National Institute for Public Health
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FRONT OFFICE FOOD AND PRODUCT SAFETY

ASSESSMENT OF PINNATOXIN-G (PNTX-G) IN SHELLFISH AND CRUSTACEANS

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Beoordeling van PnTX-G in schaal- en schelpdieren (Nederlandse samenvatting)
Pinnatoxinen (PnTXs) zijn een groep mariene biotoxines behorend tot de klasse cyclische imines die worden geproduceerd door de dinoflagellaat *Vulcanodinium rugosum* en kunnen worden aangetroffen in schaal- en schelpdieren. Dit betreft dan met name pinnatoxine G (PnTX-G). In recente jaren wordt deze PnTX regelmatig aangetroffen bij metingen door Wageningen Food Safety Research (WFSR) op verschillende productielocaties in Nederland.

In 2019 heeft het Franse Agence Nationale de Sécurité Sanitaire (ANSES) een voorlopige acute benchmarkwaarde afgeleid voor PnTX-G van 0,13 µg PnTX-G/kg lichaamsgewicht. Op basis hiervan is in Frankrijk een voorlopig maximale waarde van 23 µg PnTX-G/kg in schaal- en schelpdieren voorgesteld. De afgelopen jaren is er meer literatuur verschenen over pinnatoxinen. De Nederlandse Voedsel- en Warenautoriteit (NVWA) wil graag een review van deze literatuur om te bepalen of er actie ondernomen moeten worden. Bureau Risicobeoordeling en onderzoek (BuRO) vraagt het Front Office Voedsel- en Productveiligheid (FO) daarom een veilige waarde af te leiden voor PnTX-G in schaal- en schelpdieren.

Het FO heeft een literatuuronderzoek uitgevoerd naar recente publicaties (≥2020) over de toxiciteit van PnTX's. Daarnaast heeft het gekeken naar bestaande risicobeoordelingen. Er zijn weinig gegevens beschikbaar over de toxiciteit van PnTX's (alleen in muizen) en er zijn geen relevante nieuwe inzichten sinds de publicatie van ANSES. De beschikbare gegevens over de acute toxiciteit van PnTX-G in muizen laten zien dat er een zeer kleine marge is tussen de dosering die geen effecten geeft en de letale dosering. Het is daarom niet mogelijk een betrouwbare Acute Referentie Dosis (ARfD) af te leiden voor PnTX-G. Als gevolg daarvan is het ook niet mogelijk een betrouwbare maximale hoeveelheid in schaal- en schelpdieren vast te stellen.

Om toch een indicatie van een veilige hoeveelheid te krijgen ten behoeve van handhaving, kan de aanpak die ANSES heeft gevolgd gebruikt worden. ANSES heeft een voorlopige acute benchmarkwaarde afgeleid op basis van een acute orale muizenstudie via twee benaderingen, namelijk 1) door uit te gaan van de dosering die geen effecten gaf en daar onzekerheidsfactoren op toe te passen en 2) door een ondergrens van het 90% betrouwbaarheidsinterval van de benchmark dose ($BMDL_{10}$) af te leiden en daarop

onzekerheidsfactoren toe te passen. Het FO heeft dezelfde benaderingen gebruikt waarbij voor een aantal aspecten andere keuzes zijn gemaakt dan ANSES op basis van *expert judgement* en vernieuwde methodieken. Beide benaderingen leiden tot een vergelijkbare voorlopige acute referentiewaarde, respectievelijk 0,08 µg PnTX-G/kg lichaamsgewicht en 0,04 µg PnTX-G/kg lichaamsgewicht. Uitgaande van een grote portie van 400 g schaal- en schelpdiervlees, een lichaamsgewicht van 70 kg en een voorlopige acute referentiewaarde van 0,04 µg PnTX-G/kg lichaamsgewicht kan een voorlopige veilige hoeveelheid van 7 µg PnTX-G/kg schelpdiervlees worden afgeleid. Het moet hierbij worden opgemerkt dat PnTX-F de meest toxische analoog lijkt te zijn en daarom niet afgedekt wordt met deze indicatieve veilige hoeveelheid. PnTX-F is tot dusver niet aangetroffen. Tot slot worden enkele aanbevelingen gedaan die de huidige beoordeling kunnen verbeteren en de aanwezige onzekerheden kunnen reduceren.

Subject

Wageningen Food Safety Research (WFSR) is commissioned by the National Institute for Public Health and the Environment (RIVM), who in turn is commissioned by the Netherlands Consumer and Product Safety Authority (NVWA), to analyse bivalve molluscs from several production sites in the Netherlands for the presence of marine biotoxins. The analytical method not only detects the marine biotoxins that are regulated, but also several which are not regulated. One group of these non-regulated marine biotoxins are the pinnatoxins, including pinnatoxin G (PnTX-G). Pinnatoxins are produced by the dinoflagellate *Vulcanodinium rugosum*, and can accumulate in shellfish. In recent years PnTX-G has been regularly detected in bivalve molluscs in the Netherlands.

Pinnatoxins have been monitored in France for a longer period of time. In 2020, ANSES published an article in which a preliminary maximal PnTX-G level in shellfish and crustaceans of 23 µg/kg was derived. This level was based on a provisional acute benchmark value of 0.13 µg PnTX-G/kg body weight (bw), a body weight of 70 kg and a single consumption of 400 g meat from shellfish/crustaceans.

The highest levels of PnTX-G detected in bivalve molluscs in the Netherlands are around the preliminary maximal level derived by ANSES (WFSR, personal communication). This maximal level was based on relatively few data and several uncertainties were described by ANSES. In addition, since 2020, new literature on pinnatoxins has become available. A review of all available information is needed for the NVWA to decide whether action on pinnatoxins in shellfish in the Netherlands is warranted. The Office for Risk Assessment & Research (BuRO) asked the Front Office Food and Product Safety (FO) to investigate whether it is possible to derive a safe value for PnTX-G in shellfish and molluscs using the ANSES publication as starting point. This only concerns the acute toxicity of PnTXs; the chronic toxicity was out of the scope of the question.

Question

What is a safe value for PnTX-G in shellfish and molluscs?

Conclusion

Due to the limited available data on the acute toxicity of PnTXs, i.e. only studies in one animal species and sex (female mice) designed for the identification of LD_{50} values with a very small window between the no effect dose range and lethal dose range for PnTX-G, and no reported human intoxication data, it is not possible to derive a reliable acute reference dose (ARfD). As a consequence, it is also not possible to define a well-supported maximum level in shellfish meat.

As an indicative approach the two methods pursued by ANSES were followed by the FO. In several aspects, choices were made which differed from those of ANSES, based on expert judgement related to the uncertainties in the available data and the type of effect and updated risk assessment methodologies. Both methods result in provisional acute benchmark values that are in the same range, i.e. 0.08 µg PnTX-G/kg bw and 0.04 µg PnTX-G/kg bw. Using a large portion size of 400 g meat of shellfish, a body weight of 70 kg and a provisional acute benchmark value of 0.04 µg PnTX-G/kg bw, a provisional safe level would amount to 7 µg PnTX-G/kg total shellfish meat.

It should be noted that PnTX-F appears to be the most toxic analogue, and is therefore not covered by this provisional safe level. Thus far it has not been detected in the Netherlands.

The limited time available for this assessment did not allow the collection of detailed information on PnTXs. If desired, the authors of Munday, Selwood & Rhodes (2012) and Servent et al. (2021) can be requested to provide the study details and raw data about the acute toxicity of several PnTXs in mice and of PnTX-G in rats, respectively. These data will provide information about the acute toxicity in another animal species, i.e. rats. Also, it gives more insight into the precise dose-response relationship of PnTXs, possibly also in that of the possibly more toxic PnTX-F. This will make the assessment more robust and reduce uncertainties. In addition, using APROBA-Plus to derive a probabilistic reference value instead of a deterministic one can further refine the assessment by better capturing the relative influence of variability and uncertainties within the hazard characterization.

Introduction

Pinnatoxins (PnTXs) are a group of cyclic imines produced by the dinoflagellate *Vulcanodinium rugosum*. They are considered as emerging marine biotoxins and are not regulated. Cyclic imines, including PnTXs, and their metabolites are known to be bioaccumulative (Aráoz et al., 2020). The main analogue of PnTXs found during screening is pinnatoxin G (PnTX-G). Wageningen Food Safety Research (WFSR), commissioned by the National Institute for Public Health and the Environment (RIVM) who in turn is commissioned by the Netherlands Consumer and Product Safety Authority (NVWA), analyses bivalve molluscs from several production sites in the Netherlands for the presence of several marine biotoxins, including PnTXs. In recent years, PnTXs have been increasingly detected. Last summer, PnTX-G was detected in some samples with levels around 20 µg/kg with occasional peaks of up to 50 µg/kg.

The Agence Nationale de Sécurité Sanitaire (ANSES) derived a provisional acute benchmark value for PnTX-G of 0.13 µg PnTX-G/kg bw (ANSES, 2019; Arnich et al., 2020). Based on this, a provisional maximal level of 23 µg PnTX-G/kg was proposed using a large portion size of 400 g of shellfish meat (EFSA, 2010b) and a body weight of 70 kg.

Literature search

A literature search was conducted to identify new literature (≥2020) on the toxicity of pinnatoxins. EMBASE, Pubmed and Web of Science were searched using the search terms "pinnatoxin*" and "Vulcanodinium rugosum" (for more details see Annex 1). In total, 78 unique references were obtained. Based on title/abstract screening, seven relevant articles were identified, including the article of ANSES, (Arnich et al., 2020) and the study in mice they used to derive the provisional safe level.

Toxicology

European Food Safety Authority (EFSA)

In 2010, the European Food Safety Authority (EFSA) published a Scientific Opinion on marine biotoxins, assessing the risks to human health due to the consumption of shellfish containing different cyclic imines, a family of marine biotoxins including pinnatoxins (EFSA, 2010a). EFSA concluded that even though an acute reference dose should be established in view of the acute toxicity, this was not possible due to the lack of data. In addition, the exposure to PnTXs could not be estimated from the available data and therefore, no conclusions could be drawn.

Food Safety Authority of Ireland (FSAI)

In 2016, the Food Safety Authority of Ireland (FSAI) published a report on the occurrence of marine biotoxins and the risk of exposure of seafood consumers (FSAI, 2016). They mentioned that cyclic imines, including pinnatoxins, are not currently regulated and that up to that point no association has been found between exposure to cyclic imines and human illness.

Agence Nationale de Sécurité Sanitaire (ANSES)

In 2019, ANSES derived a provisional acute benchmark value for PnTX-G of 0.13 µg PnTX-G/kg bw based on an acute oral toxicity study in mice (Sosa et al., 2020). The value was only provisional due to insufficient data respective to the hazard characterization (ANSES, 2019; Arnich et al., 2020). ANSES identified another acute oral toxicity study in mice (Swiss albino, female) reporting oral LD_{50} values of 2800 µg/kg bw for PnTX-E, of 25.0 µg/kg bw for PnTX-F and of 150 µg/kg bw for PnTX-G after gavage and of 50.0 µg/kg bw for PnTX-F and of 400 µg/kg bw for PnTX-G after voluntary intake through feed (Munday, Selwood & Rhodes, 2012), however some important study details were missing, such as symptoms per dose group, making it less informative for a dose-response curve. Therefore, the study of Sosa et al. (2020) was chosen as the key study.

In this study, female CD-1 mice (n=3-5 per dose group; n=8 for the control group) received a single dose of 0, 8, 20, 50, 120, 220, 300, 370 or 450 µg/kg bw purified PnTX-G via oral gavage after 3-hours fasting (Sosa et al., 2020). Lethality was observed from doses of 220 µg/kg bw (3/5) and higher (5/5) within 30 minutes, and preceded by neurotoxic symptoms, including prostration, tremors, jumps, abdominal breathing, hypothermia, hind leg paralysis and cyanosis. These neurological symptoms are consistent with the mode of action of PnTXs, i.e. targeting skeletal muscle nicotinic acetylcholine receptors (nAChRs) (Aráoz et al., 2020). No clinical signs were observed at lower doses. No macroscopic organ changes were observed, and histological analysis revealed only minor changes (moderate mucosal degeneration, villous atrophy) in the small intestine of mice given doses ≥300 µg/kg bw. No differences in biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH), creatine phosphokinase (CPK), creatinine, and electrolytes (Na^+, K^+, Ca^{2+}, Cl^- and inorganic phosphorous (P_i)) were observed between treated and control mice. The oral LD_{50} value was calculated at 208 µg/kg bw (95%

confidence interval (CI): 155 – 281 µg/kg bw). The study authors also provided a provisional No-Observed-Adverse-Effect-Level (NOAEL) of 120 µg/kg bw with the notion that the LD_{50} value indicates a steep dose-response relationship.

ANSES applied two methods using the data from this study to calculate a benchmark value:

1) a maximum tolerated dose (MTD) of 120 µg PnTX-G/kg bw was selected as a reference value on which an overall uncertainty factor of 900 was applied. The overall uncertainty factor (UF) consisted of a factor 100 for inter- and intraspecies differences, a factor 3 for insufficient data and a factor 3 to take into account the severity and pattern of the dose-response curve.
2) a $BMDL_{10}$ of 69.1 µg PnTX-G/kg bw was chosen as a point of departure with an overall uncertainty factor of 525. The overall UF consisted of a factor 10 for intraspecies differences, a factor 7 for allometric scaling, a factor 2.5 for the toxicodynamic component and a factor 3 for insufficient data.

Both methods resulted in a provisional benchmark value of 0.13 µg PnTX-G/kg bw. Using a large portion size of 400 g of shellfish and a bodyweight of 70 kg, a provisional safe level was calculated at 23 µg PnTX-G/kg total meat.
ANSES notes that using mouse mortality data to derive a reference value is not common, however they considered it justified in this case because the mortality occurs very rapidly, and the range between a dose that shows no signs of toxicity and a lethal dose is small. Considering all uncertainties (type/quality of data, choice of critical effect and mode of action, choice of key study, choice of critical dose), they assigned an overall confidence level of 'moderate' to the provisional acute benchmark value.

Committee on the Toxicity (COT)
In 2023, the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) discussed the risk of PnTXs in British shellfish (COT, 2023). They concluded that it was not possible to determine the health risk due to the lack of toxicological and occurrence data. Further, they noted that, although there were no reports of adverse effects in humans, the limited information on the mechanisms of action and the observed acute effects of PnTXs in mice indicate there could potentially be a risk.

Literature
Besides the ANSES publication (Arnich et al., 2020) and the study of Sosa et al. (2020), five publications on the toxicity of PnTXs were obtained. One of these articles is a review article about cyclic imines in shellfish and two are conference abstracts; these will not be further discussed (Servent et al., 2020; Servent et al., 2024; Finch et al., 2024). One article is about the bioaccumulation and mode of action of 28-*O*-palmitoyl ester of PnTX-G (Aráoz et al., 2020). The last article (Servent et al., 2021) is on the ability of PnTX-G to distribute in the body and cross physiological barriers, including the brain-blood barrier and the placental barrier. The authors found that, after ingestion of 100 µg/kg bw (fasted state), PnTX-G is distributed widely in rats and able to cross the intestinal and blood-brain barriers. The dose of 100 µg/kg bw was chosen based on the authors' own preliminary studies, that showed that this dose was not lethal to rats. Unfortunately, this information (and possibly a dose-response curve) was not included in the publication. In addition, after an intravenous injection with 10 µg/kg bw in pregnant rats, PnTX-G was able to cross the placental barrier. The latter distribution was also observed by the same authors in a human placental model. No cases of illness in humans related to pinnatoxins have been identified (Finch et al., 2024).

Assessment

The available data on the acute toxicity of PnTXs are limited. There are only suitable data from studies in one animal species and sex available (female mice) specifically designed for the identification of LD_{50} values. There are no reports of human intoxications. No new relevant toxicological data outside the aforementioned study by Servent et al. has become available since the publication of ANSES. The available data on the acute oral toxicity of PnTX-G in mice shows that the difference between the no effect dose range and lethal dose range is very small. Therefore, deriving an acute reference dose (ARfD) is not appropriate for PnTX-G. As a consequence, it is also not possible to define a well-founded maximum level in shellfish.

As an indicative approach the risk assessment methods pursued by ANSES were followed, with some slight alterations based on expert judgement and updated risk assessment methodologies (i.e. model averaging in BMD analysis).

Method 1

Based on the absence of effects observed in mice at and below doses of 120 µg PnTX-G/kg bw, this value can be viewed as a provisional NOAEL. Besides applying a default UF of 100 for intra- and interspecies extrapolation and a UF of 3 for the limited database, as was also done by ANSES, it was considered appropriate to apply a UF of 5 instead of 3 as used by ANSES for the severity of the effect (i.e. lethality) and the steep dose-response curve (WHO-IPCS, 2020). Applying these UFs to the provisional NOAEL of 120 µg PnTX-G/kg bw leads to a provisional acute benchmark value of 0.08 µg PnTX-G/kg bw.

Method 2

The second method is based on benchmark dose analysis. ANSES used the best fitted model, the probit model, and a BMR of 10% to derive the BMDL. A BMR of 1% was regarded by ANSES as too uncertain due to the small number of mice in the key study, despite the fact that a BMR of 10% was considered high by ANSES in view of the severity of the effect (lethality). The FO performed a new BMD analysis using the same experimental data, but applying the model averaging approach (EFSA, 2017) and considering two benchmark responses (BMR), i.e. a BMR of 10% for direct comparison with ANSES and a BMR of 1%. The BMD analysis is presented in Annex 2. The uncertainty in the obtained BMD, indicated by the width of the BMD confidence interval (CI) (BMDU/BMDL) was regarded acceptable (<factor 10) when using a BMR of 10% or 1% and therefore, the results using a BMR of 1% were judged to be appropriate for use in risk assessment. The narrow BMD CI (of factor 5) at a BMR of 1% indicates that the argument of ANSES that the number of animals in the experiment will lead to a too uncertain BMD CI, is not justified.

The EFSA Guidance on the use of BMD modelling (2022) states that the use of a BMR of 10% extra risk for quantal data “might in many cases be appropriate”, and suggests that “any decision to deviate from this proposed value should be explained and documented.” Based on similar argumentation WHO proposes a BMR of 10% extra risk for quantal data, and notes that “…., there may be reasons to deviate from this default, and other BMR values may be used with a sound scientific rationale provided” (WHO-IPCS, 2020). Although mortality is a quantal endpoint, acute toxicity studies cannot be compared with the chronic/carcinogenicity studies on which EFSA based their suggested use of 10% (Allen et al., 1994; Sand et al., 2011). Fowles et al. (1999) analysed acute inhalation lethality data from 120 studies and found that BMDs “for either 1 or 5% response incidence gave results that were consistently close to the limit of experimentally detectable effects, did not exceed the LOAEL and contained a relatively small degree of uncertainty as determined by statistical confidence limits”. Thus, considering that any

mortality is, in fact, not desirable from a biological and protection-level perspective, the considerations noted in Fowles et al. (1999) related to the appropriate BMR for acute mortality, and, importantly, the fact that the $BMDL_{01}$ could be modelled with adequate accuracy based on the available data (Annex 2), a BMR of 1% is considered as the most appropriate in this assessment.

By means of comparison, using a BMR of 10% resulted in a BMDL in the same order of magnitude as ANSES, i.e. 93 µg PnTX-G/kg bw. Using a BMR of 1% gave a BMDL of 39.5 µg PnTX-G/kg bw, which falls within the experimental dose range (Annex 2). The same default UFs of 100 for intra- and interspecies extrapolation and a UF of 3 to account for the aforementioned limitations in the database were applied as in method 1. In addition, it was considered appropriate to also apply a UF for the severity of the effect and the steep dose-response. Since, in fact, an extra risk of 1% for mortality is still not considered acceptable, and due to the steepness of the dose-response relationship, a small increase in exposure will result in a large increase in extra risk. Since the BMD analysis takes into account the whole dose-response curve as opposed to a point estimation, as in the NOAEL approach, the value of the UF can be lower, i.e. 3 instead of 5. This leads to a provisional acute benchmark value of 0.04 µg PnTX-G/kg bw.

Provisional safe level
Both methods result in provisional acute benchmark values that are in the same range. Using a large portion size of 400 g of shellfish meat (EFSA, 2010b) and a body weight of 70 kg and a provisional acute reference value of 0.04 µg PnTX-G/kg bw, a provisional indicative safe level would amount to 7 µg PnTX-G/kg total meat.

This assessment focusses on PnTX-G. It should be noted that PnTX-F appears to be the most toxic analogue (ANSES, 2019; Munday, Selwood & Rhodes, 2012), and is therefore not covered by this provisional safe level for PnTX-G. Thus far PnTX-F has not been detected in the Netherlands (WFSR, personal communication).

Uncertainties
Additional UFs have been applied in addition to the default UF of 100 (for inter- and intraspecies differences) in order to address the severity of the effect and steepness of the dose-response and the aforementioned limitations in the database. This is done as a conservative approach. The size of these UFs can be reconsidered when additional toxicity data become available in the future. Below, these UFs are further discussed.

Severity of the effect and steepness of the dose-response
Since no clinical signs were observed at doses under the LD_{50} in Sosa et al. (2020), including doses higher than the $BMDL_{01}$, it may be questioned whether an additional UF is necessary. However, although a BMR of 1% extra risk was chosen as the lowest detectable effect level in the study in question (see above for discussion on why a BMR of 1% was chosen), in reality an extra risk of 1% for mortality is still not considered acceptable, as no human mortality would be the most appropriate allowed effect in reality. In addition, due to the steepness of the dose-response relationship, a small increase in exposure could result in a large increase in extra risk, which should be avoided (WHO-IPCS, 2020). To cover these uncertainties, an extra UF of 3 was included (which is lower than the UF of 5 applied in method 1, since a $BMDL_{01}$ is used). Additional toxicity data adequate for dose-response evaluation might result in a lower extra UF (or removal), if it appears that, for example, the dose-response in other species is less steep.

Relative paucity of available toxicity data

Animal data

It is noted that in female mice clinical signs were only observed at the dose level also causing mortality. It is unclear whether and which milder effects could occur at lower doses and may have been undetected or unreported, or whether, which and at what exposure level(s) effects could occur in other species or humans. In this light, it would be useful to request the authors of Munday, Selwood & Rhodes (2012) and Servent et al. (2021) to provide additional study details and raw data on the acute toxicity of several PnTXs in mice and of PnTX-G in rats, respectively. These data would provide information about acute toxicity in another animal species, to improve understanding of species sensitivity, as well as additional precision on the dose-response relationships of PnTXs. These data would make the assessment more robust and reduce the aforementioned uncertainties as a result of the relatively few available data. This could result in lowering or removing the UF of 3 added to cover the lack of additional adequate toxicity data for better characterization of the hazard.

Human data

At present, there are no reports on human intoxications with PnTXs due to the consumption of shellfish, contrary to other marine biotoxins such as saxitoxins, tetrodotoxins and various diarrhetic shellfish poisons. PnTX-G is found on average in 8.2% of the shellfish samples monitored (166 out of 2020) by WFSR above the level of 7 µg PnTX-G/kg shellfish over the course of 2020-2025. There is a clear variation among harvest areas, for some this frequency is much higher. Furthermore, compared to the other marine biotoxins there is no seasonality in occurrence of PnTX-G in shellfish, most likely due to the fact that *Vulcanodinium rugosum* is a benthic dinoflagellate (WFSR, personal communication). Based on aforementioned, it can be expected that shellfish with concentrations above the provisional safe level have been consumed.

Additional considerations

The provisional safe level has now been derived for PnTX-G, however PnTX-F has been reported to be the most toxic analogue. For PnTX-F derivation of a provisional safe level was currently not possible due to a lack of dose-response data in the available study. Thus far PnTX-F has not been detected (WFSR, personal communication).

Recommendations

The limited time available for this assessment did not allow the collection of detailed information on PnTXs. If desired, the authors of Munday, Selwood & Rhodes (2012) and Servent et al. (2021) can be requested to provide the study details and raw data about the acute toxicity of several PnTXs in mice and of PnTX-G in rats, respectively. These data will provide information about the acute toxicity in another animal species, i.e. rats. Also, it gives more insight into the precise dose-response relationship of PnTXs, possibly also in that of the possibly more toxic PnTX-F. This will make the assessment more robust and reduce uncertainties. In addition, using APROBA-Plus to derive a probabilistic reference value instead of a deterministic one can further refine the assessment by better capturing the relative influence of variability and uncertainties within the hazard characterization.

Conclusion

What is a safe value for PnTX-G in shellfish and molluscs?

Due to the limited available data on the acute toxicity of PnTXs, i.e. only studies in one animal species and sex (female mice) designed for the identification of LD_{50} values with a very small window between the no effect dose range and lethal dose range for PnTX-G,

and no reported human intoxication data, it is not possible to derive a reliable acute reference dose (ARfD). As a consequence, it is also not possible to define a well-supported maximum level in shellfish meat.

As an indicative approach, the two methods pursued by ANSES were followed by the FO. In several aspects, choices were made which differed from those of ANSES, based on expert judgement related to the uncertainties in the available data and the type of effect and updated risk assessment methodologies. Both methods result in provisional acute benchmark values that are in the same range, i.e. 0.08 µg PnTX-G/kg bw and 0.04 µg PnTX-G/kg bw. Using a large portion size of 400 g meat of shellfish, a body weight of 70 kg and a provisional acute benchmark value of 0.04 µg PnTX-G/kg bw, a provisional safe level would amount to 7 µg PnTX-G/kg total shellfish meat.

It should be noted that PnTX-F appears to be the most toxic analogue, and is therefore not covered by this provisional safe level. Thus far it has not been detected in the Netherlands.

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Annex 1 Search strategy

Embase

Session Results

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No.	Query	Results	Date
#4.	#3 AND (2020:py OR 2021:py OR 2022:py OR 2023:py OR 2024:py OR 2025:py)	24	12 Nov 2025
#3.	vulcanodinium AND rugosum	44	12 Nov 2025
#2.	#1 AND (2020:py OR 2021:py OR 2022:py OR 2023:py OR 2024:py OR 2025:py)	50	12 Nov 2025
#1.	pinnatoxin*	141	12 Nov 2025

.....

Pubmed

Search number	Query	Filters	Search Details	Results	Date
5	vulcanodinium rugosum	from 2020 - 2025	("vulcanodinium"[All Fields] AND "rugosum"[All Fields]) AND (2020:2025[pdat])	21	2025/11/27
4	vulcanodinium rugosum		"vulcanodinium"[All Fields] AND "rugosum"[All Fields]	34	2025/11/27
3	pinnatoxin*	from 2020 - 2025	("pinnatoxin*"[All Fields]) AND (2020:2025[pdat])	38	2025/11/27
2	pinnatoxin*		"pinnatoxin*"[All Fields]	86	2025/11/27
1	pinnatoxin		"pinnatoxin"[All Fields] OR "pinnatoxins"[All Fields]	86	2025/11/27

Web of Science

Date of search: 2025/11/27

Number of retrieved results: 63

Annex 2 Report of BMD analysis

Data description

The lethality data related to mice after single administration by gavage of purified PnTX G from the study of ANSES-University of Trieste-CNRS Report, 2014 (published in Sosa et al., 2020) were used in the BMD analysis (see Table A1).

Table A1. Lethality data of mice used for the BMD analysis.

Dose (µg/kg bw/day)	Lethality	Group size
0	0	8
8	0	3
20	0	3
50	0	3
120	0	3
220	3	5
300	4	5
370	5	5
450	5	5

Selection of benchmark response (BMR)

The BMR used is i) 1% (Analysis 1), and ii) 10% (Analysis 2) extra risk. The benchmark dose (BMD) is the dose corresponding with the selected BMR. A 90% confidence interval around the BMD will be estimated, the lower bound is reported as BMDL and the upper bound as BMDU.

The selected BMR does not reflect a risk which is considered acceptable in risk assessment, but is a practical choice that is measurable. A lower BMR may lead to a (much) more uncertain BMD confidence interval, and therefore a (much) lower BMDL which renders the use of the BMDL as a Point of Departure undesirable.

Software used

The analysis was carried out using the RIVM web-tool for BMD analysis, the PROASTweb version 71.1. The model averaging approach was applied (1000 bootstrap runs).

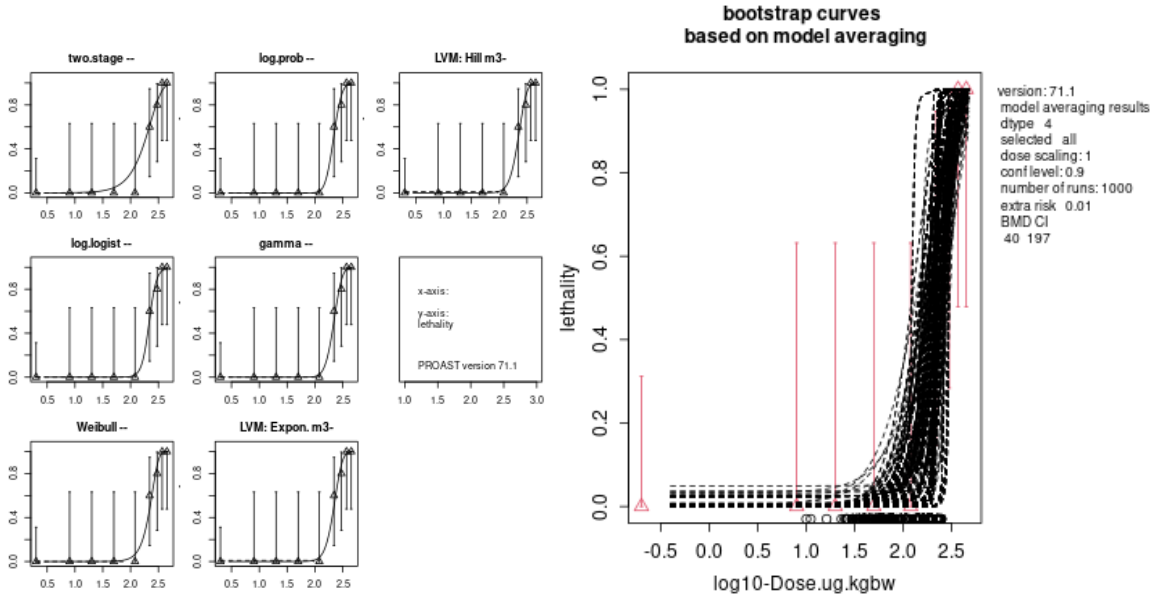
Specification of deviations from default assumptions

In Analysis 1 a BMR of 1% extra risk was used. There are no other deviations from default.

Results

Analysis 1 (BMR = 1%)

Fit of single models and analysis based on model averaging



Fitted models

Model	No.par	Loglik	AIC	Accepted	BMDL	BMDU	BMD	Conv
Null	1	-27.27	56.54	NA	NA	NA	NA	NA
Full	9	-5.87	29.74	NA	NA	NA	NA	NA
Two.stage	3	-7.35	20.7	no	NA	NA	22.7	yes
Log.logist	3	-6.37	18.74	yes	35.9	165	106	yes
Weibull	3	-6.39	18.78	yes	16.3	140	71.8	yes
Log.prob	3	-6.26	18.52	yes	45.6	172	115	yes
Gamma	3	-6.28	18.56	yes	28.2	168	105	yes
LVM: Expon. m3-	3	-6.31	18.62	yes	10.2	157	94.4	yes
LVM: Hill m3-	3	-6.31	18.62	yes	10.3	157	94.8	yes

Model weights

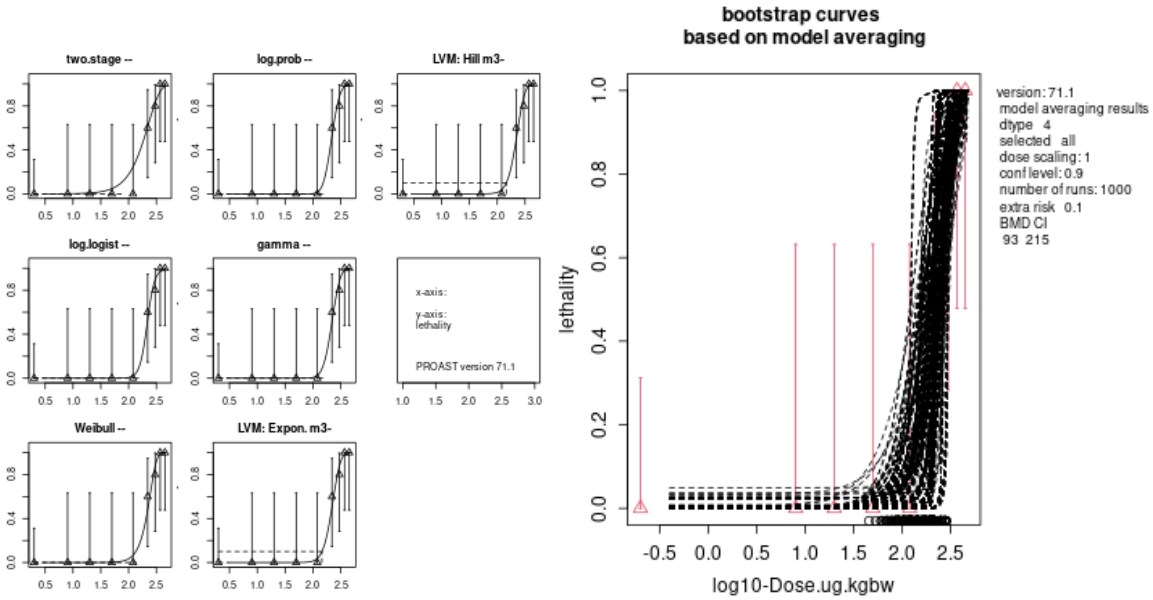
Model	Weight
Two.stage	0.0561
Log.logist	0.1495
Weibull	0.1465
Log.prob	0.1669
Gamma	0.1636
EXP	0.1587
HILL	0.1587

BMD confidence interval based on model averaging

BMDL	BMDU	BMDU/BMDL
39.5	197	5

Analysis 2 (BMR = 10%)

Fit of single models and analysis based on model averaging



Fitted models

Model	No.par	Loglik	AIC	Accepted	BMDL	BMDU	BMD	Conv
Null	1	-27.27	56.54	NA	NA	NA	NA	NA
Full	9	-5.87	29.74	NA	NA	NA	NA	NA
Two.stage	3	-7.35	20.7	no	NA	NA	73.5	yes
Log.logist	3	-6.37	18.74	yes	80.8	201	154	yes
Weibull	3	-6.39	18.78	yes	60.4	196	135	yes
Log.prob	3	-6.26	18.52	yes	81.9	202	153	yes
Gamma	3	-6.28	18.56	yes	71.1	201	149	yes
LVM: Expon. m3-	3	-6.31	18.62	yes	64.3	199	145	yes
LVM: Hill m3-	3	-6.31	18.62	yes	64.6	199	145	yes

Model weights

Model	Weight
Two.stage	0.0561
Log.logist	0.1495
Weibull	0.1465
Log.prob	0.1669
Gamma	0.1636
EXP	0.1587
HILL	0.1587

BMD confidence interval based on model averaging

BMDL	BMDU	BMDU/BMDL
93	215	2.3

Conclusion

A small BMD confidence interval indicates that the data are of sufficient quality to derive a sufficiently reliable BMD(L) at a particular BMR. The shape of the curve (at low dose but also elsewhere) is constrained by data. If there is sufficient data then this will result in precise model fits and subsequently in small BMD CIs. So, the width of the BMD CI is a measure of the quality of the data and the confidence in the result needed, the BMDL. In the case of BMR=1%, the uncertainty in the resulting BMD(L) is acceptable (< factor 10). For reference purposes we also reported the BMD_{10} CI.