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Review

Tetramine in the Salivary Glands of Marine Carnivorous Snails: Analysis, Distribution, and Toxicological Aspects

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Abstract: Focusing on tetramine, tetramethylammonium ion, contained in the salivary glands of marine carnivorous snails, this paper gives an overview of analytical methods, distribution in marine snails, and toxicological aspects. Some *Neptunea* snails have often caused food poisoning in North Atlantic and Northeast Asia regions, especially in Japan. The toxin of both *N. arthritica* and *N. antiqua* was first proven to be tetramine in 1960. Subsequent research on marine snail tetramine has progressed with the development of analytical methods. Of the various methods developed, the LC/ESI-MS method is most recommended for tetramine analysis in terms of sensitivity, specificity, and versatility. Accumulated data show that tetramine is ubiquitously contained at high concentrations (usually several mg/g) in the salivary glands of *Neptunea* snails. Tetramine is also found in the muscle and viscera of *Neptunea* snails and even in the salivary gland of marine snails other than *Neptunea* species, although mostly at low levels (below 0.1 mg/g). Interestingly, the major toxin in the salivary glands of *Fusitriton oregonensis* and *Hemifusus tuba* is distinguishable from tetramine. In tetramine poisoning, diverse symptoms attributable to the ganglion-blocking action of tetramine, such as visual disturbance, headache, dizziness, abdominal pain, and nausea, develop within 30 min after ingestion of snails because of rapid absorption of tetramine from the gastrointestinal tract. The symptoms are generally mild and subside in a short time (within 24 at most) because of rapid excretion through the kidney. However, it should be kept in mind that tetramine poisoning can be severe in patients with kidney dysfunction, as shown by two recent case reports. Finally, given the diffusion of tetramine from the salivary gland to the muscle during boiling and thawing of snails, removal of salivary glands from live snails is essential to avoid tetramine poisoning.

Keywords: marine snail; *Neptunea*; salivary gland; tetramine; toxin

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1. Introduction

A variety of toxins are distributed in marine carnivorous snails [1]. Some of them, such as tetrodotoxin [2–4] and surugatoxins (neosurugatoxin and prosurugatoixn) [5–7], are exogeneous. Apart from the exogeneous toxins, endogenous toxins are present in the venom glands, hypobranchial glands, or salivary glands of marine carnivorous snails. The most extensively studied endogenous toxins are conotoxins (or conopeptides), cysteine-rich neurotoxic peptides, found in the *Conus* venom glands [8–11]. Conotoxins are a treasure trove of new drugs and indeed ω-MVIIA (ziconotide) [12] from the venom of *C. magus* has been clinically used to treat chronic pain in serious cancer and AIDS patients [13,14]. Muricidae and other neogastropod species contain choline ester toxins, such as murexine (urocanylcholine) [15,16] and senecioylcholine [16,17], in the hypobranchial glands. As a hypobranchial gland toxin, a K channel inhibitor (6-bromo-2-mercaptotryptamine) is also known from *Calliostoma canaliculatum*, a member of the family Calliostomatidae [18]. However, hypobranchial gland toxins have not received much attention, probably because there seems to be no mechanism to release the toxins from the glands.

As for salivary gland toxins of marine snails, two classes of toxins, echotoxins and tetramine, have so far been well-characterized. Echotoxins, 25 kDa hemolytic proteins, which were purified from the highly toxic salivary gland of *Monoplex parthenopeus* (formerly

Monoplex echo) belonging to the family Ranellidae [19,20], are similar in primary structure to actinoporins, pore-forming cytolysins from sea anemones [21,22]. On the other hand, tetramine, tetramethylammonium ion $(CH_3)_4N^+$, which is mainly contained at high levels in the salivary glands of *Neptunea* snails belonging to the family Buccinidae, is a very simple compound. Of the endogenous toxins in marine carnivorous snails, tetramine is the sole toxin implicated in food poisoning, but the symptoms induced are usually mild and subside in a short time (within 24 h at most). Thus, tetramine in marine snails has attracted little attention of researchers, such as natural products chemists and toxicologists. Reflecting on this situation, there has been no review article focusing on tetramine in marine snails for more than 30 years since that of Anthoni et al. [23] published in 1989, although tetramine has been only briefly mentioned in some reviews [1,24–27] on marine toxins or mollusk toxins. However, it is worth mentioning that two serious cases of tetramine poisoning in patients with kidney dysfunction have recently been reported [28,29]. These case reports led us consider that it is timely to summarize the current findings on snail tetramine to inform researchers, clinicians, and consumers that tetramine poisoning cannot be underestimated. This review deals with analytical methods, distribution, and toxicological aspects of marine snail tetramine. The taxonomy of gastropods has been significantly revised since the 1990s. It should be noted that some of the scientific names described in this review are different from those in the original papers, since the taxonomy follows the World Register of Marine Species (WoRMS, https://www.marinespecies.org/, accessed on 10 November 2021).

2. Anatomical Descriptions of Salivary Glands of *Neptunea* Species

Both primary and accessary salivary glands are present in gastropods of the order Neogastropoda [30]. In a number of families of Neogastropoda, however, the accessary salivary glands are reduced to a single gland or absent [31]. This is the case with the family Buccinidae including *Neptunea* snails, which possess only a pair of primary salivary glands. In Figure 1, pictures of the shell and soft tissue of *Neptunea arthritica* are shown, a representative toxic species. A pair of yellowish salivary glands can be seen under the mantle. Two salivary ducts, one from each gland, run along the esophagus until opening into the roof of the buccal cavity [30]. Tetramine produced in the salivary gland is delivered through the ducts to the mouth, where it presumably acts to paralyze prey animals. Alternatively, tetramine may be secreted into the surrounding water, functioning as a defensive substance against potential predators [32]. In this way, tetramine is generally considered to play an active role as a toxic component. However, the salivary gland lacks the musculature required for the rapid ejection of saliva containing tetramine. This may imply that tetramine is simply a toxic by-product of metabolism [24,32]. The exact function of tetramine in *Neptunea* snails awaits future study.

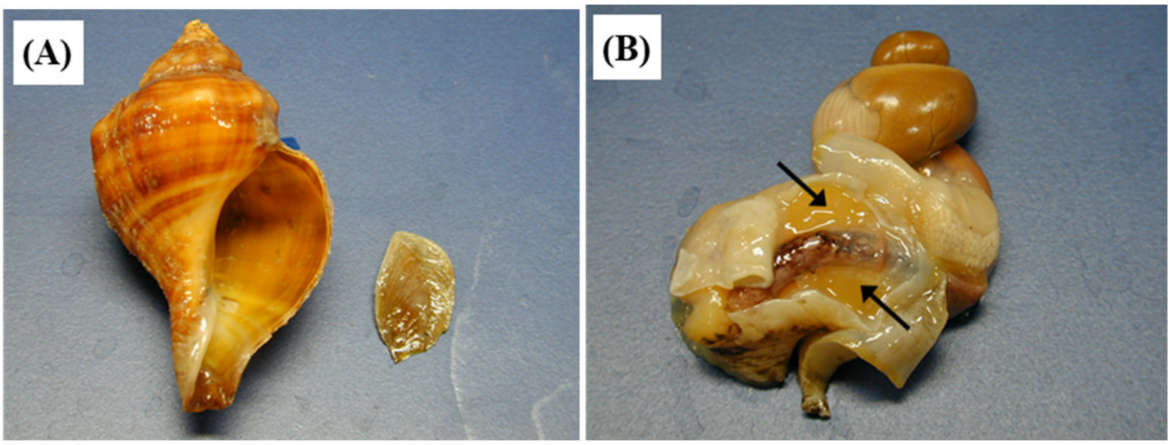

Figure 1. Pictures of the shell and operculum (**A**) and soft tissue (**B**) of *Neptunea arthritica*. Note that in (**B**), the mantle has been cut open to indicate the location (shown by arrows) of the salivary glands.

The salivary glands of *Neptunea* snails are quite large, reaching ~10 g for large species (e.g., *N. polycostata* with a shell height of ~20 cm), ~5 g for medium-sized species (e.g., *N. intersculpta* with a shell height of ~15 cm), and ~3 g for small species (e.g., *N. arthritica* with a shell height of ~10 cm). The weight ratio of the salivary gland to the soft tissue is reported to be 3–5% for *N. antiqua* [33] and similar values can be calculated for other *Neptunea* snails from the data presented in the papers: for example, 3.9–9.1% for *N. intersculpta* [34] and 3.3–6.7% for *N. polycostata* [35].

3. Analytical Methods

In the early stages of the study, tetramine was analyzed by classical methods, i.e., paper chromatography, thin layer chromatography, and paper electrophoresis [33,36–38]. Needless to say, these methods lack specificity and are low in sensitivity. Since then, the following analytical methods for tetramine have been developed one after another: bioassays using mice and killifish [39], spectrometric analysis using an ion-paring reagent [40], ion chromatography with conductivity detection [41,42], liquid chromatography with refractive detection [43], proton nuclear magnetic resonance spectroscopy [44], capillary zone electrophoresis/tandem mass spectrometry (CZE/MS/MS) [45], and liquid chromatography/electrospray ionization-single quadrupole mass spectrometry (LC/ESI-MS) [46]. Among these methods, CZE/MS/MS and LC/ESI-MS are excellent in both specificity and sensitivity. As a separation technique, LC is much more common than CZE in many laboratories. Accordingly, the LC/ESI-MS method developed by our research group [46] is recommended for specific and sensitive analysis of tetramine.

The established analytical conditions of LC/ESI-MS for tetramine are summarized in Table 1. The sample solution for analysis can be easily prepared by extracting each tissue sample with methanol, followed by defatting with hexane. At a cone voltage of 30 V, the molecular ion (*m/z* 74) showed maximum intensity and no fragment ions were substantially produced. With the increase of the cone voltage, two fragment ions, *m/z* 58 ion corresponding to $CH_2{=}N^+(CH_3)_2$ and *m/z* 42 ion to $CH_2{=}N^+{=}CH_2$, became abundant and showed the maximum intensity at 70 and 110 V, respectively. These fragment ions are useful for the identification and quantification of tetramine, especially in the LC/MS/MS system [47]. As a typical example, the LC/ESI-MS chromatogram of the sample solution prepared from the salivary gland of *Neptunea polycostata* is shown in Figure 2. It is worth mentioning that besides tetramine, three trimethylated compounds, glycine betaine $(CH_3)_3N^+CH_2COOH$, trimethylamine oxide $(CH_3)_3NO$, and choline $(CH_3)_3N^+CH_2CH_2OH$, which are widely found in biological samples, can be analyzed by monitoring molecular ions (*m/z* 118 for glycine betaine, *m/z* 76 for trimethylamine oxide, and *m/z* 106 for choline). Indeed, Figure 2 shows that glycine betaine and choline, together with tetramine, are contained in the sample solution, although trimethylamine oxide is absent.

Table 1. Analytical conditions of LC/ESI-MS for tetramine [46].

LC	Column	Nucleosil 100-10SA (0.46 × 25 cm, Macherey-Nagel)
	Injection volume	10 μL
	Eluent	0.03 M pyridine-formic acid buffer (pH 3.1) containing 20% methanol
	Flow rate	1 mL/min
MS	Ionization	Electrospray ionization
	Polarity	Positive
	Monitor ion	*m/z* 74 (molecular ion)
	Cone voltage	30 V

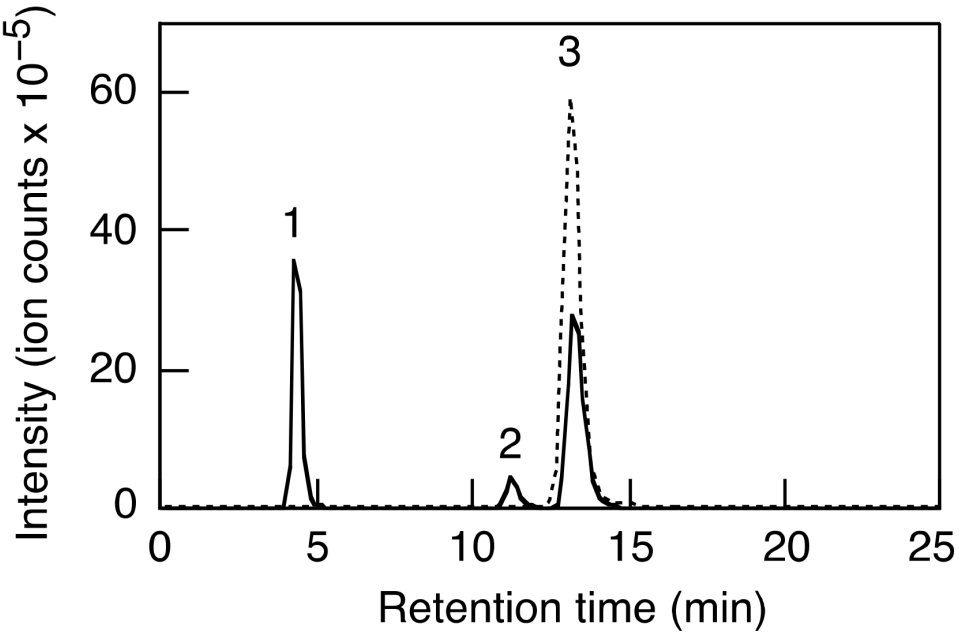

Figure 2. LC/ESI mass chromatogram of the sample solution (methanolic extract) prepared from the salivary gland of *Neptunea polycostata*. This figure corresponds to Figure 4a in the paper of Kawashima et al. [46]. Solid line: sample solution. Broken line: sample solution spiked with tetramine. Monitored at 30 V for *m/z* 118 (glycine betaine, 1), *m/z* 76 (trimethylamine oxide), *m/z* 104 (choline, 2), and *m/z* 74 (tetramine, 3). Note that there is no peak of *m/z* 76 to be observed at a retention time of 9.4 min, because of the absence of trimethylamine oxide in the sample.

The detection limit of our LC/ESI-MS method is equivalent to as low as 10 ng/g of tissue (*S/N* = 3). As described later, low concentrations of tetramine derived from the salivary gland are found in the broth after boiling of *Neptunea* snails [42,46,47]. Although the broth may be the only material to be analyzed in some cases of tetramine poisoning, our sensitive determination method makes it possible to directly analyze low concentrations of tetramine in the broth, leading to the rapid identification of tetramine as the causative toxin.

4. Distribution in Marine Snails

4.1. Marine Snails Containing High Amounts of Tetramine

Tetramine was first found in the sea anemone *Actinia equina* [48] and later in some cnidarians, such as sea anemone *Condylactis gigantea* and jellyfish *Physalia physalis* [49]. Initially, tetramine was considered to function as a major toxin in sea anemones, but it was later proved that sea anemone toxins are neurotoxic peptides [50] and cytolytic proteins [51]. Therefore, little attention has been paid by scientists to tetramine contained in cnidarians.

Study on tetramine in marine carnivorous snails was initiated in the 1950s by two research groups in Japan [37,38,52] and Norway [33,36]. In Hokkaido, Japan, food poisoning due to ingestion of the marine snail *Neptunea arthritica*, which is called 'nemuri-tsubu' (sleeping snail) since humans become sleepy when poisoned, occasionally occurred [52]. In this regard, Asano [52] demonstrated that the toxin of *N. arthritica* displaying mouse lethality is located in the salivary gland. Emmelin and Fänge [36] independently studied the salivary gland toxin of the red whelk *Neptunea antiqua* inhabiting the Northeast Atlantic Ocean and suggested that the toxin is neurin, trimethylvinylammonium ion $(CH_3)_3N^+CH{=}CH_2$, or some other quaternary ammonium compound. Shortly after this study, Asano and Itoh [38] purified the toxin of *N. arthritica* as picrate and clearly identified it as tetramine by combustion analysis, the melting point, and the infrared spectrum. In addition, they suggested the occurrence of high amounts of tetramine in the salivary glands of two other

marine snails, *Neptunea intersculpta* and *Fusitriton oregonensis*. Agreeing with these results, Fänge [33] also concluded that the toxin of *N. antiqua* is not neurin but tetramine.

Subsequently, tetramine has been detected in the salivary glands of various species of marine snails (Table 2), owing to the advances in analytical methods. Notably, all of the 14 species of *Neptunea* snails so far examined were found to contain significant levels of tetramine (several mg/g, except for the slightly lower content of 0.91–0.94 mg/g in *N. frater*), strongly suggesting the ubiquitous distribution of high levels of tetramine in the salivary glands of *Neptunea* snails. On the other hand, no high levels of tetramine have been detected in the salivary glands of buccinid species other than *Neptunea* snails, although the concentration of 0.45 mg/g determined for one specimen of *Buccinum middendorffii* was rather high. Besides the *Neptunea* snails, two species of snails also contain high amounts of tetramine in the salivary gland; one is *Fusitriton oregonensis* belonging to the family Ranellidae of the order Littorinimorpha and the other is *Hemifusus tuba* belonging to the family Melongenidae of the order Neogastropoda. The high concentrations of tetramine in the salivary glands of these two species are discussed in detail later.

Interestingly, Anthoni et al. [53] suggested that tetramine is contained not only in the salivary gland but also in other tissues (muscle, mid-gut gland, and viscera). This suggestion was confirmed with four species of *Neptunea* snails, *N. arthritica* [46], *N. intersculpta* (including *N. constricta*, a synonym of *N. intersculpta*) [34,47], *N. lamellose* [34], and *N. polycostata* [46], and even with *Buccinum middendorffii* [46]. The determined tetramine contents in the muscle, mid-gut gland, and viscera of *Neptunea* snails were less than 0.01 mg/g in many specimens. However, high concentrations of 0.18 and 0.34 mg/g were detected in the muscles of each specimen of *N. arthritica* and *N. lamellose*, respectively. The threshold for the amount of tetramine that causes poisoning in adults was estimated to be 10 mg [42,47]. Therefore, for muscles with tetramine concentrations of 0.18 and 0.34 mg/g, the threshold is reached with intakes of 55.6 and 29.4 g of muscle, respectively. Although specimens with a tetramine concentration of 0.1 mg/g or higher in the muscle may be rare, we should be careful not to overeat to avoid poisoning. It should also be pointed out that the presence of high levels of tetramine not only in the salivary gland but also in the muscle may cast doubt on the hypothesis that *Neptunea* snails carry tetramine to paralyze prey animals and/or protect against predators.

4.2. Seasonal Variation of Tetramine Concentration

Asano and Itoh [38] observed that the mouse toxicity (which can be assumed to be proportional to tetramine content) of the *Neptunea arthritica* salivary gland extract fluctuates a little throughout the year but shows no marked seasonal variation. Similarly, no seasonal variation in the tetramine concentration in the salivary gland was reported for *N. polycostata* [34]. However, opposite results have been obtained with *Neptunea antiqua*. Power et al. [32] analyzed 20 samples of *N. antiqua* from the Irish Sea each month from December 1997 to October 1998 and showed that the tetramine concentration in the salivary gland was insignificant (about 0.2 mg/g) in February and increased progressively to reach the highest value (more than 5 mg/g) in October. Based on this distinct seasonal variation in the tetramine concentration and the spawning season (from late spring to early summer) known for *N. antiqua* from the Irish Sea [54], it was speculated by Power et al. [32] that *N. antiqua* does not require high concentrations of tetramine to paralyze prey animals in spring, as the snail ceases to feed at the onset of the breeding season.

Table 2. Tetramine contents in the salivary glands of marine snails.

Order	Family	Species	Tetramine Content in Salivary Gland (mg/g)	Reference
Caenogastropoda	Batillariidae	*Batillaria multiformis*	<0.01	[55]
Littorinimorpha	Naticidae	*Neverita didyma*	<0.01	[55]
	Charoniidae	*Charonia lampas*	0.003–0.031	[19]
	Ranellidae	*Monoplex parthenopeum*	<0.01	[55]
		Fusitriton oregonensis	0.064–4.0	[35,38,56]
		Fusitriton galea	0.01	[55]
Neogastropoda	Austrosiphonidae	*Kelletia lischkei*	0.01	[55]
	Buccinidae	*Buccinum aniwanum*	0.0007	[56]
		Buccinum bayani	<0.01	[34]
		Buccinum inclytum	0.00294–0.00340	[56]
		Buccinum leucostoma	<0.01	[34]
		Buccinum middendorffi	0.0012–0.45	[46,55,56]
		Buccinum mirandum	0.04	[55]
		Buccinum opisoplectum	0.1	[55]
		Buccinum striatissimum	0.03–0.05	[55]
		Buccinum tenuissimum	0.0299–0.186	[56]
		Buccinum tsubai	<0.01	[34]
		Buccinum verkruzeni	<0.01	[34]
		Neptunea amianta	11.81	[55]
		Neptunea antiqua	0.75–4.476	[32,33]
		Neptunea arthritica	0.85–12	[34,35,38,40,41,46,55]
		Neptunea cumingii	6.3–15	[57]
		Neptunea decemcostata	1.28	[46]
		Neptunea frater	0.91–0.94	[56]
		Neptunea heros	1.95–3.73	[56]
		Neptunea intersculpta *	0.17–9.75	[34,38,40,41,43,47]
		Neptunea kuroshio	2.67–3.58	[40]
		Neptunea lamellosa	0.27–9.41	[34,53,56]
		Neptunea lyrata	0.64–14.8	[19,58]
		Neptunea polycostata	0.16–4.9	[34,35,46,56]
		Neptunea purpurea	1.72–7.4	[56]
		Neptunea vinosa	0.373–6.96	[55,56]
		Japeuthria ferrea	0.05	[56]
		Siphonalia cassidariaeformis	0.117–0.135	[56]
		Siphonalia fusoides	0.204	[56]
	Fasciolariidae	*Leucozonia smaragdula*	0.08	[56]
		Fusinus forceps salisburyi	0.0675	[56]
	Melongenidae	*Hemifusus tuba*	4.5–8.8	[55]
	Muricidae	*Drupa rubisidaeus*	0.19	[55]
		Mancinella siro	0.42	[55]
		Rapana venosa	0.0057–0.04	[19,55,56]
		Reishia bronni	0.09	[55]
	Babyloniidae	*Babylonia japonica*	0.08–0.13	[55]
		Babylonia zeylanica	0.25	[55]
	Turbinellidae	*Vasum ceramicum*	<0.01	[55]

* The data for *Neptunea constricta*, a synonym of *Neptunea intersculpta*, are included in those for *N. intersculpta*.

Seasonal variation in the tetramine content in the salivary gland was also observed with *Neptunea intersculpta*, although not as pronounced as that seen in *N. antiqua*. The tetramine concentrations of *N. intersculpta* reported by Hashizume et al. [43] were 6.03–6.59 mg/g (three specimens) in May and 3.86–4.05 mg/g (three specimens) in October while those reported by Kim et al. [47] were 5.1–8.5 mg/g (three specimens) in April and 0.17–1.1 mg/g (three specimens) in December. The spawning season of *N. intersculpta* is unknown but is assumed to be the same as that (between March and August) reported for *N. polycostata* [58,59], which lives in the same area as *N. intersculpta*. If so, the tetramine content of *N. intersculpta* is high during the spawning season and then decreases, which

is the opposite of the tendency seen in *N. antiqua*. In the case of *N. intersculpta*, only three individuals were determined for tetramine only twice a year. To verify the interesting speculation of Power et al. [32] that high levels of tetramine are not needed during the spawning season, seasonal variations in the tetramine content in the salivary glands of *N. intersculpta* and other *Neptunea* snails need to be investigated in more detail.

4.3. Tetramine in Fusitriton oregonensis and Hemifusus tuba

The detected high concentrations of tetramine in two species, *Fusitriton oregonensis* and *Hemifusus tuba*, may need to be reexamined. In the case of *F. oregonensis*, Asano and Ito [38] reported that 3–4 mg/g of tetramine is contained in the salivary gland based on the color intensity developed with Dragendorff reagent following paper chromatography of the salivary gland extract. They also described that the color developed for *F. oregonensis* with Dragendorff reagent differs somewhat from that for two species of *Neptunea* snails (*N. arthritica* and *N. intersculpta*), indicating that the toxin of *F. oregonensis* is distinguishable from tetramine. Nevertheless, the toxin of *F. oregonensis* has not been studied further and thus believed to be tetramine for many years. In 2001, about 40 years after the report of Asano and Ito [38], Tazawa et al. [35] determined tetramine in the salivary glands of *F. oregonensis* and two species of *Neptunea* snails (*N. arthritica* and *N. polycostata*) by two methods, mouse bioassay and ion chromatography, and provided interesting results. For the two species of *Neptunea* snails, both analytical methods afforded almost the same contents of tetramine (around 1.0 mg/g). In the case of *F. oregonensis*, however, the tetramine content measured by ion chromatography was only 0.064 mg/g (about one-20th that of the *Neptunea* species) while the estimated mouse toxicity was about 40 times higher than that of the *Neptunea* species. Furthermore, Yoshinaga-Kiriake et al. [56] recently quantified tetramine in the salivary glands of a number of marine snails by LC/MS/MS, the most reliable analytical method. According to their results, the tetramine contents quantified for the salivary glands of two specimens of *F. oregonensis* were 0.216 and 0.545 mg/g, being rather low compared to the values previously reported by Asano and Ito [38]. Considering these results comprehensively, *F. oregonensis* is likely to contain an unknown toxin with potent mouse lethality besides a small amount of tetramine.

As for *Hemifusus tuba*, the salivary glands of two specimens were found to be toxic to mice and contain high levels of tetramine (4.5 and 8.3 mg/g) in the course of our screening for toxins in the salivary glands of marine snails [55]. However, the estimated mouse toxicity was about one-fourth that of *Neptunea arthritica*, *Neptunea lamellose*, and *Neptunea vinosa*, in which almost the same level of tetramine as in *H. tuba* was detected. In view of the fact that the colorimetric method [40] used for the quantification of tetramine is low in specificity, there are two possibilities for the salivary gland toxin of *H. tuba*. One possibility is that a colorimetrically positive but non-toxic substance coexists with tetramine. Another possibility is that *H. tuba* lacks tetramine but instead contains an unknown toxic substance that is colorimetrically positive. In order to confirm which of these possibilities is correct, it is necessary to quantify tetramine in the salivary glands of many specimens using a more specific method (e.g., LC/MS method) than the colorimetric method.

5. Pharmacological Properties

The pharmacological properties of tetramine are detailed in the review of Anthoni et al. [23]. Although the review was published in 1989, its content is still valid. In this paper, therefore, the pharmacological properties of tetramine are only briefly described.

5.1. Absorption, Distribution, and Excretion

Based on the study with the rat jejunum, Tsubaki and Kamoi [60] reported that tetramine is rapidly and almost completely absorbed through the intestinal tract. They also showed that the absorption of tetramine from the intestinal tract is attributable to a carrier transport system as well as simple diffusion. As stated by Anthoni et al. [23], orally ingested tetramine would be absorbed from the intestinal tract within 1 h, although the concomitant

ingestion of food and water, together with the vomit reflex induced by tetramine, will reduce the rate of absorption. Tetramine thus absorbed from the intestinal tract is assumed to rapidly distribute in the entire body with a significantly elevated concentration in the liver, kidney, and urine, from the results with intraperitoneally injected mice [61] and intravenously injected rats [62]. As for the excretion of tetramine, Neef et al. [62] clarified that the only important excretory pathway is through the kidney; more than 95% of tetramine is excreted through the kidney. Their results also suggested the excretion to be a combination of glomerular filtration and carrier-mediated secretion. Importantly, tetramine is chemically unchanged in the process from absorption through the intestinal tract to excretion through the kidney [23].

5.2. Toxicity

Tetramine is similar in chemical structure to acetylcholine. Therefore, it can bind to acetylcholine receptors, thereby acting as a ganglionic blocking agent that inhibits synaptic transmission [23,63]. It induces a long-lasting depolarization blockade in autonomic nervous systems and ultimately leads to flaccid paralysis of skeletal muscle. The symptoms observed in tetramine poisoning can be mostly explained by the peripheral action of tetramine. However, some poisoning symptoms, such as headache and dizziness, indicate an action on the central nervous system. Whether or how tetramine can cross the blood–brain barrier is an important issue that remains to be addressed in the future.

When injected into mice, cats, and fish, extracts from the salivary glands of *Neptunea* snails can evoke fasciculation, convulsion, motor paralysis, and finally respiratory failure leading to death [23]. The respiratory failure developed by tetramine is likely to be related to the paralysis of respiratory muscles because of its ganglionic blocking effects [63]. For reference, the LD_{50} or lethal doses of tetramine to experimental animals are shown in Table 3. Taking into account the known differences in sensitivity to depolarizing agents between animals and humans, the lethal dose of tetramine for an adult human was estimated to be 250–1000 mg [23]. So far, there have been no deaths from tetramine poisoning due to ingestion of the salivary gland of marine snails focused on in this paper. The only fatal case of tetramine poisoning (two women died) has been caused in Sudan by ingestion of the root of the medicinal plant *Courbonia virgata*, a member of the family Capparidaceae [64].

Table 3. Lethal dose or LD_{50} of tetramine to experimental animals.

Experimental Animal	Route	Lethal Dose or LD_{50} (mg/kg)	Reference
Rat	Oral	45–50 *1	[53]
	Intraperitoneal	15 *1	[53]
Mouse	Oral	16 *2	[65]
	Intraperitoneal	11 *2	[65]
	Subcutaneous	7.4–14.7 *3	[64]

*1 Lethal dose. *2 LD_{50} calculated from the data for tetramine chloride. *3 Lethal dose calculated from the data for tetramine iodide.

6. Food Poisoning

6.1. Occurrence Situation

The red whelk *Neptunea antiqua*, which inhabits cold waters of the Northeast Atlantic Ocean, is one of the first gastropods established to contain high amounts of tetramine in the salivary gland [33]. Nevertheless, only several cases of tetramine poisoning due to ingestion of *N. antiqua* have so far been recorded in the United Kingdom [66,67] and Denmark [53]. Two species of whelks, *Neptunea decemcostata* and *Neptunea despecta tornata*, inhabiting the Northwest Atlantic Ocean, are implicated in tetramine poisoning in Atlantic Canada, although not so common [45,68]. Due to the low catch of *Neptunea* snails inhabiting the North Atlantic Ocean, they are usually sold at local stalls and fish mongers rather than at markets. This situation seems to explain why food poisoning incidents due to ingestion of *Neptunea* snails are not frequent in Europe and Canada.

On the other hand, tetramine poisoning by *Neptunea* species is very common in the Northeast Asia regions. In Japan, various species of large snails, particularly buccinid snails including *Neptunea* species, are widely distributed on the market under the generic name of "tsubu", "tsubu-gai", or "bai-gai" and are eaten raw (sashimi and sushi) or boiled. According to the food poisoning incidents compiled by the Ministry of Health, Labor and Welfare of Japan, as many as 72 incidents (154 patients and no deaths) of tetramine poisoning occurred in Japan over the last 20 years from 2001 to 2020 (Table 4). Of the food poisoning caused by natural animal toxins (720 incidents, 1273 patients, and 30 deaths) that occurred from 2001 to 2020, tetramine poisoning accounted for 10%, being ranked third after puffer fish poisoning (517 incidents, 729 patients, and 27 deaths) and ciguatoxic fish poisoning (85 incidents, 272 patients, and no deaths). *Neptunea* snails are usually used as side dishes for alcoholic drinks. As described in detail below, some symptoms (e.g., headache, dizziness, and sleepiness) in tetramine poisoning resemble those when drunk. It is thus presumed that many people, even if they are poisoned by snail tetramine, think that they got drunk earlier than usual and do not report the food poisoning to public authorities. Actual tetramine poisoning cases are likely to be significantly higher than the statistical data.

Table 4. Incidence of tetramine poisoning caused by marine gastropods in Japan over the last 20 years (cumulative 2001–2020).

Causative Gastropod	No. of Incident	No. of Patient
Neptunea intersculpta *	23	54
Neptunea arthritica	13	20
Neptunea arthritica and *Neptunea bulbacea*	1	2
Neptunea intersculpta or *Neptunea amianta*	1	1
Neptunea polycostata	3	10
Neptunea lamellose	3	8
Fusitriton oregonensis	2	17
Neptunea bulbacea	1	2
Unidentified (possibly *Neptunea* species)	25	40
Total	72	154

* Four incidents (10 patients) by *Neptunea constricta*, a synonym of *Neptunea intersculpta*, are included in incidents by *N. intersculpta*.

Very interestingly, *Fusitriton oregonensis*, the major toxin of which is assumed to differ from tetramine as described above, has caused two cases of poisoning (Table 4). For risk assessment of *F. oregonensis*, elucidation of its major toxin is urgently needed. Except for the two cases of poisoning by *F. oregonensis*, all were caused by *Neptunea* species, among which *N. intersculpta* (responsible for 23 cases) and *N. arthritica* (responsible for 13 cases) are particularly important, being implicated in half of all poisoning cases. It is interesting to note that the causative species of about one-third of poisoning cases are unknown. This is because no shells of poisoned snail were left or because it was difficult for non-specialists to accurately identify the species of *Neptunea* snails.

Tetramine poisoning was previously prevalent in northern Japan (Hokkaido and Tohoku regions), as many edible *Neptunea* snails live in cold waters. However, tetramine poisoning has recently become nationwide owing to improvements in distribution technology. In fact, 47 of the 72 cases of tetramine poisoning between 2001 and 2020 occurred outside of northern Japan. It is also worth mentioning that as many as 69 of the 72 cases occurred at home. This high incidence at home is probably due to many consumers being unaware of the toxicity of the salivary glands of *Neptunea* snails and not removing the glands. Additionally, even if consumers knew that the salivary gland is toxic, they may have removed the salivary glands not before but after boiling of the snails because they

did not know that the toxic component (tetramine) could be transferred from the salivary gland to the muscle when boiled.

6.2. General Symptoms

There have been several reports on symptoms observed in tetramine poisoning following ingestion of *Neptunea* snails (*N. antiqua* [66,67], *N. arthritica* [52], and *N. intersculpta* [47]). Regardless of the snail species, similar neurological and gastrointestinal symptoms are described in these reports. According to the most detailed report by Kim et al. [47], who interviewed 17 patients (48–80 years old) involved in mass food poisoning by *N. intersculpta* in Korea, the patients exhibited the following 15 different clinical symptoms (the number in each parenthesis is that of patients who showed the symptom): eyeball pain (17), severe headache (17), dizziness (17), abdominal pain (17), nausea (17), facial fever (16), diplopia (14), wobbling gait (12), amblyopia (9), sleepiness (9), neck stiffness (6), tingling of hands and feet (5), paralysis of arms and legs (4), vomiting (2), and urticaria (1). None of the patients suffered from diarrhea, similar to the report of Fleming [66] but dissimilar to those of Reid et al. [67] and Yeo and Lim [29].

Symptoms of tetramine poisoning develop 30–60 min after ingestion of snails because of the rapid absorption of tetramine from the gastrointestinal tract and disappear within several hours (at latest within 24 h) because of rapid excretion of tetramine through the kidney. After recovery, no particular long-term complications are observed. Thus, the symptoms of tetramine poisoning are generally mild and require little hospitalization.

6.3. Serious Symptoms in Patients with Kidney Dysfunction

Although the symptoms observed in tetramine poisoning are generally mild, two severe cases (cases 1 and 2) have been reported in patients with kidney dysfunction [28,29], as described below.

Case 1 [28]: This case was observed in a 60-year-old man with end-stage renal disease caused by diabetic nephropathy. As shown in Figure 3A, the evening before he visited the hospital, he ate eight boiled snails (probably *Neptunea arthritica*). He went to bed as usual, but the next morning he awoke with nausea, drowsiness, dyspnea, limb weakness, facial palsy, and diplopia. He could not even raise his head or get out of bed. It is very interesting that unlike usual tetramine poisoning, the incubation time (time from snail ingestion to onset) was as long as 12 h. In this regard, it was speculated that tetramine absorption may have been delayed because of diabetic gastropar. Of the symptoms observed when waking up, nausea, drowsiness, and dyspnea subsided in about 1 h while the others continued for 8 h until hemoperfusion was started. At the hospital, the patient underwent hemoperfusion, followed by hemodialysis. He was able to maintain a sitting position after hemoperfusion and stand without assistance after hemodialysis. Intensive hemodialysis may promote a rapid improvement of the symptoms of tetramine poisoning. Importantly, this case report first showed that measurement of plasma tetramine is useful in the diagnosis of tetramine poisoning. The determined plasma tetramine concentration was 2.16 μg/mL before hemoperfusion and decreased to 1.11 μg/mL after hemoperfusion and 0.38 μg/mL after hemodialysis.

Case 2 [29]: In this case, the patient was a 48-year-old woman who suffered from end-stage renal disease caused by diabetic nephropathy as in case 1. The clinical course of this patient is shown in Figure 3B. Approximately 30 min after ingestion of seven boiled sea snails (probably *Neptunea cumingii*), dizziness, blurred vision, abdominal pain, and diarrhea occurred. She visited the emergency department with complaints of general weakness, nausea, vomiting, and shortness of breath. Since she was in a state of respiratory failure, and intubation and invasive mechanical ventilation were immediately performed. The initial chest radiograph showed diffuse severe pulmonary edema not seen in usual tetramine poisoning. Then, continuous renal replacement therapy was initiated in the intensive care unit to remove blood tetramine. Her symptoms gradually improved, and on the fifth day, she left the intensive care unit because of no need for mechanical ventilation.

Continuous renal replacement therapy was switched back to peritoneal dialysis on the 10th day. She fully recovered without pulmonary edema and was discharged on the 15th day of hospitalization. As far as I know, no case of tetramine poisoning had required such a long hospital stay.

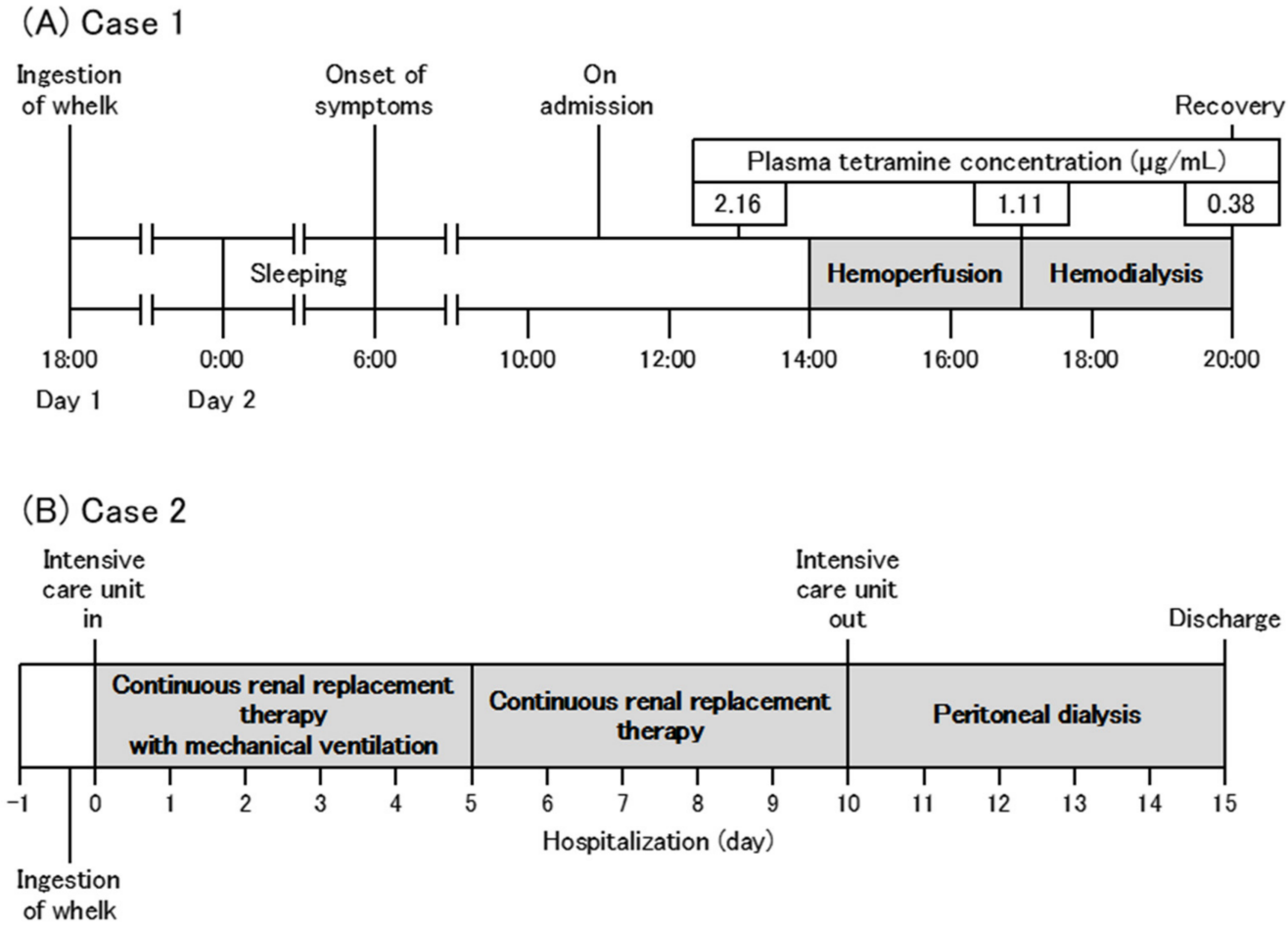

Figure 3. Clinical course of patients with end-stage kidney disease in tetramine poisoning. The shaded areas indicate how long each patient received some treatment. (**A**) Drawn by modification of Figure 2 in the paper of Takasaki et al. [28]. (**B**) Drawn by modification of Figure 3 in the paper of Yeo and Lim [29].

6.4. Prevention of Poisoning

A way to prevent tetramine poisoning is to avoid eating the salivary glands of *Neptunea* snails containing high levels of tetramine. However, such a simple thing alone cannot completely prevent tetramine poisoning. In connection with this, it should be noted that tetramine contained in the salivary glands can diffuse into other tissues during thawing of frozen snails [46] and during heating of fresh snails in boiling water [42,46,47].

We examined the diffusion of tetramine in the salivary gland into other tissues during freezing and thawing using *Neptunea polycostata* samples [46]. As depicted in Figure 4, no significant diffusion of tetramine was recognized in both frozen specimens and rapidly thawed specimens. In the case of slowly thawed specimens, however, the ratio of the tetramine amount was obviously low in the salivary gland but high in the muscle as compared to the case of live specimens; approximately 20% of tetramine contained in the salivary gland was estimated to diffuse into the muscle. These results indicate that tetramine in the salivary gland hardly diffuses into other tissues during freezing and rapid thawing but diffuses mainly into the muscle during slow thawing.

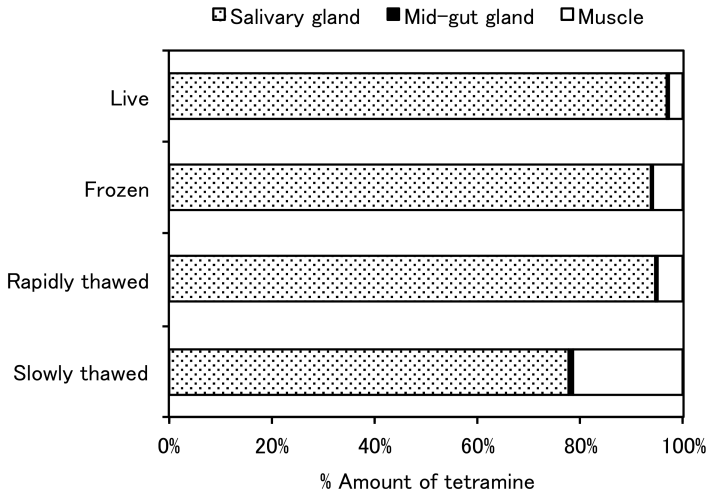

Figure 4. Tetramine contents in tissues (salivary gland, mid-gut gland, and muscle) of live, frozen, rapidly thawed, and slowly thawed specimens of *Neptunea polycostata*. Drawn from the data in Tables 1 and 3 in the paper of Kawashima et al. [46]. Three live specimens were analyzed for tetramine in the salivary gland, mid-gut gland, and muscle. Nine live specimens were frozen at −20 °C for 2 weeks and each group of three specimens was analyzed for tetramine in the salivary gland, mid-gut gland, and muscle without thawing, after rapid thawing with running water for 1 h, and after slow thawing at 4 °C for 24 h, respectively. Data are expressed as the mean of three specimens.

Much more marked diffusion of tetramine into other tissues can be induced by heating of live specimens with shells in boiling water. As seen from Figure 5 showing our results with *N. polycostata*, as much as about 50% of tetramine contained in the salivary glands diffused into the muscle during heating, although diffusion into the mid-gut gland and broth were only several %, respectively. Essentially the same results, that is, the diffusion of a significant amount of tetramine in the salivary gland into the muscle by heating, have also been reported by two other research groups [42,47].

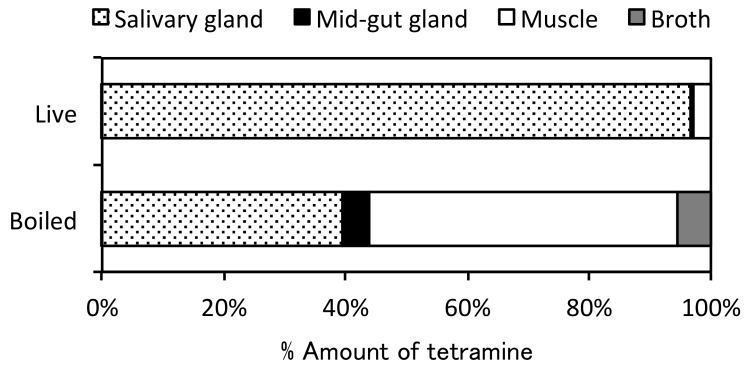

Figure 5. Tetramine contents in the tissues (salivary gland, mid-gut gland, and muscle) of live and boiled specimens of *Neptunea polycostata*. Drawn from the data in Tables 1 and 2 in the paper of Kawashima et al. [46]. A group of three live specimens was analyzed for tetramine in the salivary gland, mid-gut gland, and muscle. Another group of three live specimens with shells was heated in boiling water for 15 min and analyzed for tetramine in the salivary gland, mid-gut gland, muscle, and broth. Data are expressed as the mean of three specimens.

Based on the results described above, we propose that the best way to prevent tetramine poisoning incidents due to ingestion of *Neptunea* snails is to remove salivary glands from live specimens. If snails with salivary glands are frozen, the glands should be removed from frozen samples without being fully thawed.

7. Concluding Remarks

This review summarized the findings obtained about tetramine in the salivary glands of marine carnivorous snails, which have often caused food poisoning. Research on marine snail tetramine has progressed with the development of analytical methods, among which, the LC/ESI-MS method is most excellent in terms of sensitivity, specificity, and versatility. Apparently, high levels of tetramine are ubiquitously distributed in the salivary glands of *Neptunea* species. Although two species of marine snails, *Fusitriton oregonensis* and *Hemifusus tuba*, other than *Neptunea* specie have also been considered to contain high levels of tetramine in the salivary glands, accumulated data strongly suggest that their major toxin is distinguishable from tetramine. In particular, *Fusitriton oregonensis* has actually caused food poisoning in Japan and therefore elucidation of its toxin is an important and urgent issue in the future.

In tetramine poisoning, the symptoms observed are usually mild and transient and death is unlikely. However, it should be emphasized that the symptoms can be severe in patients with kidney dysfunction as evidenced by the two case reports presented in this review. Especially in case 2, the symptoms were so serious that they required long-term hospitalization for as long as 10 days. It is important for clinical professionals to fully recognize that tetramine poisoning can have serious consequences.

Finally, it should be noted that tetramine in the salivary gland can diffuse into the muscle during both boiling of live snails and thawing of frozen snails. Furthermore, *Neptunea* snails may rarely contain more than 0.1 mg/g of tetramine in the muscle, suggesting that even muscle can cause tetramine poisoning if consumed in large amounts (tens of grams or more). To prevent tetramine poisoning, it is important to remove salivary glands from live snails and to avoid overeating sashimi or salt-boiled meat while tasting it.

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