacol, 74*, 327-335.

Vlamis, A., Katikou, P., Rodriguez, I., Rey, V., Alfonso, A., Papazachariou, A., Zacharaki, T., Botana, A. M., & Botana, L. M. (2015). First Detection of Tetrodotoxin in Greek Shellfish by UPLC-MS/MS Potentially Linked to the Presence of the Dinoflagellate Prorocentrum minimum. *Toxins, 7*, 1779-1807.

Vranyac-Tramoundanas, A., Harrison, J. C., Sawant, P. M., Kerr, D. S., & Sammut, I. A. (2011). Ischemic cardiomyopathy following seizure induction by domoic Acid. *Am J Pathol, 179*, 141-154.

Walker, J. R., Novick, P. A., Parsons, W. H., McGregor, M., Zablocki, J., Pande, V. S., & Du Bois, J. (2012). Marked difference in saxitoxin and tetrodotoxin affinity for the human nociceptive voltage-gated sodium channel (Nav1.7) [corrected]. *Proc Natl Acad Sci U S A, 109*, 18102-18107.

Wang, J., Wang, Y. Y., Lin, L., Gao, Y., Hong, H. S., & Wang, D. Z. (2012). Quantitative proteomic analysis of okadaic acid treated mouse small intestines reveals differentially expressed proteins involved in diarrhetic shellfish poisoning. *J Proteomics, 75*, 2038-2052.

Watanabe, R., Suzuki, T., & Oshima, Y. (2010). Development of quantitative NMR method with internal standard for the standard solutions of paralytic shellfish toxins and characterisation of gonyautoxin-5 and gonyautoxin-6. *Toxicon, 56*, 589-595.

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., & Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogs. *Mar Drugs, 8*, 2185-2211.

Wingerd, J. S., Vetter, I., & Lewis, R. J. (2012). Voltage-gated sodium channels as therapeutic targets. In L. M. Botana & M. I. Loza (Eds.), *Therapeutic targets. Modulation, inhibition and activation.* (pp. 63-122). Hoboken, NJ: John Wiley & Sons.

Yang, L., Kao, C. Y., & Oshima, Y. (1992). Actions of decarbamoyloxysaxitoxin and decarbamoylneosaxitoxin on the frog skeletal muscle fiber. *Toxicon, 30*, 645-652.

Yasumoto, T., & Murata, M. (1990). Polyether toxin involved in seafood poisoning. In S. Hall & G. Stritchartz (Eds.), *Marine toxins* (pp. 120-132). Washington D.C.: American Chemical Society.

Yasumoto, T., Murata, M., Oshima, Y., Matsumoto, G. K., & Glardy, J. (1984). Diarrhetic shellfish poisoning. In E. P. Ragelis (Ed.), *ACS Symp. Series. No 262. Seafood toxins.* (pp. 207-214). Washington, D.C.: American Chemical Society.

Yasumoto, T., Murata, M., Oshima, Y., & Sano, M. (1985). Diarrhetic Shellfish Toxins. *Tetrahedron, 41*, 1019-1025.

Yasumoto, T., Oshima, Y., & Yamaguchi, M. (1978a). Occurrence of a new type of toxic shellfish in Japan and chemical properties of the toxin. In D. L. Taylor & H. H. Seliger (Eds.), *Dinoflagellate Blooms* (pp. 1249-1255). Amsterdam: Elsevier/North Holland.

Yasumoto, T., Oshima, Y., & Yamaguchi, M. (1978b). Ocurrence of a new type of shellfish poisoning in the Tohoku Distric. *Bull. Jpn. Soc. Sci. Fish., 44*, 1249-1255.

Yasumoto, T., Yotsu-Yamashita, M., Murata, M., & Naoki, H. (1988). New tetrodotoxin analogues from the newt Cynops ensicaudata. *J. Am. Chem. Soc., 110*.