

## Toxicity of the Thai Freshwater Pufferfish *Tetraodon nigroviridis* and *T. steindachneri*

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**Abstract** - The toxicity of the freshwater pufferfish *Tetraodon nigroviridis* and *T. steindachneri* was determined. All samples were toxic and marked variation of toxicity among individuals was observed. *T. nigroviridis* was the most toxic species. In both species highest concentration of the toxin was found in the skin: for *T. nigroviridis* 12-39,060 MU (mouse units) $\cdot$ g<sup>-1</sup> and for *T. steindachneri*, 103-3,657 MU $\cdot$ g<sup>-1</sup>.

Comparable to the large number of marine pufferfish, about 20 freshwater species are known in Asia; seven species are reported from Thailand. Although the toxicity of marine species has been extensively investigated, freshwater puffers have received little scientific investigation. Food poisoning caused by a freshwater puffer has been reported recently (Laobhripatr et al. 1990).

In previous papers, we reported the toxicity of *Tetraodon fangi*, *T. palembangensis* and *T. suvattii* (Saitanu and Pariyawongskul 1991; Saitanu et al. 1991). This communication is a further study on the toxicity of Thai freshwater pufferfish.

The freshwater puffers *T. nigroviridis* (local name: Pla Pakapoa Chud) and *T. steindachneri* (Pla Pakapoa Ta Daeng) were purchased from aquarium dealers in Bangkok in February and November 1989. In the laboratory, they were anesthetized with quinaldine

and dissected into various organs: skin, muscle, liver, eggs and intestine. *T. steindachneri* was too small to obtain sufficient samples from one fish. Organs of 4-8 puffers were separated and combined for one sample of each tissue.

For extraction of the toxin from the tissues, 0.03 N acetic acid was added as equal volume and weight. The samples were sonicated

Table 1. Toxin levels in various organs of freshwater pufferfish, *T. steindachneri* and *T. nigroviridis*.

Species	Sex	No.	Size			Toxicity (MU/g)				
			Weight (g)	Length (SL,cm)	Skin	Gonads*	Muscle	Intestine	Liver	
<i>T. steindachneri</i>	U	13	1.2±1.1** (0.8-3.4)	2.3±0.4 (1.9-3.1)	961±1,046 (103-3,657)	-	69±51 (13-225)	191±358 (0-1,311)	43±63 (0-200)	
	♀	12	5.1±1.8 (2.5-8.5)	4.3±0.5 (3.5-5.2)	3,231±5,001 (207-18,887)	1,084±1,196 (5-4,190)	85±131 (8-508)	174±428 (14-1,591)	163±376 (9-1,399)	
<i>T. nigroviridis</i>	U	22	6.0±1.5 (4.3-10.1)	5.0±0.5 (4.2-5.8)	6,576±11,572 (12-39,060)	-	32±33 (0-122)	16±15 (0-53)	9±14 (0-62)	

Numbers in parentheses indicate minimum and maximum values of each parameter.

\*If gonad was not found or could not be identified, sex was reported as unidentified. Eggs were found in all females.

\*\*Mean ± Standard deviation, U=unidentified sex, - = Not determined.

(Handy Sonic Model UR-20P Tomy Seiko Co., Ltd.) for 2 min. and centrifuged at 2,500 RPM for 10 min. One ml of supernatant or appropriate dilution was injected into white mice intraperitoneally. The toxicity was calculated from Kawabata's table for dose-death time of tetrodotoxin (Kawabata 1978), where 1 mouse unit (MU) was described as the amount of toxin required to kill a 20-g mouse within 30 min. after intraperitoneal injection.

The toxicity of various tissues of tested specimens is shown in Table 1. *T. nigroviridis* was the more toxic species. Skin was toxic in all puffers tested and showed the highest toxicity, while many samples of muscle, liver and intestine were nontoxic. The highest amounts of toxin in *T. nigroviridis* were found in skin (average  $\pm$  SD; 6,576 $\pm$ 11,572 MU/g) followed by muscle (32 $\pm$ 33), intestine (16 $\pm$ 15) and liver (9 $\pm$ 14). The corresponding values in *T. steindachneri* were 961 $\pm$ 1,046, 191 $\pm$ 358, 43 $\pm$ 63 and 69 $\pm$ 61 MU/g, respectively. For *T. nigroviridis*, some specimens were identifiable as females. Their toxicity is reported separately in Table 1.

Our present results support previous reports that skin of the freshwater puffer is the most toxic tissue (Laobhripatr et al. 1990; Saitanu and Pariyawongskul 1991; Saitanu et al. 1991). The results are contradictory to the toxicity of *T. leiurus* whose ovary is the most toxic organ (Kodama and Ogata 1984). The toxicity of each species shows marked variation among individuals, location and season.

Kodama et al. (1985) found that toxic species of marine puffers, after stimulation by electric shock, secrete large amounts of tetrodotoxin into the surrounding water. This may occur with *T. nigroviridis* and *T. steindachneri* in the aquarium as they are often stimulated manually. The secreted toxin could contaminate food and cause food poisoning. *T. fangi*, *T. palembangensis* and *T. suvattii* are consumed by people in rural northeastern Thailand. *T. fangi* was reported as a cause of food poisoning and the toxicity was lower than the two species reported here (Laobhripatr et al. 1990). Our results show that all freshwater puffers studied are toxic. Their consumption must be avoided.

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