

The presence of pinnatoxins in Norwegian mussels

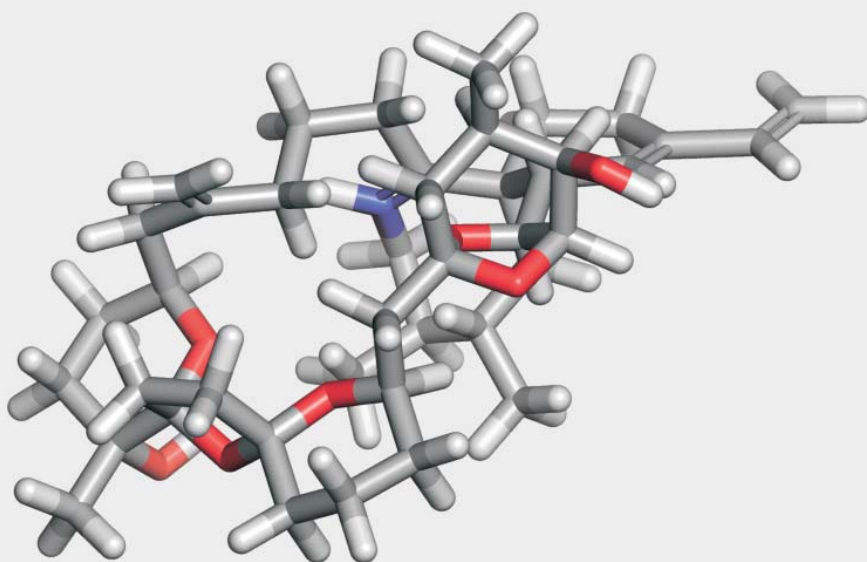
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National Veterinary Institute's Report series · 07b - 2010

Title

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Publisher

National Veterinary Institute · PO Box 750 Sentrum · N-0106
Oslo, Norway

Cover design: Graf AS

Photo frontpage: Britt I. F. Henriksen

To order

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ISSN 1890-3290 electronic edition

Suggested citation:

Miles CO, Rundberget T, Sandvik M, Aasen JAB, Selwood AI.
The presence of pinnatoxins in Norwegian mussels. National
Veterinary Institute's Report series 7b-2010. Oslo: National
Veterinary Institute; 2009.

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Veterinærinstituttets rapportserie
National Veterinary Institute's Report Series
Report 07b · 2010

The presence of of pinnatoxins in Norwegian mussels

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8. April 2010

ISSN 1890-3290 electronic edition

1. Summary

Naturally occurring algal toxins can cause poisoning in humans when they accumulate in shellfish destined for consumption. The National Veterinary Institute has now identified a hitherto unknown algal toxin in Norwegian waters that could pose a potential problem.

Pinnatoxins and pteriattoxins are toxins that were first identified by Japanese researchers in 1995, after a poisoning incident in China in 1990. It is still uncertain whether these toxins were the cause of this episode, and toxic effects on humans are unknown. These substances are structurally similar to the more familiar spirolides, produced by dinoflagellates including *Alexandrium ostenfeldii* that is frequently detected in Norwegian waters.

The National Veterinary Institute has established a sensitive analytical method for determination of pinnatoxins and spirolides. After chemical analysis of shellfish and algal extracts, toxins of the pinnatoxin group were found in shellfish and algal samples along the whole of the Norwegian coast. This is the first time that pinnatoxins have been detected in Europe. Pinnatoxins were highly toxic to animals at low doses, not just by intraperitoneal (ip) injection, but also when mice eat the toxin.

The alga that produces pinnatoxin G is still unknown, but researchers in New Zealand have managed to culture a new type of dinoflagellate that produces pinnatoxin F. There is no knowledge about the metabolism and detoxification of pinnatoxins in various species of shellfish in Norway, and it is also important to establish knowledge on its mechanisms of action, how dangerous pinnatoxins are in seafood and the appropriate limits.

2. Presence of pinnatoxins in Norwegian shellfish: background and preliminary results

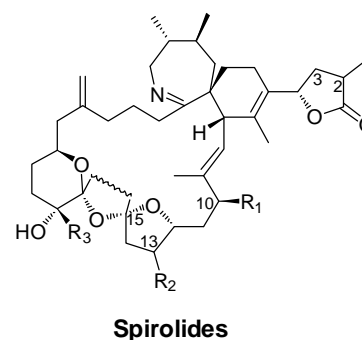
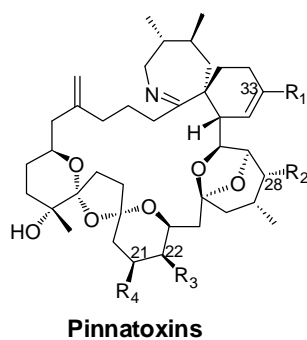
In connection with a poisoning incident in China in 1990 where shellfish consumers were poisoned after eating the shellfish species *Pinna attenuata*, Chinese researchers reported the presence of an unidentified toxin as a the possible cause (1).

Between 1995 and 2001, Japanese scientists identified a number of pinnatoxins and pteriattoxins from Japanese *Pinna* and *Pteria* species (2-5). These toxins are members of the so-called cyclic-imine group (which now includes gymnodimines, spirolides, pinnatoxins, pteriattoxins, prorocentrolides and spiro-prorocentrimines) of fast-acting toxins (6). These pinnatoxins and pteriattoxins were assumed to be metabolites of compounds biosynthesised in dinoflagellates (5).

Pinna- and pteria-toxins are highly toxic in mouse bioassays, but have not been tested orally and, as far as we know, there is no direct evidence that these toxins were responsible for the original food poisoning in China. Pinnatoxins are structurally related to the better-known spirolides (Figure 1), produced by the dinoflagellate *Alexandrium ostenfeldii* (7) that is frequently found in Norwegian waters (8).

The Veterinary Institute has, in collaboration with researchers in New Zealand and the University of Oslo, determined structures of 4 new pinnatoxins ((9) and unpublished information) isolated from several species of Australian and New Zealand shellfish after these had caused a toxic response in the mouse bioassay.

Which species of alga was producing the toxins was at the time unknown, but our colleagues in New Zealand now have in culture a new dinoflagellate species that produces one of the two groups of pinnatoxins (Pinnatoxin F) (10). The species that produces pinnatoxin G is not known. Pinnatoxin G is probably converted in shellfish to pinnatoxins A-C and pteriattoxin A-C, while F pinnatoxin is probably converted to pinnatoxins D and E (9).



	m/z [M+H] ⁺	R ₁	R ₂	R ₃	R ₄		m/z [M+H] ⁺	R ₁	R ₂	R ₂	
Pinnatoxin G	694		OH	H	H	Spirolide C	706		OH	CH ₃	CH ₃ Δ ^{2,3}
Pinnatoxin A	712		OH	H	H	13-Desmethylspirolide C	692		OH	H	CH ₃ Δ ^{2,3}
Pinnatoxins B, C	741		OH	H	H	Spirolide D	708		OH	CH ₃	CH ₃ -
Pinnatoxin F	766		H	OH	CH ₃	13-Desmethylspirolide D	694		OH	H	CH ₃ -
Pinnatoxin E	784		H	OH	CH ₃	Spirolide G	692		H	H	H Δ ^{2,3}
Pinnatoxin D	782		H	OH	CH ₃	20-Methylspirolide G	706		H	H	CH ₃ Δ ^{2,3}

Figure 1. The structures of the most common pinnatoxins and spirolides. Pinnatoxins A and G are newly identified in Norwegian waters and shellfish.

Table 1. Information on the occurrence and toxicity of pinnatoxins

Toxin	Presumed source	Where found	Toxicity (i.p., mouse, µg/kg)
Pinnatoxin A	Pinnatoxin G*	Japan New Zealand Australia Norway	<i>Pinna</i> og <i>Pteria</i> spp. Green mussels, oysters Oysters, <i>Pinna</i> sp., mussels, sediment Mussels, sea water
Pinnatoxin B/C	Pinnatoxin G*	Japan	<i>Pinna</i> sp. LD ₉₉ 22 (3)
Pinnatoxin D	Pinnatoxin F*	Japan Australia New Zealand	<i>Pinna</i> spp. <i>Pinna</i> sp., oysters Oysters LD ₉₉ 400 (2)
Pinnatoxin E	Pinnatoxin F*	New Zealand Australia Cook Islands	Oysters, green mussels, sea water, algae Oysters, <i>Pinna</i> sp., seawater, sediment Seawater LD ₅₀ 45 (9)
Pinnatoxin F	New alga (10)	New Zealand Australia Cook Islands	Oysters, algae, green mussels Oysters, <i>Pinna</i> sp., seawater, sediment Seawater LD ₅₀ 16 (9)
Pinnatoxin G	Unknown alga	New Zealand Australia Norway	Green mussels, oysters, cockles Oysters, <i>Pinna</i> sp., mussels, sea water, sediment Seawater, mussels LD ₅₀ 50 (9)
Pteriatoxin A	Pinnatoxin G*	Japan	<i>Pteria</i> sp. LD ₉₉ 100 (4)
Pteriatoxin B/C	Pinnatoxin G*	Japan	<i>Pteria</i> sp. LD ₉₉ 8 (4)

*via metabolism or hydrolysis

After obtaining standards of pinnatoxins A, E, F and G, we established a sensitive HPLC-MS/MS method for determination of pinnatoxins.

We have analyzed a range of shellfish extracts from the Norwegian School of Veterinary Science that originated from the Norwegian Food Safety Authority's monitoring programme. In addition, we analyzed extracts from passive sample collectors (a method for measuring fat-soluble algal toxins in sea water) from several localities along the Norwegian coast (12).

It turned out that there were large amounts pinnatoxin G for several samples of shellfish (up to 115 µg/kg) and that pinnatoxin G was detected in most of the extracts from shellfish and from the sample collectors.

Low levels of pinnatoxin A were also detected in mussels. There was no sign of fatty acid esters of pinnatoxins in Norwegian mussels. The foregoing HPLC-MS/MS method was also used to examine the samples for a variety of spirolides (a standard of 13-desmethylspirolide C is available). Results are shown in **Table 2** and **Figure 2**.

Table 2. Summary of LC-MS results (µg/kg shellfish) for pinnatoxin and spirolides from 166 Norwegian mussel samples in weeks 36-50, 2009.

	Spirolide C	13-Desmethylspirolide C	20-Me-Spirolide G	Pinnatoxin G
% Positive	56	22	78	67
Typical +ve levels	4-25	20-50	4-20	5-30
Maks nivå	49	226	38	115

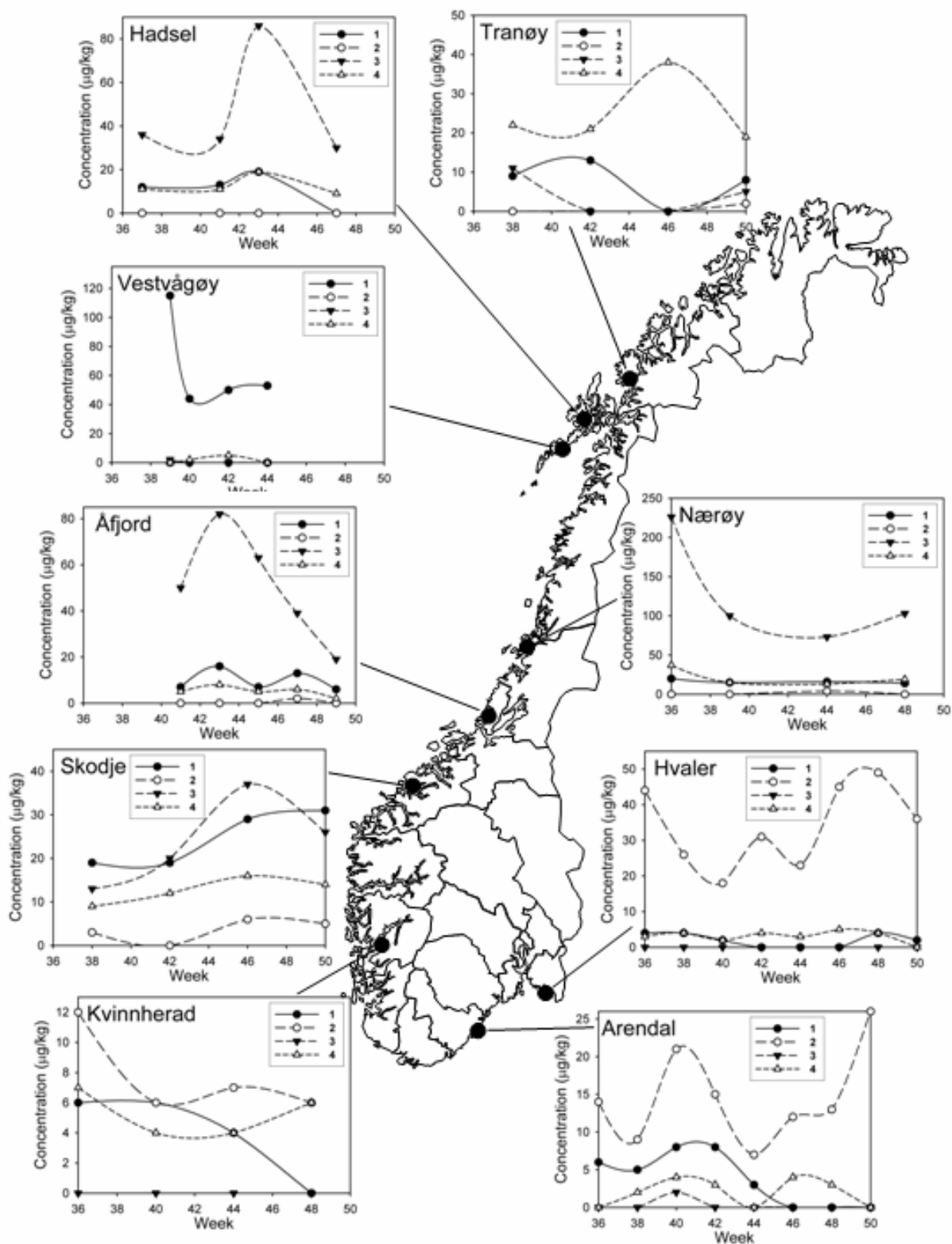


Figure 2. Concentrations of pinnatoxin G (1), spirolide C (2), 13-desmethylspirolide C (3) and 20-methylspirolide G (4) in Norwegian mussels from selected sites in weeks 36-50, 2009.

3. Toxicology

Most spirolide-toxins are highly toxic by intraperitoneal administration (i.p. mouse 10-250 µg/kg). Oral toxicity is much lower (10-100 times less toxic orally, depending on the toxin and how the toxins are administered) (13) (Table 3). On this basis, a “guidance level” of 400 µg/kg for spirolide C, 13-desmethylspirolide C and 20-methylspirolide G has been proposed (13).

Currently there is no published data for oral toxicology of pure pinnatoxins or pteriatoxins (13), but pinnatoxins and pteriatoxins are highly toxic by i.p. injection to mice (9, 13) (Table 1).

In contrast to spirolides, pinnatoxins have proven to be almost as toxic via oral dosing as they are by i.p. An extract from the pinnatoxin-producing alga from New Zealand was about 2 times less toxic per os compared to ip, and only about 5 times less toxic by feeding than by ip (10) (Table 3).

This suggests that pinnatoxin analogues may be a more serious problem in seafood than other known cyclic imines (Table 3). Converting the LD₅₀ values to µg/kg shellfish, one can see that sufficient toxin would be present in some periods such that the episode would also be expected to be detected in the mouse bioassay.

It is also possible that the occurrence of fatty acid esters of spirolides could lead to a delayed effect in the mouse bioassay (14) and could be a factor in some unexplained positive mouse bioassay results.

We have recently confirmed that pinnatoxins bind to nicotine receptors (unpublished information) activated by the neurotransmitter acetylcholine. Nicotinic acetylcholine receptors are partly responsible for transmission of nerve signals from motor neurons to muscle fibres.

Toxins in the cyclic-imine group have not yet been reviewed by EFSA.

Table 3. Toxicity (LD₅₀, mice, µg/kg*) of selected spirolides and pinnatoxin F.

Toxin	i.p.	orally	In feed
Spirolide C	7 (13)	53 (13)	~ 500 (13)
13-Desmethylspirolide C	8 (13)	125 (13)	~ 600 (13)
20-Methylspirolide G	8 (13)	88 (13)	~ 500 (13)
Pinnatoxin F	16 (9)		
Pinnatoxin E + F (1:6) [†]	13 (10)*	23 (10)*	60 (10)*

*µg/kg body weight of mice.

[†]Extract from the pinnatoxin-producing alga, with LD₅₀ values calculated from the content of pinnatoxins in the extract described in the study of Rhodes et al. (10).

2. Knowledge gaps

- Presence in Norway (when, where and in which shellfish species)
- Algal species that produce pinnatoxin G in Norway
- Metabolism and detoxification of various shellfish species in Norway
- Toxicology (mechanisms of action, how dangerous are pinnatoxins in seafood, possible regulatory limit)

3. References

1. Zheng, S. Z.; Huang, F. L.; Chen, S. C.; Tan, X. F.; Zuo, J. B.; Peng, J.; Xie, R. W., The isolation and bioactivities of pinnatoxin [in Chinese]. *Chin. J. Mar. Drugs* **1990**, *33*, 33-5.
2. Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D., Isolation and structure of pinnatoxin D, a new shellfish poison from the Okinawan bivalve *Pinna muricata*. *Tetrahedron Lett.* **1996**, *37*, 4027-30.
3. Takada, N.; Umemura, N.; Suenaga, K.; Chou, T.; Nagatsu, A.; Haino, T.; Yamada, K.; Uemura, D., Pinnatoxins B and C, the most toxic components in the pinnatoxin series from the Okinawan bivalve *Pinna muricata*. *Tetrahedron Lett.* **2001**, *42*, 3491-4.
4. Takada, N.; Umemura, N.; Suenaga, K.; Uemura, D., Structural determination of pteriatoxins A, B and C, extremely potent toxins from the bivalve *Pteria penguin*. *Tetrahedron Lett.* **2001**, *42*, 3495-7.
5. Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S. Z.; Chen, H. S., Pinnatoxin A: a toxic amphoteric macrocycle from the Okinawan bivalve *Pinna muricata*. *J. Am. Chem. Soc.* **1995**, *117*, 1155-6.
6. FAO/IOC/WHO Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Advance Prepublication Copy); FAO/IOC/WHO (http://www.fao.org/es/ESN/food/risk_biotoxin_en.stm): Oslo, Norway, Sep 26-30, 2004.
7. Cembella, A. D.; Bauder, A. G.; Lewis, N. I.; Quilliam, M. A., Association of the gonyaulacoid dinoflagellate *Alexandrium ostenfeldii* with spirolide toxins in size-fractionated plankton. *J. Plankton Res.* **2001**, *23*, 1413-19.
8. Aasen, J.; MacKinnon, S. L.; LeBlanc, P.; Walter, J. A.; Hovgaard, P.; Aune, T.; Quilliam, M. A., Detection and identification of spirolides in Norwegian shellfish and plankton. *Chem. Res. Toxicol.* **2005**, *18*, 509-15.
9. Selwood, A. I.; Miles, C. O.; Wilkins, A. L.; van Ginkel, R.; Munday, R.; Rise, F.; McNabb, P., Isolation, structural determination, and acute toxicity of novel pinnatoxins E, F and G. *J. Agric. Food Chem.* **2010**, *submitted*.
10. Rhodes, L.; Smith, K.; Selwood, A.; McNabb, P.; van Ginkel, R.; Holland, P.; Munday, R., Production of pinnatoxins by a peridinoid dinoflagellate isolated from Northland, New Zealand. *Harmful Algae* **2010**, (in press).
11. McCauley, J. A.; Nagasawa, K.; Lander, P. A.; Mischke, S. G.; Semones, M. A.; Kishi, Y., Total synthesis of pinnatoxin A. *J. Am. Chem. Soc.* **1998**, *120*, 7647-8.
12. Rundberget, T.; Gustad, E.; Samdal, I. A.; Sandvik, M.; Miles, C. O., A convenient and cost-effective method for monitoring marine algal toxins with passive samplers. *Toxicon* **2009**, *53*, 543-50.
13. Munday, R., Toxicology of cyclic imines: gymnodimine, spirolides, pinnatoxins, pteriatoxins, prorocontrolide, spiro-procentrimine, and symbioimines. In *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, 2nd ed.; Botana, L. M., Ed. CRC Press: Boca Raton, 2008; pp 581-94.
14. Aasen, J. A. B.; Hardstaff, W.; Aune, T.; Quilliam, M. A., Discovery of fatty acid ester metabolites of spirolide toxins in mussels from Norway using liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1531-7.



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The National Veterinary Institute has its main laboratory in Oslo, with regional laboratories in Sandnes, Bergen, Trondheim, Harstad og Tromsø, with about 360 employees in total.

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