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Risks for human health related to the presence of grayanotoxins in certain honey

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Abstract

The European Commission asked EFSA for a scientific opinion on the risks for human health of the presence of grayanotoxins (GTXs) in 'certain honey' from Ericaceae plants. The risk assessment included all structurally related grayananes occurring with GTXs in 'certain' honey. Oral exposure is associated with acute intoxication in humans. Acute symptoms affect the muscles, nervous and cardiovascular systems. These may lead to complete atrioventricular block, convulsions, mental confusion, agitation, syncope and respiratory depression. For acute effects, the CONTAM Panel derived a reference point (RP) of 15.3 μg/kg body weight for the sum of GTX I and III based on a $BMDL_{10}$ for reduced heart rate in rats. A similar relative potency was considered for GTX I. Without chronic toxicity studies, an RP for long-term effects could not be derived. There is evidence for genotoxicity in mice exposed to GTX III or honey containing GTX I and III, showing increased levels of chromosomal damage. The mechanism of genotoxicity is unknown. Without representative occurrence data for the sum of GTX I and III and consumption data from Ericaceae honey, acute dietary exposure was estimated based on selected concentrations for GTX I and III reflecting concentrations measured in 'certain' honeys. Applying a margin of exposure (MOE) approach, the estimated MOEs raised health concerns for acute toxicity. The Panel calculated the *highest concentrations* for GTX I and III below which no acute effects would be expected following 'certain honey' consumption. The Panel is 75% or more certain that the *calculated highest concentration* of 0.05 mg for the sum of GTX I and III per kg honey is protective for all age groups regarding acute intoxications. This value does not consider other grayananes in 'certain honey' and does not cover the identified genotoxicity.

Keywords: grayanotoxins, grayananes, *Rhododendron* honey, Ericaceae

Requestor: European Commission

Question number: EFSA-Q-2020-00509

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated the risks for human health related to the presence of grayanotoxins (GTXs) in honey from Ericaceae plants, herein reported as 'certain honey'. This risk assessment was extended to cover all structurally related grayananes co-occurring with GTXs in 'certain honey' and, if available, other foods.

Grayananes are polyhydroxylated diterpenes that are produced by some species of the Ericaceae family, particularly in the genera of *Agarista*, *Craibiodendron, Kalmia, Leucothoe, Lyonia, Pieris* and *Rhododendron*. Presently, approximately 180 naturally occurring grayananes have been identified. Oral exposure to grayananes is associated with acute intoxication in humans. Acute symptoms affect the muscles and the nervous and cardiovascular systems with bradycardia and hypotension being most prominent. These may lead to complete atrioventricular block, convulsions, mental confusion, agitation, syncope and respiratory depression.

Grayananes can be present in the nectar of the flowering plants and when collected by honeybees, they can accumulate in honey. Some grayanane-producing plant species are used for commercial honey production, the most important for this opinion being the *Rhododendron* species *R. ponticum*, *R. luteum* and *R. ferrugineum*. The first two species are particularly abundant in Turkey and the latter species occurs in the Alps and Pyrenees. Other *Rhododendron* or Ericaceae species may be used for honey production as well but not all of them have been described to contain grayananes (e.g. Heather honey). Non-*Rhododendron* honey from *Agarista salicifolia* is associated with grayanane acute intoxications in humans on the French island of La Réunion.

Approximately 20 grayananes have been identified in or associated with honey. GTX I and GTX III are the grayananes most studied with respect to toxicological effects as well as their occurrence in 'certain honey', but other analogues, such as rhodojaponin III, may also contribute to the effects observed.

Oral toxicokinetic studies with GTX I and III in experimental animals are scarce and do not allow conclusions on the bioavailability of GTX I and III following exposure to 'certain honey'. In case reports on human intoxications related to consumption of 'certain honey', GTX I and GTX III were detected in human blood and urine, showing that they are absorbed in the GI-tract.

The Panel noted that most toxicodynamic studies on experimental animals use i.p. injection. However, no toxicokinetic study dealing with i.p. injection of grayananes in experimental animals was identified and, therefore, it was not possible to compare the grayanane bioavailability after oral and *i.p*. exposure to 'certain honey' or the pure substance(s).

No information on the biotransformation of grayananes in mammals is available to EFSA. Based on data on the *ent*-kaurane diterpenoid oridonin, no metabolic cleavage of the grayanane rings is anticipated.

Acute animal toxicity studies reported cardiovascular effects, decreased respiratory rate and impairment of the nervous system shortly after exposure to grayananes or 'certain honey'. Among these, blood pressure reduction and bradycardia were most representative of the symptoms of human intoxication.

Based on their structure–activity relationships, grayananes can be divided into four groups. The two most potent groups showed three structural alerts, and the number of alerts is inversely proportional to the LD_{50} values. Relative potency factors (RPFs) were derived for each group as follows: 2 for Group 1A, 1 for Group 1B, 0.1 for Group 2 and < 0.01 (negligible potency) for Group 3. A potency factor of 1 was assigned to GTX I and III which have similar toxicity.

The limited information from acute, subacute and subchronic studies in rodents suggests that several biochemical parameters are altered following exposure to individual grayananes and/or 'certain honey', sometimes in a dose-dependent manner. None of these changes, however, were considered as toxicologically relevant because the magnitude of the changes was small. In most of these studies, when examined, no histological changes were reported.

From single dose studies in experimental animals, a $BMDL_{10}$ of 15.3 μg/kg bw was derived, based on the reduction of heart rate after i.p injection of GTX III in rats. This reference point (RP) was applied to the sum of GTX I and III, considering that these two substances have similar toxic potency.

No RP for subacute and/or subchronic exposure could be identified.

Histological findings in rat testis from repeated dose i.p. injection of GTX III at doses from 0.1 to 0.8 μg/kg bw suggest potential effects on the testes, but the available data are limited.

Induction of chromosomal damage as shown in an *in vivo* micronucleus (MN) test in male mice indicates genotoxicity for GTX III and honey containing GTX I and III. In the same honey sample, the analysis of chromosomal aberrations confirmed this result. The mechanism of genotoxicity is unknown.

With regard to observations in humans, case reports are available on intoxications due to ingestion of grayananes containing honeys, plants, plant parts or their preparations. Focus is put on reports with relevance for the risk assessment giving information on dose effect relationships after oral intake of 'certain honey'.

Acute intoxications reported in the last decades from European countries are mainly associated with imported honey from Turkey or Nepal, but some have also been caused by honey from other countries. No intoxication cases have been reported for honeys from EU origin.

Rhododendron honeys have a traditional use as folk medicine, e.g. against gastrointestinal disorders, hypertension, heart failure, diabetes, asthma, cough and as an aphrodisiac.

Known amounts of ingested *Rhododendron* honey associated with acute intoxication in humans vary and are reported to range from 5 to 150 g with levels of GTX I and/or GTX III ranging from 0.5 to 54 mg/kg. The majority of intoxications are reported to refer to males aged between 40 and 65 years, the higher prevalence being explained by the higher use of 'certain honey' as a sexual stimulant as well as a higher rate of hypertension in males of this age group. It was noted, however, that not all reported intoxications relate to the use of *Rhododendron* honey as folk medicine but cover also consumption of honey as conventional food.

Resulting symptoms on the nervous and cardiovascular systems occur within minutes and up to 5 hours. Poisoning symptoms may persist from a few hours to a few days. In general, patients recover within 1–2 days.

Even though more than 1,000 cases of intoxication after ingestion of 'certain' Ericaceae honeys by individuals were retrieved in published literature only a few of these case reports provide quantitative information on the content of grayananes in the honey.

In acute poisoning cases observed with *Rhododendron* honeys, the estimated intake on a single occasion of GTX I or GTX III as part of the mixture of grayananes present in the honey range from 0.6 to 17 μg/kg bw or 3.9 to 24 μg/kg bw, respectively, and from 4.8 to 40 μg/kg bw for the sum of GTX I and GTX III. A possible contribution of other grayananes to the development of adverse effects cannot be quantitatively assessed due to insufficient information on their occurrence and on their toxicological profile. From cases of intoxications following the ingestion of *Rhododendron* honey, in which GTX I and III could not be detected, it can be concluded that other grayananes also cause adverse effects on the muscles and the nervous and cardiovascular systems. An NOAEL for any GTX or for total grayananes could not be identified from the data available for human case studies.

For human chronic exposure, reported symptoms do not differ from those of acute exposure. However, information on dose–effect relationships for long-term intake of grayananes could not be identified.

Results from one study suggest that patients with cardiovascular diseases, when receiving anti-ischaemic or antihypertensive therapy (e.g. beta-blockers), are at higher risk of developing adverse cardiovascular effects when exposed to grayanotoxins compared to healthy subjects.

The mechanism of acute intoxication relates to grayanane binding to sodium voltage gated channels in their open state and preventing their inactivation. This results in a persistent activation of excitable cells like neurons, muscle cells, cardiomyocytes and is consistent with the promotion of acetylcholine release from the vagus nerve. The use of specific sodium channel blockers and antagonists of acetylcholine receptors counteracts grayananes toxicity, supporting the involvement of these mechanisms in their toxicity.

As a result of the indicated *in vivo* genotoxicity for the pure GTX III and for one honey sample containing GTX I and III, a health-based guidance value (HBGV) cannot be derived for grayananes. Due to the lack of information on the underlying mode of action of genotoxicity and the lack of data on chronic toxicity and carcinogenicity, the Panel was unable to assess the risk related to chronic/repeated exposure.

In addition, due to the lack of specific occurrence data on grayananes, the lack of consumption data for *Rhododendron* honey and/or other honeys containing grayananes in the EFSA databases and the scarcity of data in the literature, dietary exposure was estimated based only on selective concentrations for GTX I and III in 'certain honey'.

No information was available regarding GTX occurrence in foods other than honey in the EU.

Regarding the risks for acute toxicity, the Panel assessed the health concern using the MOE approach. MOEs were calculated based on the RP of 15.3 μg/kg bw for the sum of GTX I and III and

the exposure at selected concentrations reflecting the highest measured concentration for GTX I in a honey sample of EU origin (0.1 mg/kg) and concentrations measured for the sum of GTX I and III in 'certain honeys' (1 and 10 mg/kg). The estimated MOEs were below 100, and thus raised health concerns for acute toxicity.

To estimate the *highest concentration(s)* for the sum of GTX I and III in 'normal' honey below which no acute effects on heart rate and blood pressure would be expected following acute consumption of 'certain honey', the highest reported P95 intake of honey consumers was combined with the RP of 15.3 μg/kg bw for the sum of GTX I and III and an MOE of 100 or greater was applied.

For toddlers, the lowest value was estimated as 0.05 mg GTX I and III/kg honey based on the P95 consumption of 3 g honey per kg bw. This should protect also other age groups with equal or lower relative honey consumption.

When *Rhododendron* honey is consumed by adults as traditional folk medicine, the Panel calculated *highest concentrations* for the sum of GTX I and III based on portions of *Rhododendron* honey from labels of products sold on the Internet and originating from Nepal or Turkey. The *highest concentration* was estimated to be 0.16 mg GTX I and III/kg honey for the general adult population and corresponds to consumption of 3 tablespoons (66 grams) of honey. It was noted that the honey portions on the products' labels used in the calculations are specific to these products and are not necessarily representative of all commercially available products. In addition, it is uncertain if all consumers follow the recommendations on the products' labelling.

Uncertainty analysis was performed to identify and quantify sources of uncertainty affecting the risk assessment and combine them to assess the overall certainty of the main conclusions. Sources of uncertainties related to hazard identification and characterisation of grayananes and consumption of honey containing grayananes in the EU were listed and described. Since the Panel did not identify non-standard uncertainties in the genotoxicity assessment, no further uncertainty analysis was undertaken for this.

The Panel quantified by expert judgement the combined impact of the identified sources of uncertainty on the RP for acute toxicity, the relative potency of other grayananes and the P95 of honey consumption for EU toddlers and adults.

Based on these judgements, the Panel is 85% certain that the RP is protective, i.e. would not be lower if the uncertainties affecting it were resolved, and 70–95% certain that the proposed RPFs are conservative.

The Panel is 75% or more certain for toddlers and adults that the MOEs presented in the Opinion would not be lower if the uncertainties relating to the RP for acute toxicity and exposure based on selected concentrations and the 95th percentile of acute consumption in the EFSA database were resolved. This conclusion is also applicable for all the other age groups.

The Panel is also 75% or more certain that the *highest concentration* of 0.05 mg/kg in honey calculated for toddlers is protective for all age groups, i.e. will not result in a concern for acute effects at the 95th percentile of acute consumption. The Panel is 85% certain that, when *Rhododendron* honey is consumed as folk medicine, the *highest concentration* of 0.16 mg/kg, based on a portion size of 66 g, would not lead to a concern for acute effects. It was noted that larger portion sizes would result in proportionately lower *highest concentrations*.

These conclusions do not cover the genotoxicity endpoint. In addition, they do not consider the impact of other co-occurring grayananes in 'certain honey' which cannot be analysed presently. When analytical standards for other grayananes become available, it may be possible to refine the assessment and include those.

Recommendations

The following needs have been identified to improve the risk assessment for humans and reduce the uncertainties:

- Analytical standards are required for grayananes other than GTX I and III to enable accurate measurement of these substances in 'certain honey' and other foods, in particular for rhodojaponin III.
- Validated analytical methods should be developed for the quantification of total grayananes in 'certain honey' with LOQs per grayanane at 0.01 mg/kg or lower.
- There is a need for occurrence data on grayananes in honey and commodities thereof from Ericaceae species. Quantitative data should be collected for at least GTX I and GTX III.

- Integration of the monitoring of grayananes in *Rhododendron* honeys on the EU market in the national control activities is recommended, especially from specific regions of production (e.g. Alps and Pyrenees).
- Investigations of the market share of *Rhododendron* honeys in the EU market are needed.
- Mechanistic studies are required to understand the mode of action of genotoxicity.
- The short- and long-term effects of orally administered grayananes on the reproductive system need to be investigated with single and repeated-dose toxicity studies across a broader dose range (> 0.8 μg/kg bw).
- Experimental studies via the oral route on the effects of acute and chronic exposure/ carcinogenicity to grayananes are required.
- Observations/surveys in humans on the acute effects of grayananes in the low-dose range and on the NOAEL are desirable.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

BACKGROUND

Grayanotoxins are produced by plants in the Ericaceae family. While many species of this family contain grayanotoxins, only a few contain significant levels. Bees that collect pollen and nectar from these grayanotoxin-containing plants often produce honey that also contains grayanotoxins. Consumption of this honey may cause grayanotoxin poisoning in humans. As the presence of grayanotoxins is related to very specific honeys, not widely produced, measures taken in certain Member States at national level appear to be effective in avoiding poisoning cases in the EU. However, it is appropriate that EFSA performs a risk assessment in order to consider the need for taking additional risk management measures at EU level to ensure a high level of public health protection.

TERMS OF REFERENCE

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide an opinion on the risks for human health related to the presence of grayanotoxins in certain honey.

1.1.1. Interpretation of the Terms of Reference

The scientific opinion on grayanotoxins (GTXs) in certain honey shall cover the potential risks for human health following exposure to honey and honey products originated from Ericaceae family plants which contain GTXs and other structurally related substances of concern (i.e. grayananes of concern).

For the purpose of the present assessment, the term 'grayananes' shall refer to GTXs and other structurally related substances of concern and the term 'certain honey' shall refer to honey and honey products originated from Ericaceae family plants and containing grayananes. The terms 'mad honey' or 'toxic honey' shall refer to honey containing grayananes in sufficient amounts to cause intoxication in humans.

In particular, the CONTAM Panel should address the questions by the requestor as follows:

- Identify the grayananes of concern present in honey and honey products;
- Evaluate the toxicity of grayananes of concern present in 'certain honey' (i.e. honey and honey products originated from Ericaceae family plants);
- Evaluate the composition and levels of grayananes of concern in 'certain honey' based on data from monitoring and the literature, including information on occurrence and consumption of 'certain honey' from countries outside the EU;
- Estimate the dietary exposure of the EU population to grayananes of concern present in 'certain honey';
- Assess the human health risks for the EU population, including the consumption patterns of honey containing grayananes by specific groups of the population;
- If there is information on the presence of grayananes in other food products originating from the Ericaceae family, this will be assessed as appropriate.

In the absence of representative data for grayanane occurrence in honeys marketed in the EU, and providing the available data will allow the derivation of health-based guidance value(s) (HBGVs) for these substances, a backward calculation will be performed in order to estimate the *highest concentrations* of grayananes per eating portion that can be present in 'certain honey' below which no health effects are expected.

1.2. Supporting information for the assessment

1.2.1. Chemistry

Grayananes are polyhydroxylated diterpenes belonging to the chemical group of grayanoids. Grayanoids are present in Ericaceae plants. The most important genera producing these substances are *Agarista*, *Craibiodendron, Kalmia, Leucothoe, Lyonia, Pieris* and *Rhododendron* (Li et al., 2013, 2019; These et al., 2015). Presently, approximately 300 natural grayanoids have been isolated from Ericaceae plants (Li et al., 2013, 2019).

The biogenetic precursor of grayanoids is assumed to be *ent*-kaurane (Li et al., 2013, 2019; Hanson, 2016). Studies on the biosynthesis of grayanoids suggest at least six possible bio-transformations of *ent*-kaurane resulting in the formation of six groups of substances: grayananes, 3,4-seco-grayananes, 9,10-seco-grayananes, 1,5-seco-grayananes, kalmanes and leucothanes (Li et al., 2013) (Figure 1).

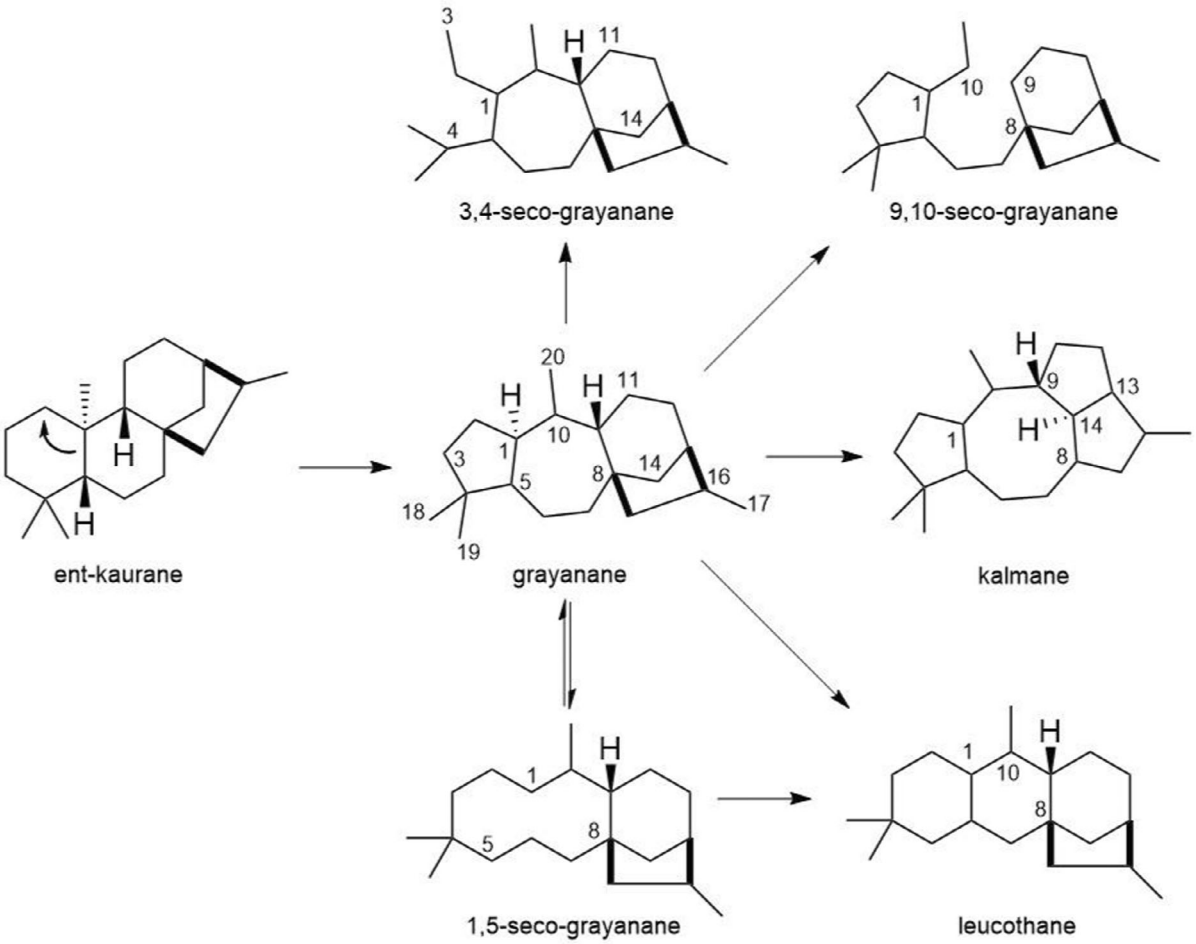


Figure 1: Simplified scheme for the formation and diversification of grayanoids in plants (Adapted from Li et al., 2013)

The substances of interest for this assessment belong to the group of grayananes and constitute the largest group with over 180 structures described (Li et al., 2013, 2019). Grayananes have as basic structure an A-nor-B-homo-*ent*-kaurane skeleton. Their structure is characterised by a *trans* junction between rings A and B and a *cis* junction between rings B and C. Their stereochemistry is shown in Figure 2. Grayananes may also contain additional structural groups which influence their toxicological properties and potency (see Appendix A and Section 3.1.5).

Based on their structure, grayananes are further divided to the following subgroups: non-epoxidised grayananes, 2,3-epoxy-grayananes and 5,9-epoxy-grayananes (Li et al., 2013). GTXs belong to the subgroup of non-epoxidised grayananes. The detailed structures, synonyms, sources and chemical properties of grayananes are shown in Appendix A.

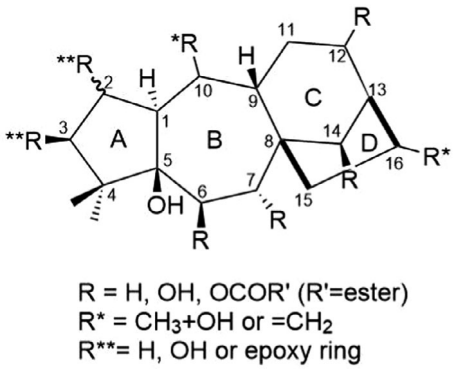


Figure 2: Generalised structure and stereochemistry of grayananes under assessment

The nomenclature of grayananes is complex and ambiguous. Thus, structurally similar grayananes are reported with various names such as grayanotoxins, asebotoxins, rhodojaponins, andromedotoxins, andrometodols, pieristoxins, etc. (Li et al., 2013; These et al., 2015). The naming in most cases refers to the plant species from which the compound was first isolated and structurally described. Almost all numbered grayananes have been first described for *Leucothoe grayana* (Li et al., 2013).

Some grayanane names, such as andromedotoxin/rhodotoxin, lyoniatoxin or rhomotoxin, have been discontinued, because later research found them to be identical to grayananes described earlier from other species. GTX I was the first grayanane isolated in pure form (Tallent et al., 1957). Its molecular structure was elucidated in 1970 by X-ray crystallography (Narayanan et al., 1970).

It has been reported that oral exposure to plants or plant parts containing grayananes may result in acute intoxications in animals and humans. Bees pollinating grayanane-containing Ericaceae may also produce honey containing grayananes, causing intoxication in humans (see Section 3.1.3).

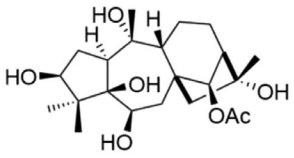
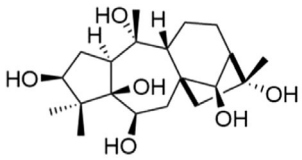
Additionally, grayananes have been described to exhibit antifeedant and insecticidal properties (Li et al., 2017). The Panel noted that patent applications for the use of Chinese azalea extract (CN1218635C, 2001[1]) and a preparation of rhodojaponin III with chlorpyrifos (CN101124911B, 2007[2]) for use as insecticide have been previously submitted in China. In 2016, an application for the use of grayanane-containing honey as rodenticide was submitted to the European Commission and considered by EFSA (EFSA, 2017). This is discussed in Section 1.2.4.

For the purpose of the present assessment only grayananes which occur in honey and honey products will be evaluated. If, however, there is information on the presence of grayananes in other food products originating from Ericaceae, this will be assessed where appropriate.

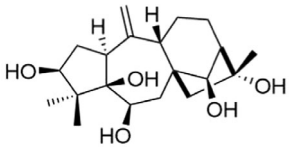
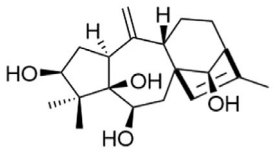
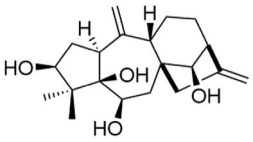
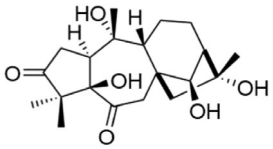
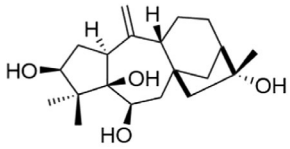
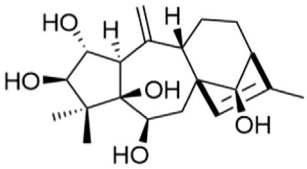
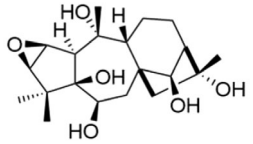
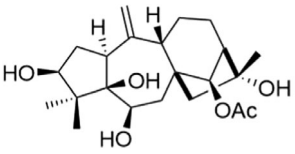
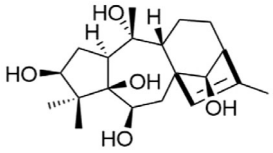
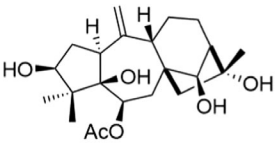
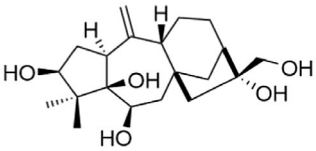
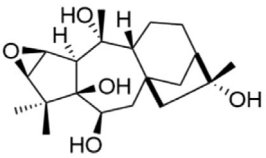
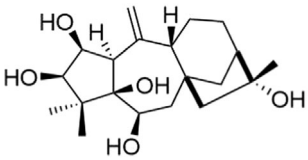
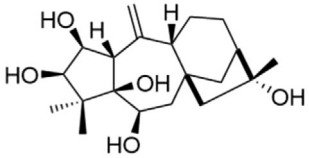
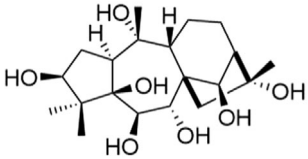
Appendix A reports the compounds of Ericaceae origin identified to date and includes, for each compound, the trivial, IUPAC names and synonyms, physical properties and Ericaceae species. For most of these substances, no data on occurrence in honey or other dietary sources are available. Substances which have been identified or tentatively associated with honey (see Section 3.2) are shown in Table 1.

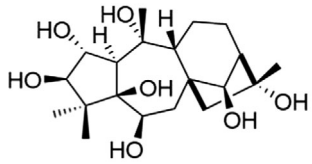
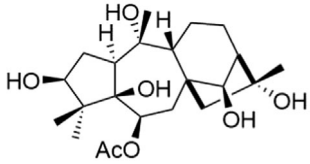
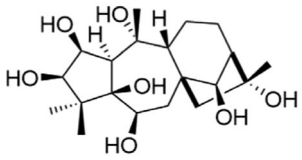
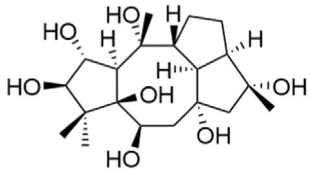
The assessment does not consider synthetic compounds derived from the grayanane backbone.

Table 1: Grayananes identified or tentatively associated with honey

Grayanane[(a)] **Molecular formula** **CAS no.**	**Structure**	**Grayanane[(a)]** **Molecular formula** **CAS no.**	**Structure**
A. Grayananes for which reference standards are commercially available[(b)]			
GTX I $C_{22}H_{36}O_7$ 4720-09-6		GTX III $C_{20}H_{34}O_6$ 4678-45-9	

[1] https://patents.google.com/patent/CN1218635C/en#citedBy
[2] https://patents.google.com/patent/CN101124911B/en

Grayanane[a] Molecular formula CAS no.	Structure	Grayanane[a] Molecular formula CAS no.	Structure
B. Grayananes for which reference standards are not commercially available, but which have been identified in honey[c]			
GTX II[d] $C_{20}H_{32}O_5$ 4678-44-8		GTX VII $C_{20}H_{30}O_4$ 30460-59-4	
GTX VIII $C_{20}H_{30}O_4$ 30460-60-7		GTX XVII $C_{20}H_{30}O_6$ 59740-27-1	
GTX XVIII $C_{20}H_{32}O_4$ 70474-76-9		Rhodomollein XIX $C_{20}H_{30}O_5$ 629615-33-4	
Rhodojaponin III[e],[f] $C_{20}H_{32}O_6$ 26342-66-5			
C. Grayananes for which reference standards are not commercially available, but which are associated with honey[c]			
GTX IV $C_{22}H_{34}O_6$ 30272-17-4		GTX VI $C_{20}H_{32}O_5$ 30460-36-7	
GTX XVI $C_{22}H_{34}O_6$ 59236-87-2		Craiobiotoxin I $C_{20}H_{32}O_5$ 864679-89-0	
Craiobiotoxin II $C_{20}H_{32}O_5$ 864679-90-3		Craiobiotoxin V $C_{20}H_{32}O_5$ 864679-93-6	
Craiobiotoxin VIII $C_{20}H_{32}O_5$ 864680-12-6		Kalmitoxin I $C_{20}H_{34}O_7$ 56663-60-6	

Grayanane[(a)] Molecular formula CAS no.	Structure	Grayanane[(a)] Molecular formula CAS no.	Structure
Rhodojaponin VI $C_{20}H_{34}O_7$ 37720-87-9		Rhodomollein XIII $C_{22}H_{36}O_7$ 54781-72-5	
Rhodomollein XVIII $C_{20}H_{34}O_7$ 292164-20-6		Rhodomollein XIV $C_{20}H_{34}O_7$ 798558-84-6	

(a): For information on the botanical sources of grayananes and their physico-chemical properties, see Appendix A.
(b): Recently, a reference standard on rhodojaponin II became commercially available.
(c): Based on the elementary composition calculated for compounds present in a sample of contaminated *Rhododendron* honey from the Black Sea region in Turkey, These et al. (2015) suggest a list of possible grayanane analogues: In group B, grayanane analogues are shown that have an elementary composition matching with that of one or more of the chromatographic peaks obtained by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) analysis of the sample and for which in the literature only one plausible grayanane analogue is known for that specific elementary composition (or two analogues in case two peaks with the same elementary composition are present). In group C, compounds are shown that have an elementary composition matching with that of one or more of the peaks, but for which more than one plausible grayanane analogue is known. For these compounds, more information is required to ascertain which analogue is actually present in the sample. See also Appendix A for information on grayananes identified in Ericaceae spp.
(d): Structure confirmed based on Lee et al. (2008).
(e): Synonyms: Rhomotoxin, desacetylpieristoxin B.
(f): Based on Scott et al. (1971). These et al. (2015) did not identify this substance in the honey sample they analysed.

1.2.2. Sources

1.2.2.1. Grayananes in plants

Grayananes have been found in plants or parts of plants (leaves, berries, flower nectar and pollen) of some, but not all, genera of Ericaceae, such as *Agarista, Craibiodendron, Kalmia, Leucothoe, Lyonia, Pieris* or *Rhododendron* (Gunduz et al., 2008; EFSA, 2009; Li et al., 2013, 2019; These et al., 2015; Hanson, 2016). Many of these species are also cultivated as ornamental plants (Koca and Koca, 2007; Gunduz et al., 2008). *Rhododendron* is a very large genus, comprising over 1,000 species[3] (Egan, 2015; Shrestha et al., 2018). The other genera comprise between 5 and 40 species.

Geographical origin of grayanane-containing species

An overview (non-exhaustive list) of Ericaceae species that have been investigated with respect to the presence of grayananes is presented in Annex B. The large majority of toxic Ericaceae species are found in the northern hemisphere, in Europe, Asia and North America. Only a limited number of grayanane-containing species are found in Africa, South America and Australia. The largest diversity of grayanane-containing species is present in China and the Himalayan region, followed by south-east Asia and Japan (Shrestha et al., 2018).

Several *Kalmia* species that occur in North America, such as *K. latifolia* (mountain laurel) and *K. angustifolia* (sheep laurel) are well known to cause intoxications in sheep and goats when foraged (Furbee and Wermuth, 1997; Puschner et al., 2001).

In the French island of La Réunion, grayananes have been found in a number of endemic plant species, especially *Agarista salicifolia*.[4]

In Turkey, *Rhododendron* species *R. luteum* and *R. ponticum* are the most common toxic species (Gunduz et al., 2008) and both grow in the Black Sea region (Turkey, 2022 under Documentation as provided to EFSA). *R. ponticum* is particularly invasive (Koca and Koca, 2007).

[3] Global Biodiversity Information Facility (GBIF) website, https://www.gbif.org/species/2883026
[4] Préfet de la Réunion. Direction de l'alimentation, de l'agriculture et de la foret, Service de l'alimentation. Arrêté préfectoral n° 2013-1977. Réglementant la vente de miel en gaufres à la Réunion. http://administration.reunion.gouv.fr/spip.php?article2460

In Europe, *R. ponticum* grows naturally in the high and humid mountains of Spain and Portugal. In France, *R. ponticum* mostly develops in the north-western part of the country and Aquitaine (Manceau, 2015). In the UK and Ireland, *R. ponticum* was introduced as ornamental plant in the late 1800s (Egan et al., 2016). Although the native *R. ponticum* in Spain and Portugal are endangered species, the introduced populations in Great Britain and Ireland are invasive.

Levels of grayananes in plants

The nature and concentrations of grayananes vary considerably from one species to another, but also within and between populations of the same species.

Sahin (2014) studied the GTX III content of *R. ponticum* and *R. luteum* leaves and flowers originating from the Turkish Black Sea region and found that *R. luteum* contained considerably higher GTX III concentrations than *R. ponticum*, in both flowers and leaves (See Table 2 and data submitted by Turkey, 2022 under Documentation as provided to EFSA).

Table 2: GTX III concentrations in flowers and leaves of *R. ponticum* and *R. luteum* in different locations of the Black Sea Turkish region

Rhododendron ponticum		***Rhododendron luteum***	
Flower (mg/kg dw[(a)])	**Leaf (mg/kg dw)**	**Flower (mg/kg dw)**	**Leaf (mg/kg dw)**
< LOQ (=0.04) – 22.9	< LOQ (=0.04) – 36.3	1.74–760	3.35–154

(a): dry weight.

R. ponticum cultivated in botanical gardens in Germany is reported to contain GTX I from 1,300 to 2,300 mg/kg fresh weight (Lechtenberg et al., 2014).

In a study on *R. ponticum* nectar from different geographical origins, the GTX I concentrations in the Iberian nectars were found up to 5,670 mg/kg on a dry weight basis, which are much higher than concentrations reported in the Turkish honey from the same species (Egan et al., 2016). According to Stout (2022, see Documentation as provided to EFSA), the lower levels of GTX I in the Turkish *Rhododendron* nectar might explain why honeybees are able to tolerate the toxins and produce honey in Turkey. Another explanation may relate to the ability of Turkish subspecies of honeybees to metabolise/detoxify the toxins. There is presently no evidence, however, to confirm these statements.

When comparing the GTX concentrations between nectars of *R. ponticum* from introduced populations in Ireland (mostly chemotype B, GTX III dominant) and the native populations in Portugal and Spain (chemotype A, GTX I dominant), no statistically significant variations in the levels of GTX III were observed. On the other hand, the concentrations of GTX I in the introduced populations were found to be significantly lower than in the native populations (mean 810 mg/kg dw for the Irish populations vs. 1,460 mg/kg dw for the Iberian populations) (Egan et al., 2016).

EFSA did not identify studies on the occurrence of grayananes in *Rhododendron* pollen; therefore, the grayanane content in *Rhododendron* pollen is unknown.

Rhododendron species with low or not known content of grayananes

R. ferrugineum grows at high altitudes in the Alps and Pyrenees and is used for the production of honey in the EU. Seephonkai et al. (2021) did not detect grayananes in *R. ferrugineum* extracts analysed by thin layer chromatography (TLC) (limit of detection (LOD) not known). On the TLC plate, the spots of the chloroform-soluble fraction of the plant did not coincide with those of solutions of GTXs I-IV, XIV, XVIII, grayanoside A and C, the aglycone of grayanoside C and kalmitoxin I.

Analysis by GC-MS of extracts of *R. ferrugineum* and *R. hirsutum* (which also flourishes in the Alps) did not show the presence of GTX I. The presence of grayananes cannot be completely excluded in this case, considering the low sensitivity of the method (LOD of 30 mg/kg) (Lechtenberg et al., 2014).

Lucatello et al. (2022) recently reported the presence of low levels of GTX I in Italian alpine *Rhododendron* honey. The presence of GTX I in these honeys was attributed to *R. ferrugineum*, which is an abundant species in the areas of collection.

Other *Rhododendron* species found in Europe are *R.* × *intermedium* which is a hybrid of *R. ferrugineum* and *R. hirsutum* and flourishes in the Alps (Bruni et al., 2016; Sosnovsky et al., 2017), and *R. myrtifolium* in the Carpathians (Sosnovsky et al., 2017). No information on the presence of grayananes in these species is available.

1.2.2.2. Grayananes in honey

For the purpose of the present assessment, the term 'certain honey' shall refer to honey and honey products originated from Ericaceae family plants and containing grayananes. The terms 'mad honey' or 'toxic honey' shall refer to honey-containing grayananes in sufficient amounts to cause intoxication in humans (see Section 3.1.3). These definitions will be used throughout this opinion.

The Panel is aware that the terms 'mad honey' or 'toxic honey' are not exclusive to the honey rich in grayananes but have been previously associated with honeys containing hazardous substances other than grayananes (Zhang et al., 2016). As these other honeys are not derived from Ericaceae species, these are out of the scope of the present assessment and will not be discussed further.

The most commonly studied GTXs in honey are GTXs I, II and III. In a study by These et al. (2015) and more recently by Mulder and de Vries (2022 under Documentation as provided to EFSA), several other grayananes were tentatively identified in honey which may also contribute to the overall risk following oral consumption of 'mad honey' (see Table 1 and Section 3.2).

The presence of other substances in 'mad honey' which might exhibit similar effects to grayananes was considered by the Panel, but no evidence supporting the presence of other potential toxins in Ericaceae honey showing toxicity in humans was identified (Machado et al., 2020; Alkan et al., 2020).

Qualitative characteristics of Rhododendron honeys

Data on the qualitative characteristics of *Rhododendron* honeys suggest that these honeys meet the EU standard[5] requirements for honey (Oddo et al., 2004; Akgün et al., 2021; Alkan et al., 2020).

Although 'mad honey' is reported to have a sharp and bitter taste (Gunduz et al., 2007; Mayda et al., 2018), honey produced from EU *Rhododendron* species has not (Oddo et al., 2004; Italy, 2022 under Documentation as provided to EFSA).

Rhododendron honeys can be monofloral (i.e. containing at least 45% *Rhododendron* pollen; Mayda et al., 2018) or polyfloral. Monofloral honeys such as buckwheat honey and chestnut honey may also contain *Rhododendron* pollen (Panseri et al., 2013, Mayda et al., 2018). Results from analysis of chestnut honey samples submitted by Turkey in the framework of the EFSA *call for stakeholder inputs in relation to the mandate on grayanotoxins in 'certain honeys'* did not show the presence of grayanotoxins in any of the samples analysed (Turkey, 2022 under Documentation as provided to EFSA).

Pollen from *Rhododendron* species has characteristic shape and size and can be distinguished by scanning electron microscopy (SEM). It presents in the form of relatively large tetrads (four individual pollen fused together) of 30–70 μm (Alkan et al., 2020; Sarwar and Takahashi, 2013). Pollen analysis supports the identification of territorial origin of the honey based on co-occurring 'accompanying species' and the degree of purity of the honey samples (Stakeholder and focal points inputs on grayanotoxins, 2022, under Documentation as provided to EFSA). The amount of pollen transported by bees depends on the size of the flower (which can vary geographically, even within species), the bee body size and foraging behaviour (Stout et al., 2015).

Honeys known to contain grayananes

Most 'mad honey' intoxications reported in the literature relate to consumption of *Rhododendron* honeys from the Turkish Black Sea region or Nepal (see Section 3.1.3). In the French island of La Réunion, honeys associated with grayanane intoxications in humans are produced from foraging of endemic plant species, especially *Agarista salicifolia*.[6]

In 370 BC, the Greek historian Xenophon was the first to report intoxications from 'mad honey' consumed by soldiers in their returning journey through Turkey (401–400 BC) (Furbee and Wermuth, 1997; Gunduz et al., 2008; Dusemund et al., 2012).

In Turkey, honey produced in springtime is generally more toxic and may contain higher concentrations of grayanotoxins than the honey produced in other seasons. The ratio of GTXs in honey depends on the temperature (> 12°C in the night) and the precipitation during the flowering period which may increase the water content in the nectar but decreases its yield. Another important factor is the wind direction: the northern humid air from the Black Sea increases the yield; the southern dry air flow instead, decreases the nectar density (Turkey, 2022 under Documentation as provided to EFSA).

[5] Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ L 10, 12.1.2002, p. 47–52.
[6] Préfet de la Réunion. Direction de l'alimentation, de l'agriculture et de la foret, Service de l'alimentation. Arrêté préfectoral n° 2013-1977. Réglementant la vente de miel en gaufres à la Réunion. http://administration.reunion.gouv.fr/spip.php?article2460

Data from the 2022 European Commission's Honey Market Presentation show that in 2021, 173,284 tons of honey of all origins were imported from third countries in the EU. Most imports were from Ukraine (31.1%) and China (27.7%), followed by Mexico (8.9%) and Argentina (8.3%) (European Commission, 2022).

From 2016 to 2020, only a small amount of honey (7–41 tons) was exported from Nepal worldwide (FAOSTAT data), and only 2.7% (4,676 tons) of the 2021 total honey imports in the EU was from Turkey (European Commission, 2022). Although information on the ratio of *Rhododendron* honey to the total imported honey from all sources is not available to EFSA, the total imports of honey from Turkey suggest that the amount of *Rhododendron* honey reaching the EU market should be low. This should also be the case for honey imported from Nepal.

The absence of intoxication cases from *Rhododendron* honeys of EU origin suggests that *R. ponticum* is not among the species intentionally used in apiculture in Europe. This is also the case for Portugal and Spain, despite Spain being the country with the most hives in Europe (European Commission, 2022), and despite grayanane-containing *R. ponticum* flourishing in the Iberian mountains (Egan et al., 2016).

EFSA is not aware if grayananes are toxic to honeybees foraging native *R. ponticum* species in Portugal and Spain but is aware that honeybees do not forage on the introduced species of *R. ponticum* in Ireland, because of the toxicity of the nectar. Therefore, it is unlikely that the Irish honeybees are exposed to the nectar toxins (Stout et al., 2006; Tiedeken et al., 2016) which may explain why honey from north-western Europe has not raised concerns related to GTX occurrence.

Finally, in a recent search for products labelled as 'mad honey' in the Mintel Global New Products database (GNPD),[7] EFSA did not find the marketing of such products in the EU. 'Mad honey' was found to be marketed, though, outside the EU and on the internet.

Rhododendron and other Ericaceae *honeys with low or not known amounts of grayananes*

Honey from *R. ferrugineum* which flourishes in the Pyrenees and the Alps is a domestic *Rhododendron* honey, produced and marketed in the EU. Italian beekeepers bring bees above 1,600 metres in the period between mid-June and the first days of July when the *Rhododendrons* blossom. EU *Rhododendron* honey is a seasonal product with a high degree of purity which presents a delicate and slightly floral/fruity fragrance (Stakeholder and focal points inputs on grayanotoxins, 2022, under Documentation as provided to EFSA).

Based on the available information, it was long thought that honey from *R. ferrugineum* would not contain grayananes (see Section 1.2.2.1; Lechtenberg et al., 2014; Seephonkai et al., 2021). However, a recent study by Lucatello et al. (2022) quantified GTX I and semi-quantified GTX III in Italian alpine *Rhododendron* honey. Of the 125 samples analysed by LC-MS/MS, 30% contained GTX I in a concentration ranging from 0.012 to 0.104 mg/kg (LOQ: 0.01 mg/kg). Some regional variation in the percentage of positive samples was observed: honey originating from Valle d'Aosta and Piemonte contained GTX I above the LOQ in 60% and 49% of the samples, respectively, while for honey from Lombardi and Trentino Alto Adige, this was the case for 14% and 21%, respectively. Most of the samples containing GTX I in levels exceeding 0.04 mg/kg were from the Piemonte and Valle d'Aosta regions. In these areas, *R. ferrugineum* was the most abundant *Rhododendron* species.

No intoxications were reported from honey produced from *Calluna vulgaris* (Heather honey) (Hanson, 2016). In Romania, honey produced by *Calluna vulgaris* in the Apuseni Carpathians area at 1,000–1,500 metres altitude is called Live Black Grass Honey. The honey is produced and collected in small quantities during the last summer period where the highest seasonal temperatures are recorded (Stakeholder and focal points inputs on grayanotoxins, 2022; see Documentation as provided to EFSA).

1.2.2.3. Grayananes in dietary sources other than honey

Only a limited number of Ericaceae species are known to produce grayananes (Koca and Koca, 2007) (Annex B). Cranberries (*Vaccinium macrocarpon*), blueberries (*V. myrtillus*) and lingonberries (*V. vitis idaea*), strawberries from *Arbutus unedo* species are popular foodstuffs originating from plants of the Ericaceae family for which there is no indication of the presence of grayananes (Hanson, 2016). In a study on the safety of cranberry leaves to be used as extracts in beverages, no GTX III was detected (LOD of 0.2 mg/kg) in any of the 75 samples collected at different locations and time points in the USA (Booth et al., 2012).

[7] https://www.gnpd.com/sinatra/gnpd/search/#

There is some evidence on the presence of GTXs in dietary supplements and processed foods (wine) from third countries (Republic of Korea; see Section 3.2) (Hwang et al., 2018), but no information is available regarding GTXs occurrence in similar foods in the EU.

Herbal teas originating from different *Rhododendron* species to which the EU consumers might be exposed may also contain grayananes. Many *Rhododendron* species have been used in alternative medicine, and other ethnopharmacological applications e.g. traditional Chinese or Ayurvedic medicine (Popescu and Kopp, 2013; Dampc and Luczkiewicz, 2015; Shi et al., 2021). See also Annex B. The investigation of the biological and potential medicinal properties of *Rhododendron species*, in particular their diterpenoid constituents, still constitutes a very active field of research (Li et al., 2013, 2019; Wang et al., 2014). Although the presence of such products in the EU market is unknown, EU consumers may still be exposed by purchase of such products online.

1.2.3. Sampling and methods of analysis

For reliable quantification of grayananes, reference standards of high purity are required. Only for GTXs I and III, a limited number of suppliers could be identified. Recently, a new reference standard became available for rhodojaponin II. For all other GTXs and related compounds (Appendix A), no reference standards are currently available.

1.2.3.1. Extraction and clean-up

Grayananes are often isolated from plants by methanol or chloroform extraction followed by further purification and crystallisation (Koca and Koca, 2007). GTXs can be extracted from honey using aqueous solutions, often in combination with methanol as organic modifier to improve extraction (Lee et al., 2008; Kaplan et al., 2014; Kurtoglu et al., 2014). Optionally an organic or inorganic acid is added (These et al., 2015; Aygun et al., 2018) and the sample may be heated to facilitate dissolution of the honey matrix (Sibel et al., 2014; Silici et al., 2016). Solid-phase extraction (SPE) on C18 or polymeric phase cartridges has been used to clean the sample (Lee et al., 2008; Kurtoglu et al., 2014; Sibel et al., 2014; These et al., 2015; Silici et al., 2016). Determination of GTXs in honey using a dilute-and-shoot approach has been described (Kaplan et al., 2014). Extraction of GTXs from biological matrices such as blood and urine require purification and concentration by SPE (Cho et al., 2014; Aygun et al., 2018). Hwang et al. (2018) extracted dietary supplements and homemade wine with methanol and used SPE for sample clean-up (Hwang et al., 2018).

1.2.3.2. Detection/quantification methods

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is most commonly used for the analysis of GTXs in honey and in other biological matrices (Holstege et al., 2001; Lee et al., 2008; Kaplan et al., 2014; Kurtoglu et al., 2014; Sibel et al., 2014; Silici et al., 2016; Aygun et al., 2018; Hwang et al., 2018). Typically, the GTXs are analysed in positive electrospray ionisation mode (ESI), but analysis in negative ESI has also been reported (Zou et al., 2014; Sahin et al., 2015; Silici et al., 2016). Fragmentation spectra of GTXs generally exhibit several losses of water and sodium adducts are often reported (Holstege et al., 2001; Cho et al., 2014; These et al., 2015; Hwang et al., 2018). Quantitative LC-MS/MS analysis of GTXs and related compounds is hampered by the lack of available analytical standards. Most literature reports therefore only include GTXs I and III. Alternatively, LC-HRMS has been used (Zou et al., 2014; These et al., 2015; Hwang et al., 2018). Using LC-HRMS, it could be shown that a sample of 'mad honey' contained up to 15 grayanoid compounds that were tentatively identified and quantified (These et al., 2015). Kurtoglu et al. (2014) reported limits of detection (LOD) in positive ESI for GTXs I and III of 0.2 and 0.1 mg/kg, respectively, in honey. Limits of quantification (LOQ) reported were 0.6 and 0.3 mg/kg, respectively. Kaplan et al. (2014) reported a decision limit (CCα) of 0.012 and 0.010 mg/kg for GTXs I and III, respectively, in honey. An LOD of 0.013 mg/kg and an LOQ of 0.039 mg/kg in honey for grayanotoxin III in negative ESI has been reported (Sahin et al., 2015). For GTXs I and III, based on the lowest validated level, an LOQ of 1 μg/L in rat whole blood could be established (Cho et al., 2014).

1.2.4. Previous risk assessments

In 2010, as a consequence of a case of poisoning reported to the Hessian ministry of consumer protection, the German Federal Institute for Risk Assessment (BfR) evaluated the health risks of honeys which contain GTXs (BfR 2010a and 2010b). Four hours after consumption of an unknown amount of a honey originating from the Turkish Black Sea region, a consumer had developed

disturbance of consciousness and bradycardia requiring medical treatment. Symptoms faded within 24 hours. An analysis performed by the Hessian food control authority revealed that the honey in question contained GTX III in a concentration of 43 mg/kg and pollen originating mainly from *Castanea sativa* and *R. ponticum*. The possible presence of GTX I had not been investigated. BfR only associated the part of the honey derived from *R. ponticum*, and not that derived from *C. sativa*, with the poisoning. Based on a review of the relevant literature on the toxicity in humans and experimental animals, the BfR concluded that a No-observed-adverse-effect level (NOAEL) for GTX III could not be identified. Also, the limit values for GTX I and III in honey, below which they would not be associated with health concerns, could not be derived due to missing information on dose–effect relationships of these substances and their interactions between each other and with other plant components in honeys. According to the evaluation of human data on intoxication due to honeys containing GTXs, intake amounts that have led to poisoning vary considerably and were as low as 5 g of these honeys per person. In recent years, intoxications with GTXs-containing honeys in German-speaking countries were exclusively associated with consumption of Turkish honeys. Based on the available knowledge at that time, the BfR drew the conclusion that *Rhododendron* honey from regions of the Turkish Black Sea coast cannot be regarded as safe. Furthermore, the BfR noted in view of the global occurrence of *Rhododendron* species and other species of the Ericaceae family which contain GTXs, that poisoning would only be expected through honeys collected where such plants dominate the vegetation and not where they are only cultivated as ornamental plants.

In 2013 and 2016, the European Commission submitted to EFSA requests related to an application on the approval of *Rhododendron* honey as 'basic substance' for plant protection purposes in line with Art. 23 of Regulation (EU) No 1107/2009. The applicant's intention was to use honey from *Rhododendron* containing GTXs as a rodenticide. Concentration ranges from 10 to 60 mg/kg for GTX I and 10–300 mg/kg for GTX III in *Rhododendron* honey were proposed by the applicant (EFSA, 2017).

Following consultation with member states, EFSA identified a number of limitations such as the variability of occurrence levels of GTXs and/or the lack of specifications for the product to be marketed which does not allow a consistent composition and/or identify impurities of potential concern to be established. At the time of the evaluation, no information on the marketing of GTXs-containing *Rhododendron* honey in the EU was available neither regarding the composition of such products nor provisions for maximum levels of GTXs in honey from *Rhododendron* (EFSA, 2017). Other limitations relate to the lack of data to support that the available methods of analysis are valid and fit for purpose, and the lack of a proper scientific report to demonstrate, among others, that after being exposed to *Rhododendron* honey in the field, mice die without undergoing unnecessary suffering.

With regard to the effects in humans, EFSA stressed that honey originating from *Rhododendron* spp. containing GTXs cannot be considered compliant with the EU food law since food poisoning was associated with the presence of GTXs in *Rhododendron* honey (also called 'mad honey') (EFSA, 2017). Additionally, note was taken that plant parts of such *Rhododendron* spp. contain natural substances of possible concern for human health as reported in the EFSA Compendium of botanicals (EFSA, 2012).

The Panel noted that in addition to *Rhododendron* spp., plant parts of *Andromeda* spp., *Kalmia latifolia*, *Lyonia* spp., *Pieris formosa* and *Pieris japonica* also contain GTXs and are reported in the EFSA Compendium of botanicals.[8]

1.2.5. Legislation

No maximum levels for GTXs in honey from Ericaceae are set in the EU and no specific regulatory provisions are applicable for GTXs and other grayanane analogues in 'certain honey'. Among Member States, France regulates comb honey ('miel en gaufre') from *Agarista salicifolia* and other endemic plants containing GTXs at regional level (La Réunion) and for the flowering season only (from 1 November to 31 March).[9] In 2010, Germany (BfR) issued a recommendation discouraging consumption of *Rhododendron* honey originating from the Turkish Black Sea coast. This provision, however, is not implemented in a regulation.

[8] https://www.efsa.europa.eu/en/data-report/compendium-botanicals

[9] Préfet de la Réunion. Direction de l'alimentation, de l'agriculture et de la foret, Service de l'alimentation. Arrêté préfectoral n° 2013-1977. Réglementant la vente de miel en gaufres à la Réunion. http://administration.reunion.gouv.fr/spip.php?article2460

2. Data and Methodologies

The EFSA risk assessment on grayananes in 'certain honey' was developed applying a structured methodological approach, which implied developing a priori the protocol or strategy of the full risk assessment and performing each step of the risk assessment in line with the strategy and documenting the process. The protocol in Annex A to this Opinion contains the method that was proposed for all the steps of the risk assessment process, including any subsequent refinements/ changes made.

Information on chemistry, formation in plants, natural sources, analytical methods, a previous BfR assessment, an EFSA assessment on the use of 'mad honey' as plant protection product and legislation was gathered from the literature, assessments by EFSA and other bodies (by checking their original websites of the relevant organisations), from current EU and Member State legislation and a *call for stakeholder and focal points inputs in relation to the mandate on grayanotoxins in 'certain honeys'* launched by EFSA in January 2022 (see Documentation as provided to EFSA). Literature searches were conducted to identify new information in reviews and peer-reviewed publications. Details of the literature searches are given in the external scientific report (DTU, 2020) and the protocol (Annex A).

2.1. Hazard identification and characterisation

Information relevant for the sections under hazard identification and characterisation was identified in the framework of a literature search performed on grayanotoxins (DTU, 2020) and additional literature searches conducted during the risk assessment in order to ensure that the most recent information on grayananes is considered in this Opinion (see also Protocol in Annex A).

The selection of the scientific papers for inclusion or exclusion was based on consideration of the extent to which a study was relevant to the assessment or on general study quality considerations (e.g. sufficient details on the methodology, performance and outcome of the study, on dosing, substance studied and route of administration and on statistical description of the results), irrespective of the results. Limitations in the information used are documented in this Scientific Opinion and considered in the uncertainty analysis.

Benchmark dose (BMD) analysis was carried out using the PROAST software package developed by RIVM (version 70.0) on the web-app 'EFSA-Proastplatform' based on the EFSA Scientific Committee guidance (2017a). To judge the width of the BMD credible interval, the set of criteria and cut-off values described in the latest EFSA Guidance on BMD modelling (EFSA Scientific Committee, 2022) were applied.[10]

2.2. Occurrence data

EFSA gathers data on the occurrence within the call for continuous collection of chemical contaminants occurrence data in food and feed that is issued every year. European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on grayananes.

No data on grayananes occurrence in honey, honey products or other foods were submitted to EFSA following the continuous call for data on chemical contaminants, including grayanotoxins.

A literature review was carried out by an external contractor in order to collect published data (DTU, 2020) and results are presented in Section 3.2.1. Collection and appraisal of occurrence data were carried out as described in the EFSA Protocol (Annex A).

Quantitative results on the occurrence of GTX I, II, and III in 'certain honey' are available in the literature. Some authors analysed honey for one grayanane only (GTX I or GTX III), others for GTX I and III (see Section 3.2.1, Tables 14 and 15). The Panel noted that only eight samples from Nepal were analysed for GTX I, II and III. There are no studies that provide quantitative concentrations of other co-occurring grayananes in the honey samples. Presently, only semi-quantitative information from a limited number of studies is available for grayananes other than GTX I, II and III in 'certain honey' (Kerkvliet, 1981; These et al., 2015; Mulder and de Vries, 2022 under Documentation as provided to EFSA). These other grayananes may contribute to the overall toxicity of these particular

[10] The latest EFSA Guidance on BMD modelling published in 2022 that introduced Bayesian model averaging was published on 25 October 2022. However, its implementation in the EFSA 'R4EU BMD analysis platform' was not available before 16 November 2022 (https://zenodo.org/record/7334435#.Y5osYXbMLD4). Due to the timelines for discussion and adoption of the present Opinion, it was not possible to perform the BMD modelling using this newer version.

honeys. In the absence of analytical standards for all grayananes (except for GTX I and III), the available information cannot be used for exposure assessment purposes. In addition, Nepalese honeys appear to have a different grayanane composition than honeys from the Turkish Black Sea Region which does not support the merging of analytical results for these two different types of honey.

2.3. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. It was first built in 2010 (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011). The latest version of the Comprehensive Database updated in July 2021 was used.[11] Within the dietary studies, subjects are classified in different age classes as follows:

- Infants: > 16 weeks of age to < 12 months old
- Toddlers: ≥ 12 months to < 36 months old
- Other children: ≥ 36 months to < 10 years old
- Adolescents: ≥ 10 years to < 18 years old
- Adults: ≥ 18 years to < 65 years old
- Elderly: ≥ 65 years to < 75 years old
- Very elderly: ≥ 75 years old

Additional surveys provided information on specific population groups: 'Pregnant women' (from Austria, Cyprus, Spain, Latvia, Portugal and Romania), 'Lactating women' (from Estonia and Greece) and Vegetarians (from Romania).

For the acute assessment, food consumption data were available for 51 surveys from 24 countries. The mean and P95 acute consumption (expressed in g per day and g/kg bw per day) were calculated from the distributions of the total daily consumption of each subject in each dietary survey and age class.

For the chronic assessment, food consumption data were available for 47 surveys from 22 countries surveys based on at least two survey days. The mean and P95 chronic consumption (expressed in g per day and g/kg bw per day) were calculated from the distributions of the total daily consumption of each subject in each dietary survey and age class.

In Annex C.1, these dietary surveys and the number of subjects available for the acute exposure assessment are described.

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

It was noted that honey from *Rhododendron* spp. is not specifically addressed in the EFSA food consumption database where most of the eating events relate to the general term 'honey'. Since consumption of *Rhododendron* honey is not reported, a proxy of the consumption of *Rhododendron* honey was used. The Panel decided to use consumption data for honey by merging all consumption events related to 'Honey', 'Honey, monofloral', 'Honey, polyfloral', 'Honey, blended' and 'Minor honey types' (as reported in FoodEx2).

Acute consumption data of 'normal honey' were used for risk characterisation based on a margin of exposure (MOE) and backward calculations under scenario A 'consumption of *Rhododendron* honey as 'normal' honey' (see Section 2.6, 3.4.1 and 3.5.1).

Additionally, a consumption scenario was based on recommended doses for consumption taken from 'mad honey' product labels. A search was made to investigate availability of 'mad honeys' accessible to the EU consumers via the internet market. Examples of recommended doses for consumption were taken from five product labels retrieved from the market (all produced outside the EU) and used in the risk assessment (see Section 3.5.2). The Panel is aware that these portions, as provided on the product labels, are specific to these products and are not necessarily representative of all commercially available 'mad honeys'. In addition, it is uncertain if all consumers follow the

[11] Available online: https://www.efsa.europa.eu/en/data-report/food-consumption-data#the-efsa-comprehensive-european-food-consumption-database

recommendations on the labelling. On the labels, portions were reported in teaspoons or tablespoons; therefore, standard conversion factors of 8 g for 1 full teaspoon of honey and of 22 g for a full tablespoon of honey (LARN, 2014)[12] were applied. Portions in grams were divided by 70 kg as a standard adult body weight. These consumption data were used in backward calculations for risk characterisation under scenario B '*Rhododendron* honey as traditional folk medicine in adults ('mad honey')' (see Sections 2.6 and 3.5.2).

2.4. Food classification

Consumption data present in the EFSA comprehensive consumption database were classified according to the 'Exposure hierarchy' of the FoodEx2 classification system (EFSA, 2015). This is based on a food list of 2,673 entries, referred as 'basic FoodEx2 code', aggregated into food groups and broader food categories in a hierarchical parent–child relationship (up to seven levels). In addition, a catalogue of 28 'facets' is available in order to describe further characteristics of the foods, such as physical state (e.g. powder, liquid) or processing technology (e.g. grinding, milling, crushing).

2.5. Exposure assessment

Because of the lack of specific occurrence data on grayananes and because of lack of consumption data for *Rhododendron* honey and/or other honeys containing grayananes in the EFSA databases and the scarcity of data in the literature, dietary exposure was estimated based on selected concentrations for GTX I and III reflecting concentrations measured in 'certain honey' (see Section 3.4.1).

2.6. Risk characterisation

The general principles of the risk characterisation for chemicals in food as described by the WHO/IPCS (2009) were applied as well as the different EFSA guidance documents relevant to this step of the risk assessment (see Annex A on the Protocol) and the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a).

The acute consumption P95 estimates and selected concentrations for the sum of GTX I and III reflecting the concentrations measured in 'certain honey' were used to derive MOEs based on a reference point (RP) for acute effects derived from BMD analysis. In addition, backward calculations were performed in order to estimate the *highest concentration* of grayananes in 'certain honey' that is unlikely to lead to acute health concerns within the two specific consumption scenarios A and B (see Sections 2.3 and 3.4). When reference is made to these concentrations, the terms *calculated highest concentration(s)* or *highest concentration(s)* will be used in *italics* throughout this opinion in order to distinguish them from the measured highest concentrations in Section 3.2.1.

A comprehensive risk assessment for chronic exposure could not be performed due to the lack of data (see Section 3.4).

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics

Toxicokinetic data are scarce and, therefore, will be considered as a whole and not subdivided into Absorption, Distribution, Metabolism and Elimination (ADME). Four animal and three human studies were identified. The studies are reported per substance, starting with the most potent compound.

In a study using ^{3}H-rhodojaponin III (rhomotoxin) injected intravenously (*i.v.*) into mice the highest radioactivity was found in the gall bladder and bile followed by the liver and kidney. The amount of radioactivity in the gall bladder and bile was four times that found in liver and five times that in the kidney. 52.4% of the dose was excreted by 6 h via the urine and faeces. Approximately 60% was found bound to plasma proteins at 30 min (Liu et al., 1986).

Dong et al. (2014) determined rhodojaponins I, II and III[13] in the plasma of male Sprague-Dawley rats, following oral administration of 21 and 113 mg/kg bw extract of *Rhododendri mollis* flos

[12] https://sinu.it/wp-content/uploads/2019/07/20141111_LARN_Porzioni.pdf

[13] Rhodojaponins I, II and III used as standards in the method of analysis were isolated from extract of *Rhododendri mollis* flos and identified by high-resolution mass spectroscopy and NMR. Their purity determined by LC was higher than 98%.

containing 0.37% rhodojaponin I, 0.53% rhodojaponin II and 0.11% rhodojaponin III, respectively. Blood samples were collected and analysed at 0, 10, 20, 30, 60, 90, 120, 240, 360, 480 and 720 min of exposure. It was noted that a solution of carboxy methyl cellulose was added as a vehicle which might impact the bioavailability of the substances. The kinetic data of rhodojaponin I, II and III in blood are reported in Table 3.

Table 3: Kinetic parameters for rhodojaponin I, II and III in blood of male SD rats (n = 12) (Dong et al., 2014)

Parameter	Rhodojaponin I		Rhodojaponin II		Rhodojaponin III	
Dose (mg/kg bw)[(a)]	0.08	0.42	0.11	0.60	0.02	0.12
C_{max} (ng/mL)	50 ± 10	340 ± 50	50 ± 10	180 ± 50	40 ± 10	190 ± 30
T_{max} (min)	45.0 ± 21.2	54.0 ± 48.3	60.0 ± 32.0	72.0 ± 34.2	52.5 ± 21.2	50.0 ± 36.7
$T_{1/2}$ (min)	63.7 ± 18.2	147 ± 37.8	134 ± 66.1	216 ± 164	83.7 ± 39.6	220 ± 91.1
AUC (ng min/mL)	4,350 ± 480	56,700 ± 10,330	6,090 ± 2,680	47,030 ± 3,410	3,150 ± 860	46,040 ± 8,210

(a): Dose calculated based on the data provided.

Assuming that $AUC_{12h} \approx AUC_{\infty}$ (Mei et al., 2006)[14] and based on the results from Table 3, the Panel estimated the ingested doses for rhodojaponins I, II and III. The ratios of the AUC/dose per substance indicate that rhodojaponin III is approximately three times more bioavailable than rhodojaponin I and three to five times more bioavailable than rhodojaponin II. For rhodojaponin III, the % bioavailability based on AUC was estimated between 3 and 7%.

In addition, the Panel also compared the bioavailability of rhodojaponins I, II and III at the time window associated with the maximum concentration of the substances in blood, considering that the risk from acute effects of grayananes is of relevance to the present risk assessment. By comparing the C_{max}/dose, the Panel noted that rhodojaponin III, with an estimated bioavailability between 10% and 13% based on Cmax, is approximately two to three times more bioavailable than rhodojaponin I and four to five times more bioavailable than rhodojaponin II.

Cho et al. (2014) measured kinetic parameters for GTX I and GTX III[15] in rat whole blood using LC-MS/MS with an LOQ of 1 ng/mL. Male SD rats were exposed to 'mad honey' at 11.5 mg/kg bw (corresponding to 0.30 mg/kg bw GTX I and 0.35 mg/kg bw GTX III), or to pure GTX III at 0.35 mg/kg bw, by gavage. Blood samples (approximately 0.5 mL) were taken at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 240 and 360 min after dosing. The dose and kinetic data are presented in Table 4. The Panel noted that this implies GTX I and III levels in honey of 26 and 30 g/kg, respectively, which is orders of magnitude higher than normally observed. It is unclear whether the rats received more honey or whether the actual dose of GTX I and III was much lower.

Table 4: Kinetic parameters for GTX I and III in whole blood of male SD rats (n=6) (Cho et al., 2014)

Parameter	GTX I (honey)	GTX III (honey)	GTX III (reference standard)
Dose (mg/kg bw)	0.30 ± 0.00	0.35 ± 0.00	0.35 ± 0.00
C_{max} (ng/mL)	8.7 ± 1.3	10.3 ± 1.4	23.0 ± 1.0
T_{max} (min)	20.0 ± 14.6	20.0 ± 15.3	45.0 ± 24.6
$T_{1/2}$ (min)	256 ± 50.2	580 ± 18.2	351 ± 15.2
Area Under Curve (AUC_{6h}) (ng min/mL)	2,842 ± 46.8	6,836 ± 729	12,872 ± 1,372
Mean Residence Time (min)	367 ± 56.6	701 ± 152	511 ± 171
Cl (mL/min)	0.106 ± 0.001	0.051 ± 0.030	0.027 ± 0.017

[14] The authors suggest that when $C_{12h}/C_{max} < 80\%$, the AUC_{12h} can be considered representative of the AUC_{∞} (Mei et al., 2006).

[15] GTX III was administered in the form of a hemi (ethyl acetate) complex with formula $C_{20}H_{34}O_6 \cdot ½C_4H_8O_2$ and molecular weight 414.5. The molecular weight of GTX III is 370.5.

Due to the limitations identified in the study by Cho et al. (2014), no conclusions can be drawn on the bioavailability of GTX I and III following exposure to 'certain honey'.

Accidental poisoning of two mini pigs, kept as pets, with *Pieris japonica* plants resulted in quantifiable levels of GTX I in multiple tissues and body fluids. The highest content was observed in the gastric contents (26.8 ng/g) followed by the bile (19.9 ng/mL) and then the liver (16.8 ng/g) (Pischon et al., 2018).

Aygun et al. (2015, 2018) undertook two separate observational studies to determine the association between GTX levels in blood and urine and the clinical data. In the more recent study (Aygun et al., 2018), concentrations of GTX I and III were measured in 25 patients, presented to the Emergency Medicine Department with symptoms of intoxication (see Table 5 and Section 3.1.3). Due to few cases reported in the study by Aygun et al. (2015), the data reported for blood are not considered for toxicokinetics. Aygun et al. (2018) reported honey intakes between 3 and 30 g. Assuming an average honey intake of 10 g, the Panel estimated that the intake of GTX I and III would be, respectively, 87 and 276 μg for a person weighing 70 kg, or 1 and 4 μg/kg bw based on the mean concentrations of GTX I (8.73 μg/g) and GTX III (27.6 μg/g) in the honeys consumed. When assuming a urine volume of 0.25 L, the average amount of GTX I and III excreted in urine would be, respectively, 7.5 and 97.5 μg, i.e. 9 and 35% of the ingested dose.

Table 5: Mean content of GTX I and III in blood and urine of humans after consumption of 'mad honey' by 25 patients (Aygun et al., 2018)

Toxin	Blood (ng/mL)	Urine (μg/mL)	Honey (mg/kg)
GTX I	4.8 ± 5.6	0.03 ± 0.04	8.7 ± 6.0
GTX III	6.6 ± 8.4	0.39 ± 0.46	27.6 ± 19.0

In a small retrospective observational study aimed at determining the relationship between blood toxin level and clinical response, Choi et al. (2017) measured GTX I and III in six patients (four male and two female) who had consumed *Rhododendron* liqueur (also known as Doo-Gyeon Ju). Initial blood and follow-up (20 h) levels were measured. In the liqueur, the content of GTX I and III was 1.44 and 16.9 ng/mL, respectively. It was estimated from the study that the levels of GTX I and III in blood required to cause hypotension were between 2.52 and 4.55 ng/mL and 17.5 and 27.3 ng/mL. While these concentrations in blood are consistent with other reported data for GTX I and III, there are inconsistencies in the concentrations of the GTX I and GTX III in the liqueur and the subsequent calculations.

No information on the biotransformation of grayananes in mammals is available to EFSA. Tian et al. (2015) investigated, and De Sousa et al. (2018) reviewed the metabolism of diterpenes. For the *ent*-kaurane diterpenoid oridonin, 16 phase I metabolites were identified, following oral administration of the substance in rats. Some of these metabolites were conjugated with glucuronic acid, but no metabolites with an altered diterpene backbone structure were identified.

Considering that grayananes and oridonin are polyhydroxylated diterpenes sharing the same biogenetic precursor (*ent*-kaurane), no metabolic cleavage of the grayanane rings is anticipated. This is supported by studies on the stability of grayananes in rat blood which suggest that the *ent*-kaurane structure is stable (Cho et al., 2014).

Summary on toxicokinetics

In summary, there are few studies on the toxicokinetics of grayananes and they are limited. Thus, the Panel could not draw conclusions on the oral bioavailability of grayananes following exposure to 'certain honey'.

In case reports on human intoxications related to consumption of 'mad honey', GTX I and GTX III were detected in human blood and urine, showing that they are absorbed from the GI-tract.

The Panel noted that most toxicodynamic studies on experimental animals use *i.p.* injection (see Section 3.1.2). However, no toxicokinetic study dealing with *i.p.* injection of grayananes in experimental animals was identified and, therefore, it was not possible to compare the grayanane bioavailability after oral and *i.p.* exposure to 'mad honey' or the pure substance(s).

No information on the biotransformation of grayananes in mammals is available to EFSA. Based on data on the *ent*-kaurane diterpenoid oridonin, no metabolic cleavage of the grayanane rings is anticipated.

3.1.2. Toxicity in experimental animals

This section provides information on both the toxicological and pharmacological effects related to the exposure of animals to grayananes alone, 'certain honey', extracts of 'certain honey' or other extracts. Data are organised in different subsections addressing acute toxicity (i.e. LD_{50} and single dose exposure), and repeated dose toxicity. Only information relevant for the risk assessment is reported in detail.

Among the studies focusing on 'certain honeys', their or other extracts, only those providing quantitative results on the occurrence of grayananes in the tested matrix have been considered further.

3.1.2.1. Acute single-dose toxicity studies

Grayanane acute LD_{50} estimation

Nine acute single-dose studies compared the toxicity of different natural grayananes by the derived LD_{50} values. Results are summarised in Table 6. For each single grayanane analysed, the botanical origin of the substances, route of exposure and species exposed are also reported. Grayananes are grouped in the table according to the route of exposure, starting from oral administration and animal species. In each group, grayananes have been ranked from the most to the least potent.

LD_{50} values have been derived in mice by oral (Hikino et al., 1979), intraperitoneal (*i.p.*) (Scott et al., 1971; Fukuda et al., 1974; Hikino et al., 1976) (Table 6) or subcutaneous (*s.c.*) injection (Kohanawa, 1956) and in guinea pigs by intravenous (*i.v.*) injection (Hotta et al., 1980) (see Table in Appendix B). EFSA did not identify studies deriving LD_{50} values for grayananes in rats.

The main route of exposure used to derive an LD_{50} in mice was *i.p*. Results obtained from *i.p*. studies by different authors analysing the same substance in mice are generally consistent (Table 6). Taking into account all these studies, asebotoxin III was found to be the most potent grayanane. Among grayananes unequivocally identified in honey (see Table 1), the most potent appears to be GTX III, followed by GTX I and II. The potency of GTX II, however, is one order of magnitude lower than the potencies of GTX I and III.

In two studies in mice, Hikino et al. (1976, 1979) used the same source (*L. grayana*), strain (dd) and vehicle (3% gum arabic solution) to derive LD_{50}s for GTX I and GTX III, based on two different routes of administration: *i.p*. and oral. The oral LD_{50} values appeared 3.8 and 5.8 times higher than the *i.p*. LD_{50} values for GTX I and GTX III, respectively (see Table 6). The Panel noted that use of a similar experimental design in the two studies by Hikino et al. reduces the uncertainty in comparing the oral vs. the *i.p*. LD_{50} values for the two grayananes. Such comparison, however, incorporates multiple uncertainties associated with testing male mice only, the gross endpoint assessed in the studies (mortality), the bioavailability of grayananes in the oral studies and the lack of toxicokinetic studies allowing comparison of oral vs. *i.p*. bioavailability (see Section 3.1.1). Because of these limitations and information from other studies reporting results from both *i.p*. injection and oral administration (Popescu and Kopp, 2013; Sibel et al., 2014), the Panel did not extrapolate from *i.p*. to oral dose but considered the information by Hikino et al. (1976, 1979) in the uncertainty analysis.

Disturbance of cardiac rhythm (ventricular fibrillation) and depression of respiration are considered the main causes of death (Scott et al., 1971; Fukuda et al., 1974). At lower doses, bradycardia was observed rather than ventricular fibrillation. Animals that did not die after 1 h recovered (Hikino et al. 1976). The signs of toxicity following GTX I and GTX III administration were similar and independent of the route of administration (Hikino et al., 1979).

After *i.v*. exposure to grayananes, guinea pigs experienced respiratory failure, hypersecretion obstructing the airways and convulsions (Hotta et al., 1980) (see Table B.1 in Appendix B). These effects are consistent with those observed in mice.

The Panel noted that these experiments have been performed mainly in males. Thus, LD_{50} values might be overestimated assuming that females are slightly more sensitive to the acute toxic effect than males (OECD Guidelines 420, 2022). Finally, the purity of the compounds tested in the studies is often not reported.

'Mad honey' acute LD_{50} estimation

Only one study provides an indication of an LD_{50} value for 'mad honey' in mice: It relates to *i.p.* injection of 34 g/kg bw of 'mad honey' extract (from British Columbia) in mice (Scott et al., 1971). The grayanane concentrations in honey were estimated to be 3 and 7 mg/kg for GTX II and III, respectively, together with an unknown amount of rhodojaponin III.[16] Using the data reported by Hikino et al. (1976) (Table 6), the Panel calculated the total grayanane content in honey to be approximately 8.4 mg/kg (range: 7.3–9.3 mg/kg). No GTX I was identified in the 'mad honey' extract (Scott et al., 1971). It was noted that the purity of the compounds tested is not reported and the number of animals in the study is small (three animals).

Table 6: LD_{50} values for naturally occurring grayananes of potential concern in mice. Compounds are ranked in the table based on their potency

Compound		Species/strain/sex	Route	LD_{50} (mg/kg bw)	Reference
Trivial name	**Origin**				
GTX III	*L. grayana*	Mice dd, M	Oral	4.9	Hikino 1979§
GTX I	*L. grayana*	Mice dd, M	Oral	5.1	Hikino 1979§
Asebotoxin III	*P. japonica*	Mice dd, M	*i.p.*	0.1 (0.09–0.11)	Hikino 1976(a),§
Rhodojaponin III	*R. japonicum*	Mice, Kunming, M&F	*i.p.*	0.27 (0.18–0.35)	Li et al. 2015
		Mice dd, M	*i.p.*	0.4 (0.36–0.43)	Hikino 1976(a),§
Asebotoxin I	*P. japonica*	Mice dd, M	*i.p.*	0.51 (0.47–0.55)	Hikino 1976(a),§
Rhodojaponin V	*R. japonicum*	Mice dd, M	*i.p.*	0.75 (0.71–0.83)	Hikino 1976(a),§
GTX III	*Not provided*	Mice ddY, M	*i.p.*	0.8	Fukuda et al. 1974
	L. grayana	Mice dd, M	*i.p.*	0.84 (0.77–0.9)	Hikino 1976(a),§
	Standard	Mice, M	*i.p.*	0.908 (0.805–1.03)	Scott 1971(a)
Lyoniol B	*Not provided*	Mice dd, M	*i.p.*	0.61 (0.56–0.67)	Hikino 1976(a),§
	Not provided	Mice ddY, M	*i.p.*	0.8	Fukuda et al. 1974
GTX VI	*L. grayana*	Mice dd, M	*i.p.*	0.83 (0.75–0.89)	Hikino 1976(a),§
GTX I	Standard	Mice, M	*i.p.*	1.28 (1.11–1.49)	Scott 1971(a)
	L. grayana	Mice dd, M	*i.p.*	1.31 (1.19–1.44)	Hikino 1976(a),§
	Not provided	Mice ddY, M	*i.p.*	1.5	Fukuda et al. 1974
Rhodojaponin VI	*R. japonicum*	Mice dd, M	*i.p.*	1.23 (1.21–1.26)	Hikino 1976(a),§
		Mice, Kunming, M&F	*i.p.*	1.79 (1.32–2.17)	Li et al. 2015
Kalmitoxin I	*P. japonica*	Mice dd, M	*i.p.*	2.28 (2.09–2.49)	Hikino 1976(a),§
Lyoniol A	*L. ovalifolia*	Mice dd, M	*i.p.*	3.01 (2.70–3.25)	Hikino 1976(a),§
	Not provided	Mice ddY, M	*i.p.*	5.8	Fukuda et al. 1974
Pieristoxin G	*P. japonica*	Mice dd, M	*i.p.*	6.52 (5.82–7.17)	Hikino 1976(a),§
GTX II	Standard	Mice, M	*i.p.*	No toxicity up to 4	Scott 1971(a)
	Not provided	Mice ddY, M	*i.p.*	10	Fukuda et al. 1974
	L. grayana	Mice dd, M	*i.p.*	26.1 (23.7–28.7)	Hikino 1976(a),§
GTX V	*L. grayana*	Mice dd, M	*i.p.*	> 100	Hikino 1976(a),§
3-epi-GTX III	*L. grayana*	Mice dd, M	*i.p.*	> 100	Hikino 1976(a),§
GTX XVI	*L. grayana*	Mice dd, M	*i.p.*	> 100	Hikino 1976(a),§
Rhodomollein XIII	*L. grayana*	Mice dd, M	*i.p.*	> 100	Hikino 1976(a),§
Leucothol B	*L. grayana*	Mice dd, M	*i.p.*	> 100	Hikino 1976(a),§

§: extract, purity not provided.
(a): 48 h.

[16] In the paper referred to as desacetyl pieristoxin B.

Other single-dose toxicity studies with individual grayananes

Symptoms after ingestion of honey containing grayananes in humans occur after minutes to hours and are attributable to effects on the cardiovascular and nervous system (see Section 3.1.3). The Panel paid particular attention to animal models addressing endpoints representative of human intoxication and affected shortly after the exposure, i.e. effects on the nervous system (motor activity and coordination, paraesthesia, muscular activity, salivation) and the cardiovascular system (decreased blood pressure and heart rate) (Moran et al., 1954; Cotten et al., 1956; Li et al., 1982; Mao et al., 1982; Ohgaki et al., 1988; Turkmen et al., 2013; Doğanyiğit et al., 2020).

The effects on the nervous and cardiovascular system are summarised in Tables 7 and 8, respectively. Both tables provide the major characteristics of the experimental design, type and characteristics of grayananes analysed, the most effective dose for each time of exposure selected, the ED_{50} value (when available) and the effects observed. Observations were grouped per representative endpoint and results were ranked from the most to the least potent grayanane.

Potential pharmacological effects of grayananes have also been investigated. However, these studies were considered not relevant to the present assessment and will not be considered further (Ohgaki et al., 1987; Gunduz et al., 2012a; Kuru et al., 2014; Li et al., 2015; Niu et al., 2016, 2018; Sun et al., 2019; Zheng et al., 2019).

Neurotoxicity studies (Table 7)

In two studies by Ohgaki et al. (1988), male mice were exposed via *i.p*. to two different doses of GTX III per animal group and per study: 0.1 and 0.25 mg/kg bw. The results showed a significant decrease in locomotor activity (Ohgaki et al., 1987) and coordination (Ohgaki et al., 1988). The effect occurred within 30 min after administration of both doses and remained significant up to 1 h at the highest dose only (Ohgaki et al., 1987). Higher levels of GTX I (0.25 mg/kg bw, i.p.) were required to induce the same reduction in locomotor activity as GTX III (0.1 mg/kg bw). The effects observed were considered significant within a timeframe of up to 1 h following exposure to GTX I (or GTX III). The highest effect was observed within 30 min of exposure. After 1 h of exposure, mice had fully recovered. When GTX II was administered i.p. at 0.25 mg/kg bw, no effect was observed (Ohgaki et al., 1987).

Other effects following i.p. exposure to GTX I, II, III and lyoniol A and B in rodents are piloerection, increased salivation and retching, all occurring between 5 and 30 min, depending on the dose (Fukuda et al., 1974; Fukumoto et al., 1993). In mice, increased grayanane exposure results in washing, grooming, abnormal gait and salivation (Fukuda et al., 1974). According to Fukuda et al. (1974), ataxia is also observed with increasing doses in mice (doses not reported). Salivation was observed in rats exposed to GTX III (Fukumoto et al., 1993).

The studies reporting effects on the nervous system did not allow the identification of an NOAEL since adverse effects were observed at all tested doses, and part of the data is reported as ED_{50} only (Fukuda et al., 1974; Ohgaki et al., 1987, 1988; Fukumoto et al., 1993).

Table 7: Summary of grayananes affecting the nervous system in animal studies[(a)]

Experimental design	Grayanane	Dose mg/kg bw	Time	Effects on the nervous system	Reference
Locomotor activity					
Mice, Std-ddy, M *i.p.* n = 3 *per* group Vehicle: saline	GTX III[(b)]	0.1; 0.25	Within 30 min	Reduction of locomotor activity (0.1 vs. 0.25 mg/kg bw, ns) and coordination The effect is reverted at 60 min, only for 0.1 mg/kg bw (see below)	Ohgaki et al. (1987) Ohgaki et al. (1988)
		0.1	60 min	No effect	
		0.25		Reduction of locomotor activity	

Experimental design	Grayanane	Dose mg/kg bw	Time	Effects on the nervous system	Reference
Mice, Std-ddy, M *i.p.* n = 3 *per* group Vehicle: saline	GTX I[(b)]	0.25	Within 30 min	Reduction of locomotor activity	Ohgaki et al. (1987)
			60 min	No effect	
Mice, Std-ddy, M *i.p.* n = 3 *per* group Vehicle: saline	GTX II[(b)]	0.25	Up to 60 min	No effect	Ohgaki et al. (1987)
Cholinergic effect					
Mice, ddY *i.p.* n = 10–15	Lyoniol B	0.11	Within 30 min	Salivation (ED_{50}) and retching	Fukuda et al. (1974)
Mice, ddY *i.p.* n = 10–15	GTX III	0.15	Within 30 min	Salivation (ED_{50}) and retching	Fukuda et al. (1974)
Mice Std-ddy, M *i.p.* n = 3 *per* group Vehicle: saline	GTX III[(b)]	0.1; 0.25	Not reported	Salivation, vomiting, paralysis of the hind paw	Ohgaki et al. (1988)
Wistar rats, M *i.p.* n = 4 *per* group; control n = 3 vehicle: EtOH/ saline	GTX III[(c)]	0.8	8–15 min	Salivation	Fukumoto et al. (1993)
		2.8	5–10 min	Salivation plus loss of power, strength and respiratory inhibition after 20 min	
Mice, ddY, M *i.p.* n = 10-15	GTX I	0.22	Within 30 min	Salivation (ED_{50}) and retching	Fukuda et al. (1974)
Mice, ddY, M *i.p.* n = 10–15	Lyoniol A	0.7	Within 30 min	Salivation (ED_{50}) and retching	Fukuda et al. (1974)
Mice, ddY, M *i.p.* n = 10–15	GTX II	10–15	Within 30 min	Salivation (ED_{50}) and retching	Fukuda et al. (1974)
Mice ICR, M *i.p.* n = 3 *per* group	GTX II[(d)]	10	Immediately after dosing	Piloerection also coupled with sedation and ptosis	Terai et al. (2003)

M: male; vehicle: absent when not reported; ns: not significant.
(a): All studies include control groups.
(b): Standard provided by another researcher. Standard was identified by NMR, MS and IR.
(c): Standard purified from leaves of *L. grayana*.
(d): Standard derived from dehydration of GTX III.

Cardiovascular toxicity studies (Table 8)

The main cardiovascular effects reported after administration of GTX I (Moran et al., 1954; Cotten et al., 1956), GTX III (Turkmen et al., 2013) and rhodojaponin III (Li et al., 1982; Mao et al., 1982) are decreased blood pressure and bradycardia. Most studies have been performed via i.v. injection in dogs; only one study describes i.p. injection in rats (see Table 8). In order to measure blood pressure, heart rate and contractile force, animals were anesthetised to be cannulated. Grayananes tested in dogs were all injected through the femoral vein.

Reduction of blood pressure and heart rate after i.v. injection of rhodojaponin III in dogs was immediate (0.15–5 min), dose dependent and occurred at 0.004 mg/kg bw (Li et al., 1982; Mao et al., 1982). No decrease in blood pressure was observed at 0.002 mg/kg bw rhodojaponin III, while no data were provided for heart rate (Mao et al., 1982). Decreased heart rate and blood pressure were

also observed after i.v. injection of 0.005 and 0.008 mg/kg bw GTX I in dogs and i.p. injection of 0.2–0.8 mg/kg bw GTX III in rats (Moran et al., 1954; Cotten et al., 1956; Turkmen et al., 2013).

In a study by Turkmen et al., (2013), four groups of six male Sprague Dawley rats were exposed to a single i.p. dose of GTX III[17] at levels 0 (control group), 0.2, 0.4 and 0.8 mg/kg bw. Blood pressure and heart rate were monitored continuously over 2 h with a 20 min baseline measurement before treatment. Values obtained at intervals of 30 min over a timeframe of 90 min after GTX III administration were taken into account to allow comparison with baseline values and between the different doses tested (Turkmen et al., 2013). The Panel noted that heart rate decreased with time in a dose-dependent manner. In particular, 0.4 and 0.8 mg/kg bw GTX III reached the maximal heart rate decrease after 40 and 20 min of exposure, respectively; this effect is mirrored at 60 and 30 min for each dose. This decrease was most notable in the two highest doses.

Yilmaz et al. (2017) studied the variations in respiratory rate, heart rate and arterial blood pressure in six groups of seven male Wistar albino rats exposed to a single i.v. dose of GTX III at levels 0 (control group), 0.005, 0.010, 0.020, 0.050 and 0.10 mg/kg bw. The three parameters were continuously monitored for 60 min. No effect on any parameter was observed at the lowest dose, i.v. (0.005 mg/kg bw). For all other doses, a dose-depended decrease was observed for the first 15 minutes of exposure. Thereafter, recovery to control values occurred for doses at 0.010 and 0.020 mg/kg bw whereas the two highest doses were lethal. The Panel noted that the NOAEL in the study is one order of magnitude lower than the lethal dose. Limited statistical information was reported in the study.

Table 8: Summary of grayananes induced acute cardiovascular toxicity in animal studies[(a)]

Experimental design	Grayanane	Dose mg/kg bw	Time	Cardiovascular system	
Dogs *i.v.* n = 6 *per* group	Rhodojaponin III	0.0035; 0.008	Not reported	Reduces blood pressure (BP) and blood pressure elevation due to carotid pressor reflex (antihypertensive effect). The effect is dose dependent: BP: 0.0035 mg/kg bw: −18%* 0.008 mg/kg bw: −41%* BP elevation: 0.0035 mg/kg bw: −8%* 0.008 mg/kg bw: −41%*	Li et al. (1982)
Dogs *i.v.* n = 3–10 according to experimental group	Rhodojaponin III	0.001; 0.002	0.15–5 min	Not significant decrease blood pressure (BP lowering average rate %: 4.6%; 12.4%, respectively) Heart rate not measured	Mao et al. (1982)
		0.0035; 0.008; 0.02		Decreased blood pressure (BP lowering: average rate %: 60.5%, 64 %, respectively) and heart rate (lowering average rate %: 39%, 70%, respectively). The effect does not significantly increase with the dose (up to 0.02 mg/kg bw)	

[17] GTX III was administered in the form of a hemi (ethyl acetate) complex with formula $C_{20}H_{34}O_6 \cdot 1/2C_4H_8O_2$ and molecular weight 414.5. The molecular weight of GTX III is 370.5.

Experimental design	Grayanane	Dose mg/kg bw	Time	Cardiovascular system	
Dogs *i.v.* n = not reported	GTX I	0.005	5 min	Decreased blood pressure, contractile force and heart rate	Cotten et al. (1956)
Mongrel dogs *i.v.* n = 2–6 according to experimental group	GTX I	0; 0.008	Not reported	Decreased blood pressure	Moran et al. (1954)
Sprague Dawley rats, M i.p. n = 4 per group Vehicle: 2% EtOH N.B. controls were injected only with saline	GTX III[(b)]	0; 0.2; 0.4; 0.8	30, 60 and 90 min	Decreased heart rate in a dose-response manner at 60 min of exposure	Turkmen et al. (2013)
Wistar albino rats, M i.v. n = 7 per group	GTX III[(c)]	0; 0.005; 0.010; 0.020; 0.050; 0.100	0, 2, 5,10,15, 20, 25, 30, 40, 50 and 60 min	Decreased heart rate, respiratory rate and blood pressure in a dose-dependent manner No effect observed at 0.005 mg/kg bw Animals died at doses 0.05 mg/kg bw and 0.10 mg/kg bw	Yilmaz et al. (2017)

M: male; i.v.: intravenous; vehicle: absent when not reported.
*: % of inhibition calculated on reported pressure average values (mmHg).
(a): Where separate controls are not reported, cardiovascular parameters were measured before and after treatment in the same animal.
(b): Standard obtained from Sigma-Aldrich.
(c): Standard obtained from Enzo Life Sciences.

Other toxicity studies

Lipid peroxidation, increase in malondialdehyde coupled to a decrease of catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) were observed in mouse liver and kidney as well as in lung, heart, spleen, testes, epididymis, brain and erythrocytes, between 1 and 24 h after exposure to GTX III i.p at 0.01 mg/kg bw (Cakmak-Arsalan et al., 2020a,b) and at 0.015 mg/kg bw (Sibel et al., 2014). These observations were not coupled to clinical biochemistry parameters or to histological data.

Serum clinical biochemistry parameters indicative of liver damage are reduced after 1 h exposure to GTX I (0.25, 0.5, 1 mg/kg bw) in rats, with 0.25 mg/kg bw GTX I the most effective dose (Ascioglu et al., 2000). No effect was observed for GTX III up to 0.8 mg/kg bw both in rats and mice (Silici et al., 2016; Fukumoto et al., 1993). A significant increase in serum albumin and aminotransferases occurred after 1 h exposure to 2.8 mg/kg bw GTX III in mice (Fukumoto et al., 1993). Histopathological changes in the liver are described by Ascioglu et al. (2000) after exposure to 1 mg/kg bw GTX I. Neither Silici et al. (2016) or Fukumoto et al. (1993) provided data on liver histology. The lack of indication of incidence and severity grade and the absence of biochemical signs of liver toxicity and histopathological data make it difficult to evaluate whether the extent of these changes is significant. Histopathological examinations are available from another study (Kukner et al. 2016), but no significant increase of incidence in the liver congestion, steatosis and inflammatory cells was observed after 24 h from a single dose of 0.01 mg/kg bw GTX III i.p in mice. Overall, examination of clinical biochemistry parameters and histological observations after GTX I and GTX III administration provided inconsistent data (Fukumoto et al., 1993; Ascioglu et al., 2000; Silici et al., 2016).

Effects on the reproductive system

Only one i.p. study was identified with GTX III in male Sprague Dawley rats (Doğanyiğit et al., 2019). Following 24 h of a single administration at doses 0, 0.1, 0.2, 0.4 and 0.8 μg/kg bw, tissue irregularities in testis, appearance of apoptotic cells and decreased tubule diameter were documented but did not show a dose-response. No increase of male sex hormones was observed in the serum. These findings are incomprehensive, and no conclusions can be drawn. The Panel noted, however, that the potential effects of grayananes on the reproductive system require further investigation.

Other single-dose studies with 'mad honey' and other matrices

Details on studies with 'certain' Ericaceae honey and an extract from *Rhododendri Mollis* Flos are reported in Appendix B, Table B.2. Only those studies for which it was possible to estimate the exposure to grayananes are reported.

Single-dose toxicity studies with different doses of honey containing grayananes or extracts of grayanane-containing honey were performed in rats of both sexes (Onat et al., 1991a,b; Kuru et al., 2014; Sibel et al., 2014; Silici et al., 2016; Eraslan et al., 2018), male mice (Cakmak-Arsalan et al., 2020a,b; Kukner et al., 2016). The honey or honey extracts were exclusively derived from *Rhododendron* spp. (Sahin et al., 2018).

Apart from the Chinese drug Nao-Yang-Huaif extract, obtained at the Shanghai market, all other honeys came from the Turkish Black Sea region (Sahin et al., 2018; Onat et al., 1991a,b). Only five out of nine available studies provided quantitative results on the grayanane concentrations in honey, namely GTX I and GTX III. In summary, honey from the Black Sea region has been administered to animals by gavage with grayanane concentrations in honey ranging from 18 to 34 mg/kg for GTX I and from 5.9 to 39.9 mg/kg, for GTX III. The tested honeys have not been analysed for the presence of other grayananes.

Effects on the cardiovascular and respiratory system were observed in albino rats after i.p. injection of *Rhododendron* honey extracts obtained from *R. ponticum* honey which had previously led to human intoxication with severe bradycardia and hypotension. No indication of the grayanane content was provided (Onat et al., 1991a,b). Male and female albino rats exposed to 50, 1,000, and 5,000 mg/kg bw of the *R. ponticum* honey extract, via i.p. injection, displayed a dose-dependent reduction of both heart and respiratory rate. The effects were significantly different from the controls at dose levels of 1,000 and 5,000 mg/kg bw 15 min after exposure to the honey extract. No effects were observed at the lowest dose. The observed bradycardia and respiratory rate depression were restored by a subsequent i.p. injection of atropine (Onat et al., 1991a,b).

Effects in rodent liver from single-dose exposure to 'mad honey' involved altered lipid/protein ratio and lipid peroxidation (Sibel et al., 2014; Eraslan et al., 2018; Cakmak-Arslan et al., 2020a). Kukner et al. (2016) reported significant congestion and steatosis in the absence of necrosis. The data refer to a single sample of 'mad honey'. Oxidative stress has been observed in the kidney, lung, heart, spleen, testes, epididymis and brain (Sibel et al., 2014; Silici et al., 2016; Eraslan et al., 2018; Cakmak-Arslan et al., 2020b). It was noted that most of the studies assessing 'mad honey' also investigated the single grayananes (see subsection on single-dose studies on grayananes). Overall, these studies were considered of limited value due to experimental deficiencies and were, therefore, not useful for risk assessment. In addition, these limited observations in liver and kidney in rodents have not been confirmed in humans and will not be considered further.

Regarding matrices other than honey, Dong et al. (2014) investigated the effects of a mixture of rhodojaponins I, II and III[18] in male Sprague-Dawley rats following oral administration of 21 and 113 mg/kg bw extract of *Rhododendri mollis* flos containing 0.37% rhodojaponin I, 0.53% rhodojaponin II and 0.11% rhodojaponin III, respectively. The Panel estimated the intake for the low- and high-dose groups to be 0.08 and 0.42 mg/kg bw rhodojaponin I, 0.11 and 0.60 mg/kg bw rhodojaponin II and 0.02 and 0.12 mg/kg bw rhodojaponin III. Severe, reversible, poisoning symptoms were reported in the high-dose group (vomiting, muscle rigidity, convulsion). These effects were not noted in the low-dose group. Death occurred in 6 out of 20 rats treated with the highest dose. Heart rate, left ventricular systolic and left ventricular diastolic pressure decreased suggesting cardiac dysfunction. Plasma levels of LDH and CK-MB considerably increased in the treated animals whereas only a small increase was observed in AST levels compared to the control group.

Details of these studies are reported in Appendix B, Table B.2.

[18] Rhodojaponins I, II and III used as standards in the method of analysis were isolated from extract of *Rhododendri mollis* flos and identified by high-resolution mass spectroscopy and NMR. Their purity determined by LC was higher than 98%.

Summary of acute animal toxicity studies after exposure to grayananes or preparations containing grayananes

Acute toxicity studies have been performed mainly in male rodents exposed to grayananes via i.p. injection or to 'mad honey'/'mad honey extracts'/other preparations by oral administration. Despite two different routes of exposure to grayananes have been used, similar acute effects were observed. These observations support a role for grayananes in 'mad honey' toxicity.

Among the different effects reported in acute animal toxicity studies, impairment of the nervous system and cardiovascular effects are those considered the most relevant, since occurring shortly after exposure (within 30/60 min) and are consistent with human intoxications due to 'mad honey' intake. In addition to these effects, different blood chemistry parameters indicative of kidney and liver damage were also altered. Nevertheless, these results were often inconsistent, not supported by histological changes and not representative of human intoxication.

Studies reporting effects on the nervous system (reduced locomotor activity and occurrence of cholinergic effects) do not allow the identification of an RP.

Available data allowed the calculation of an RP only for GTX III. The same value applies also for GTX I which appears to have similar toxicity. To derive RPs for the other grayananes relative potency factors (RPFs) should apply (see Section 3.1.5).

3.1.2.2. Repeated dose toxicity studies

Repeated dose studies with individual grayananes

Short-term exposure

Hikino et al. (1979) examined the following clinical parameters in rodents following exposure to GTX I and GTX III by oral gavage for 3 months at dose levels 0, 0.05, 0.25 and 1.0 mg/kg bw per day in mice, and at dose levels 0, 0.05 and 0.25 mg/kg bw per day in rats: total leucocyte and erythrocyte counts, serum glutamic–oxalacetic transaminase (GOT) and glutamic–pyruvic transaminase (GPT), alkaline phosphatase (AIP) and lactate dehydrogenase (LDH). Some of those parameters changed in a dose-dependent manner. Macroscopic examinations in livers, kidneys and spleens at necropsy did not show essential differences in the organ appearance and no notable organ alterations were observed in the histological examinations of the control group and groups of animals exposed to GTX I or III. Decrease in liver and spleen weights was observed following administration of *Rhododendron* extracts and the toxins at 1 mg/kg bw.

Ascioglu et al. (1996) examined the effects of i.p. injection of GTX I (0, 0.075 and 0.15 mg/kg bw per day) on Swiss albino male rats every day for 3 months on hepatic, renal and heart function. Urine analysis, serum analysis and histopathological examination of the liver, kidney and heart were performed. The highest dose of GTX I decreased the mean body weight gain, caused proteinuria and haematuria, increased glutamic pyruvate transaminase (GPT) and LDH4 activities and decreased total serum protein. No histopathological changes were observed.

In a study on male Sprague Dawley rats, the cardiotoxic effects (Doğanyiğit et al., 2020) and testicular effects (Doğanyiğit et al., 2019) of exposure to GTX III were investigated. Rats were exposed to GTX III via i.p. injection every day for 3 weeks. Doses administered were 0.1, 0.2, 0.4 and 0.8 μg/kg bw per day. Changes in brain natriuretic peptide (BNP), interleukin IL-1β, IL-6 and tumour necrosis factor-α (TNF-α) and apoptosis in heart tissue were reported. These changes, however, were small, variable and the dose–response relationship was unclear. Histological changes in the testes were evaluated by Johnsen's Testicular Biopsy Score (JTBS).[19] After an initial decrease in the score, the response curve flattened. The diameter of the seminiferous tubules decreased at the two highest doses. Vacuolisation of the testes was seen at 0.1, 0.4 and 0.8 μg/kg bw per day. No statistically significant changes in the levels of LH, FSH or testosterone were observed in the serum and the testes, respectively. Apoptosis was observed in the testicular cells.

No teratogenic effect was observed after i.p. injection of GTX I (0.15 mg/kg bw per day) in mice for three consecutive days (group 1: 6–8 Gestation Days (GD); group 2: 9–11 GD, group 3: 12–14 GD) and injection into the embryogenic coelom one day after egg incubation in chicks (Kobayashi et al., 1990).

[19] https://www.karger.com/Article/Abstract/178170

Repeated dose studies with *Rhododendron* Honey

Two repeated dose toxicity studies with 'mad honey' were identified.

Following exposure of Sprague-Dawley female rats to 'mad honey' by oral gavage for eight consecutive days at doses of 0, 0.3, 0.6, 1.2 and 2.4 g/kg bw per day (equivalent to 0.012, 0.024, 0.048 and 0.096 mg GTX III /kg bw per day) a dose-response related increase in a number of clinical chemistry parameters such as AST, ALT, LDH, ALP, CK and CK-MB was observed in the serum (Sahin et al., 2016). There were no further examinations to support that these alterations were toxicologically relevant.

In a study on male Wistar rats, Eraslan et al. (2018) found significant variations in the levels of oxidative stress markers in animals exposed to 'mad honey' by gavage at 5 g/kg bw per day for 60 days. The honey contained GTX I and III at 34 and 6.5 mg/kg, respectively. Thus, the Panel estimated the daily exposure to be 0.17 mg/kg bw per day GTX I and 0.03 mg/kg bw per day GTX III. Malondialdehyde and nitric oxide increased in the liver, kidney, brain, testes, heart and plasma. Superoxide dismutase increased in the same organs except for the brain where a decrease was seen. Catalase increased in the heart and erythrocytes whereas it decreased in the liver, kidney, brain and testes. GSH peroxidase increased in the liver, brain and erythrocytes and decreased in the kidney, testes and heart tissues. 4-Hydroxynonenal increased in the serum.

Summary of repeated-dose toxicity studies

The limited information from subacute and subchronic studies in rodents suggests that several biochemical parameters are altered following exposure to individual grayananes and/or 'mad honey', sometimes in a dose-dependent manner. None of these changes, however, were considered as toxicologically relevant because the magnitude of the changes was small. Histological examinations were only performed in some of the studies showing changes in biochemical parameters in the liver, kidney or heart, and no histological changes were observed in any of these organs.

Histological findings in the testes from repeated dose *i.p*. injection of GTX III in rats at doses from 0.1 to 0.8 μg/kg bw per day for 3 weeks suggest effects on the testes.

Based on the above, no RP for subacute and/or subchronic exposure could be identified from these studies.

3.1.2.3. Genotoxicity studies

Cucer and Eroz (2010) investigated the mutagenic effects of GTX II and GTX III on cultured human lymphocytes. 10 male and 10 female subjects aged between 26 and 45 donated blood which was used for the micronucleus (MN) test and chromosome breakage analysis. GTX II and GTX III were added to the cultures at 0.35 and 3.52 mg/mL for 72 h. An increase in micronucleated cells was seen with age, but no genotoxic effects of GTX II and GTX III were observed in either the MN assay or in the number of cells with chromosomal breaks in comparison with the negative control. The positive control (mitomycin C) did show higher values in both assays.

In a study on male Wistar rats, Eraslan et al. (2018) evaluated the effect of 'mad honey' on oxidative stress, hepatic metabolism and genotoxic damage under acute (12.5 g honey/kg bw single dose, n = 12), subacute (7.5 g honey/kg bw per day for 21 days, n = 12) and subchronic (5 g honey/kg bw per day for 60 days, n = 12) dosing conditions. The concentration of GTX I and GTX III was, respectively, 34 and 6.5 mg/kg in the 'mad honey' used. Thus, the Panel estimated the daily exposure, given by gavage, in the acute study to be 0.43 mg/kg bw GTX I and 0.08 mg/kg bw GTX III, in the subacute study 0.26 mg/kg bw GTX I and 0.05 mg/kg bw GTX III, and in the subchronic study 0.17 mg/kg bw GTX I and 0.03 mg/kg bw GTX III. Multiple markers of oxidative stress were measured in liver, kidney, brain, heart, testes and plasma or erythrocytes (results reported in Section 3.1.2.2). The authors report positive results in the Comet assay expressed as increases in tail intensities. These were observed in the kidney and heart (acute study), in the blood, liver and kidney (subacute study) and in the liver and kidney (subchronic study). There were no changes in the incidence of micronucleated cells with any treatment. The reporting of genotoxicity data was however poor, including major deviations from the recommended reporting of the primary comet assay descriptors. Overall, the evidence for genotoxicity was unclear.

A more recent study by Rasgele et al. (2021) measured the genotoxic effects of *Rhododendron* honey using three *in vivo* mammalian bioassays, e.g. chromosome aberration (CA) and micronucleus (MN) assays in bone marrow cells and induction of sperm abnormalities. Male mice (*M. musculus*) were exposed to honey at 25, 50 and 75 mg/kg bw by gavage for 24 and 48 h. *Rhododendron* honey

contained 30 mg/kg GTX I and 7 mg/kg GTX III. Thus, the Panel estimated the exposure to GTX I from honey to be, respectively, 0.75, 1.50 and 2.25 μg/kg bw, and exposure to GTX III was, respectively, 0.18, 0.35 and 0.53 μg/kg bw. In addition, GTX III was also given at 0.01 mg/kg bw by i.p. injection. A significant increase in the number of cells with CAs was observed at all the tested concentrations of *Rhododendron* honey, while negative results were obtained with GTX III. The mitotic index was not changed in any experimental group. There was a concentration-related increase in the percentage of total sperm abnormalities, but this increase was not statistically significant compared to the control. In the MN assay, the highest dose of *Rhododendron* honey and the isolated GTX III increased the number of micronucleated polychromatic erythrocytes (MNPCE) with a decrease in the ratio of polychromatic/normochromatic erythrocytes (PCE/NCE) at both times indicating a genotoxic effect in the presence of bone marrow toxicity. The Panel noted that the study was clearly positive and there was sufficient evidence of bone marrow toxicity because exposure to GTX III (0.01 mg/kg) and *Rhododendron* honey (75 mg/kg) decreased the ratio of PCE to NCE for the two exposure periods analysed (EFSA Scientific Committee, 2017b). Although the study showed some deviation from the recommendations in the OECD TG 475 guideline (e.g. GTX III injected *i.p.*, reporting of the results), the data were clearly positive for (i) a dose-related increase in the percentage of bone marrow cells with DNA breaks, i.e. chromatid fusions, following *in vivo* exposure to RH; (ii) increase of micronuclei at the highest dose of *Rhododendron* honey and at 0.01 mg/kg bw GTX III following both a 24 and 48 h treatment.

Predictive tools were also used to identify structural alerts for genotoxicity and generate predictions on the mutagenic potential of grayananes. For this purpose, structures of three non-epoxidised grayananes (GTX I, GTX II and GTX III) and two epoxidised grayananes (asebotoxin III and pieristoxin G) were selected for assessment. No structural alerts for mutagenicity were identified in ToxTree and QSAR toolbox for the three non-epoxidised grayananes. Regarding GTX I and III, the predictions of the four VEGA models were inconsistent suggesting that GTX I and GTX III are non-mutagenic but with a low reliability (score of 0.53). The result for GTX II is that the substance is unlikely to be mutagenic with an overall consensus score of 0.75. The presence of the epoxide on the molecules of asebotoxin III and pieristoxin G was identified as a structural alert. Whereas the three non-epoxidised grayananes had negative (even though partially inconsistent) predictions, the potentially reactive epoxide moieties were flagged for two substances. The above pattern of QSAR predictions does not allow to derive conclusions on the genotoxicity of grayananes.

In summary, there is positive evidence for the induction of chromosomal damage by grayananes present in *Rhododendron* honey (given by gavage) and GTX III (given *i.p.*) from an *in vivo* MN study. This is confirmed by a chromosomal aberration assay performed by the same authors on the same honey sample (Rasgele et al., 2021). In line with the Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA, 2011), if the first *in vivo* test is positive, the substance should be considered as an *in vivo* genotoxin. Presently, there is no evidence allowing to conclude that grayananes act via a direct or indirect mode of action.

3.1.3. Observations in humans

The data presented here reflect the existing knowledge from case reports on pharmacological and toxic effects after ingestion of grayananes containing honeys, plants, plant parts or their preparations. Furthermore, studies in volunteers investigating the pharmacological effects of grayananes are addressed. In the following sections, focus is put on reports with relevance for the risk assessment of the intake of grayananes with food, e.g. giving information on dose–effect relationships.

3.1.3.1. Case reports on acute intoxications due to ingestion of 'certain honey'

Information from the literature

Acute intoxications in humans due to consumption of honey containing grayananes have been mainly associated with honeys originating from the Turkish Black Sea region also referred to as 'mad honey', Pontic honey or Turkish wild honey. In this area, toxic *Rhododendron* species (*R. ponticum* and *R. luteum*) containing grayananes are widespread (e.g. Gossinger et al., 1983; Koca and Koca, 2007; Gunduz et al., 2008; Demir and Kahveci, 2012; Jansen et al., 2012). However, acute poisoning cases have also been associated with ingestion of certain Ericaceae honey from other countries, e.g. in North America (Gunduz et al., 2008; Jansen et al., 2012), from Nepal (Jauhari et al., 2009; Shrestha et al., 2009; Jansen et al., 2012; Sohn et al., 2014; Lim et al., 2016) or from Reunion Island (honey collected from *Agarista salicifolia* (synonym: *Agauria salicifolia*)) (Weber et al., 2009). Acute intoxications

reported in the last decades from European countries or South Korea are associated with imported honey from Turkey or Nepal, respectively (e.g. BfR, 2010b; Jansen et al., 2012; Ullah et al., 2018).

Known doses of ingested *Rhododendron* honey causing acute intoxications vary (see also Table 9). In the study of Yilmaz et al. (2006), doses ranged between 5 and 30 g; however, honey intakes as high as 150 g leading to poisoning are also described (e.g. Aygun et al., 2015; Jauhari et al., 2009).

The majority of intoxications refer to males aged between 40 and 65 years (Silici and Atayoglu, 2015; Ullah et al., 2018). The higher prevalence may be explained according to Ullah et al. (2018) by the higher use of 'mad honey' as a sexual stimulant as well as a higher rate of hypertension in males of this age group. Paediatric case reports of *Rhododendron* honey intoxication are scarce. In the case of a 5-month-old male, vomiting and drowsiness occurred 4 h after he was given two teaspoons of 'mad honey' against coughing (Bilir et al., 2016). He recovered after fluid replacement. No further details on origin and composition of the honey ingested are reported. Yavuz et al. (1991) mention the intoxication of a 7-month-old infant without giving any details. Uzun et al. (2013) described that, 2 h after consumption of 150 mL of 'mad honey', a 15-year-old boy experienced hypotension (75/50 mm Hg) and sinus bradycardia (pulse: 45/min) apart from weakness and dizziness. He was treated in an emergency department and discharged one day later.

Resulting symptoms, which occur within minutes up to 5 hours, are mainly associated with the binding of grayananes to the voltage-dependent sodium channels leading ultimately to depolarisation of cell membranes (see Section 3.1.4) and thus affect nerves, muscle (skeletal and heart) and the central nervous system. Although the symptoms of 'mad honey' poisoning resemble those of the 'cholinergic toxidrome' (Ullah et al., 2018; Gunduz et al., 2012b) investigations in humans could not confirm that both syndromes share a common underlying mechanism (Gunduz et al., 2012b). Initial symptoms of acute poisoning are dizziness, nausea, vomiting, salivation, perspiration and weakness, followed by diarrhoea, paraesthesia, blurry vision and typically hypotension and bradycardia. Higher doses may lead to complete atrioventricular block, convulsions, mental confusion, agitation, loss of consciousness and respiratory depression (e.g. Koca and Koca, 2007; Gunduz et al., 2008; Bostan et al., 2010; Demir et al., 2011; Jansen et al., 2012; Erenler, 2016; Aygun et al., 2018; Ullah et al., 2018). Chest pain, hyperthermia, hypothermia and throat burn have also been reported (Yilmaz et al., 2006; Aygun et al., 2016; Tekinsoy et al., 2017; Ullah et al., 2018).

Hospitalisation in cases of *Rhododendron* honey acute intoxications is most frequently due to its cardiac manifestation. Sinus bradycardia is described to be the most frequent cardiac rhythm disorder that has been observed in *Rhododendron* honey poisoning (e.g. Okuyan et al., 2010; Bayram et al., 2012; Erenler, 2016). In addition, apart from varying degrees of atrioventricular blocks (e.g. Cagli et al., 2009; Erenler, 2016; Ullah et al., 2018), second-degree heart block (Weiss et al., 2010), nodal rhythms (Biberoglu et al., 1988, Gunduz et al., 2006; Aliyev et al., 2009a; Okuyan et al., 2010), atrial fibrillation (Bayram et al., 2012; Cakar et al., 2011; Kalkan et al., 2012; Osken et al., 2012; Sohn et al., 2014), blocked left bundle branch with extreme QT prolongation (Sayin et al., 2011) or with ST segment elevation (Sayin et al., 2012), asystole (Gunduz et al., 2007), interatrial block (Ösken et al., 2021), Wolff–Parkinson–White syndrome (Biberoglu and Komsuoglu, 1988) and myocardial infarction (Akinci et al., 2008; Ocak et al., 2013) have been reported following *Rhododendron* honey ingestion.

Severity of symptoms and the onset of signs were reported to depend on the amount of honey consumed (Jauhari et al., 2009; Bostan et al., 2010). Atropine is administered intravenously for emergency medical care in severe cases of poisoning (e.g. Özhan et al., 2004; Gunduz et al., 2007, 2008; Bostan et al., 2010; Jansen et al., 2012). Symptoms may persist from a few hours to a few days (e.g. Aygun et al., 2018). In general, patients recover within 1–2 days (e.g. Jauhari et al., 2009; Jansen et al., 2012). Though no fatalities from *Rhododendron* honey poisonings have been reported in modern times (Gunduz et al., 2008; Bostan et al., 2010; Silici and Atayoglu, 2015; Ullah et al., 2018), they may be life-threatening in individuals with cardiac complications (e.g. Koca and Koca, 2007; Gunduz et al., 2008; Ullah et al., 2018).

Rhododendron honey from the Black Sea region has a traditional use as folk medicine e.g. against gastrointestinal disorders, hypertension, heart failure, diabetes, asthma, cough and as an aphrodisiac (e.g. Ullah et al., 2018; Okuyan et al., 2010; Ugur et al., 2019). Gunduz et al. (2006, 2007, 2008) reported that in this area, inhabitants are familiar with these uses of *Rhododendron* honey and its symptoms of poisoning and are able to distinguish these honeys from others, since they induce a sharp burning sensation in the throat. However, acute intoxications with *Rhododendron* honeys have also been reported from consumptions which are in general typical for honey and not related to any uses as alternative medicine, such as at breakfast e.g. on slices of bread or in tea (Kerkvliet, 1981; Nassibou et al., 2020).

From the literature search on human observations more than 1,000 cases of intoxications after ingestion of certain Ericaceae honeys by individuals (e.g. Sohn et al., 2014; Silici and Atayoglu, 2015; Ullah et al., 2018), families or groups (e.g. Jauhari et al., 2009; Weber et al., 2009) were retrieved. Details will only be given below for reports in which analytical data on the presence of grayananes in the ingested honey were available or where systematic studies investigating the toxicological effects in patients were conducted.

Only a few of these published intoxication cases provide quantitative information on the content of grayananes in the honey (Kerkvliet, 1981; Aygun et al., 2015; These et al., 2015; Nassibou et al., 2020). Details of these reports are shown in Table 9.[20] Information on underlying diseases of the patients is only given in one of the cases, information on medication of the patients is completely missing.

[20] From the report by Aygun et al. (2015), the GTX content in the honey consumed was quoted as indicated in the text of the publication, since in its abstract and Table 5, the indication of the units was obviously subject to an error.

Table 9: Reports on cases of acute intoxications with Ericaceae honey allowing to estimate the intake of grayananes

Affected subjects (age, bw, medical history when reported)	Type of honey as described (geographical and botanical origin when reported)	Intake of honey in g per person	Analytes, measured concentration of grayananes in the honey (mg/kg)	Estimated single dose of grayananes (μg/kg bw)[a]	Levels in blood (b) and urine (u)	Onset of symptoms (min/hours after consumption)	Symptoms/ Outcome (blood pressure (bp, in mmHg) and heart rate (hr, in beats/ min) when reported)	Reference
4 male students	Honey from Eastern Nepal[b], habitat of *R. arboreum* and *R. campanulatum*	Day 1: 10 g/ pers. Day 2: 20–100 g/pers. Day 3: 'a little in the tea'	GTX I, II, III: n.d. Two 'grayanotoxin analogues' (not identified): 30 mg/kg	Two 'grayanotoxin analogues' (not identified, other than GTX I, II, III): Day 1: 4.3 μg/kg bw Day 2: 8.6–43 μg/ kg bw Day 3: < 4.3 μg/kg bw	n.r.	Day 2: 25 min	Day 1: headache and feeling 'high' Day 2: all experienced pain in the chest and around the heart, tingling in the fingers and other extremities, looseness of some muscles, feeling 'high', dizziness, bradycardia (hr: 38), fainting. After 3 h, the symptoms disappeared Day 3: headache and feeling 'high'[c]	Kerkvliet, 1981
1 male, 56-year-old	'Wild Turkish honey'	Two tablespoons, amounting to approx. 25 g/pers.	GTX III[d]: 54.0 mg/kg Total grayananes[e]: 358 mg/kg	GTX III: 19.3 μg/kg bw Total grayananes[e]: 128 μg/kg bw	n.r.	2 h	Presented in hospital with severe bradycardia and loss of consciousness causing traffic accident	These et al., 2015

Affected subjects (age, bw, medical history when reported)	Type of honey as described (geographical and botanical origin when reported)	Intake of honey in g per person	Analytes, measured concentration of grayananes in the honey (mg/kg)	Estimated single dose of grayananes (μg/kg bw)[(a)]	Levels in blood (b) and urine (u)	Onset of symptoms (min/hours after consumption)	Symptoms/ Outcome (blood pressure (bp, in mmHg) and heart rate (hr, in beats/ min) when reported)	Reference
1 male, 67-year-old	'Mad honey' from Arsin, Turkish Black Sea region, habitat of *R. ponticum* and *R. luteum*	108 g/pers.	GTX I: 0.61 mg/kg GTX III: 2.53 mg/kg GTX I+III: 3.13 mg/kg	GTX I: 0.94 μg/kg bw GTX III: 3.90 μg/kg bw GTX I+III: 4.84 μg/kg bw	GTX I: b: 86.2 ng/mL u: 0.16 mg/mL GTX III: b: 8.19 ng/mL u: 1.90 mg/mL	2 h	Presented in emergency department with dizziness, nausea, hypotension (bp: 60/40), bradycardia (hr: 42)	Aygun et al., 2015
1 male, 51-year-old	'Mad honey' from Sürmene, Turkish Black Sea region, habitat of *R. ponticum* and *R. luteum*	150 g/pers.	GTX I: 7.77 mg/kg GTX III: 11.0 mg/kg GTX I+III: 18.8 mg/kg	GTX I: 16.7 μg/kg bw GTX III: 23.6 μg/kg bw GTX I+III: 40.3 μg/kg bw	GTX I: b: 19.3 ng/mL u: 0.58 mg/mL GTX III: b: 2.77 ng/mL u: 1.91 mg/mL	2 h	Presented in emergency department with dizziness, nausea, hypotension (bp: 90/60), bradycardia (hr: 40)	Aygun et al., 2015
1 male, 70-year-old	'Mad honey' from Of, Turkish Black Sea region, habitat of *R. ponticum* and *R. luteum*	100 g/pers.	GTX I: 0.45 mg/kg GTX III: 2.98 mg/kg GTX I+III: 3.44 mg/kg	GTX I: 0.64 μg/kg bw GTX III: 4.25 μg/kg bw GTX I+III: 4.90 μg/kg bw	GTX I: b: 8.13 ng/mL u: 0.19 mg/mL GTX III: b: 7.01 ng/mL u: 0.80 mg/mL	2 h	Presented in emergency department with dizziness, nausea, hypotension (bp: 80/50), bradycardia (hr: 35)	Aygun et al., 2015
1 female, 40-year-old	'Mad honey' from Yomra, Turkish Black Sea region, habitat of *R. ponticum* and *R. luteum*	16 g/pers.	GTX I: 9.90 mg/kg GTX III: 16.9 mg/kg GTX I+III: 26.8 mg/kg	GTX I: 2.26 μg/kg bw GTX III: 3.86 μg/kg bw GTX I+III: 6.12 μg/kg bw	GTX I: b: 8.91 ng/mL u: 0.86 mg/mL GTX III: b: 1.69 ng/mL u: 3.38 mg/mL	30 min	Presented in emergency department with dizziness, nausea, hypotension (bp: 80/40), bradycardia (hr: 35)	Aygun et al., 2015

Affected subjects (age, bw, medical history when reported)	Type of honey as described (geographical and botanical origin when reported)	Intake of honey in g per person	Analytes, measured concentration of grayananes in the honey (mg/kg)	Estimated single dose of grayananes (µg/kg bw)[a]	Levels in blood (b) and urine (u)	Onset of symptoms (min/hours after consumption)	Symptoms/ Outcome (blood pressure (bp, in mmHg) and heart rate (hr, in beats/ min) when reported)	Reference
1 female, 32-year-old, bw: 65 kg, medical history: ulcerative colitis	'Mad honey' of Nepalese origin, 'handmade without specific identification'[f]	20 g/pers.[g]	GTX I: 25.8 mg/kg[h]	GTX I: < 10 µg/kg bw[i]	GTX I: b: 2.60 ng/mL	2 h (same on 2nd exposure 2 days later)	Nausea, vomiting, flushing and perioral paraesthesia lasted about 6 hours. Two days later, the honey was consumed again with same symptoms appearing and presentation to the emergency department: sinus bradycardia (hr: 30), normal ECG, arterial hypotension (bp: 80/40). Symptoms spontaneously faded within 24 hours	Nassibou et al., 2020

n.r.: Not reported; n.d.: Not detected.
(a): In cases, where the body weight is unknown, a body weight of 70 kg is assumed.
(b): The honey was rich in pollen and presumably obtained by pressing the combs; composition of pollen (excluding *Betula*): Rosaceae 54%, *Senecio* 16%, *Rhododendron* 14%, *Alnus* 6%. *Prunus, Daphne* and *Magnolia* contributed less than 1%.
(c): At the time of the analysis, only GTX III could be unambiguously identified in the honey since it was the only compound commercially available as a reference standard.
(d): Not considered in the assessment of acute dose–effect relationships, since it is unclear if the effects are only associated with the honey intake on the third day or also a consequence of the exposure in the previous 2 days.

(e): For other grayananes than GTX III, a semi-quantitative approach was taken, assuming that their mass spectrometric response was comparable with that of GTX III. The following estimates were made (mg/kg honey): grayanotoxin XVII (6.2), C20H31O6 (12.1), rhodojaponin VI (1.4), rhodomollein XVIII or kalmitoxin I (9.5), rhodomollein XIX (8.3), GTX VII (116.3), GTX VIII or XIX (5.8), GTX II or VI (2.0), craiobiotoxin I, II, V or VIII (18.6), GTX XVIII (39.8), C20H33O4 (3.2), GTX I or rhodomollein XIII (58.3), GTX XIII or XIV (12.2), GTX IV, IX, X or XVI (10.5). Based on these levels and the content of GTX III, the estimates of the concentration in honey and of the resulting single dose for total grayananes were calculated.
(f): The botanical origin of the Nepalese honey not defined but assumed to be derived from Ericaceae.
(g): As estimated by the authors; unclear if the amount is consumed at a single occasion or in total on two occasions.
(h): As estimated by the authors.
(i): At the time of the analysis, only GTX I could be identified since it was the only compound commercially available as a reference standard.

Information from other sources

EFSA launched a *call for stakeholder inputs in relation to the mandate on grayanotoxins in 'certain honeys'*[21] requesting information on 'mad honey' intoxications registered in their countries. In Germany, five cases of acute intoxications associated with consumption of honey containing grayananes have been registered from 1990 to 2021 in the BfR National Case Database of Poisonings. Three of these honeys had Turkish origin and two were of unknown origin. Grayanotoxins were identified (but not quantified) in the five honey samples. All subjects were male adults (Germany, 2022, under documentation provided to EFSA). In France, five cases of 'mad honey' intoxications were reported from 2000 to 2020 and involved four male and one female adults. The two more recent cases were honeys from Nepal (2019–2020) and the other three from Turkey. None of the samples had been purchased in Europe (France, 2022, under documentation provided to EFSA). According to the Turkish Ministry of Health National Poison Information Centre, 54, 94 and 187 cases on honey and its derivatives were reported in 2018, 2019 and 2020, respectively (Turkey, 2022, under documentation provided to EFSA).

3.1.3.2. Studies on cases of acute intoxications due to ingestion of 'certain honey'

Acute exposure to 'mad honey'

Several studies were performed in Turkish hospitals to investigate the clinical picture in cases in which patients came to the emergency department due to 'mad honey' intoxication.

Sumerkan et al. (2013) analysed the data from 246 patients (58 females; age 56 $\pm$ 15 years; 188 males, age 52 $\pm$ 15 years) diagnosed with 'mad honey' intoxication between January and December 2011. On admission, 61 patients (28%) had experienced severe bradycardia (heart rate $\leq$ 40 beats per min (bpm)), 33 patients (20%) severe hypotension (systolic blood pressure $\leq$ 60 mmHg) and four patients (1.6%) presyncope or syncope.

From smaller studies, the multiple clinical manifestations are reported as summarised in Table 10 (Yavuz et al., 1991; Yilmaz et al., 2006; Okuyan et al., 2010; Uzun et al., 2013; Yaylaci et al., 2014, 2015; Bilir et al., 2017; Tekinsoy et al., 2017; Aygun et al., 2018).

Table 10: Range of symptoms in affected patients

Symptoms observed	Affected patients (% range)
Hypotension	87–100%
Bradycardia	43–95%
Nodal rhythm	3.7–21%
Atrial fibrillation	1.2–12.5%
First degree atrioventricular block	12.5–14.6%
Complete atrioventricular block	2.2–36%
Dizziness	51.7–100%
Nausea/vomiting	27.6–100%
Blurred vision	15.9–88%
Exhaustion/weakness/fatigue	35–100%
Change of consciousness	12.5–67%
Syncope	12–44%

In two of these studies the relationship between the poisoning symptoms and the grayanotoxin levels in the blood of the patients were analysed (Tekinsoy et al., 2017; Aygun et al., 2018). In the study of Tekinsoy et al. (2017), the blood analyses in the group of 36 patients with the diagnosis of 'mad honey' intoxication revealed concentrations of grayanotoxins (type not specified) with an average of 7.9 ng/mL (range: 0.00–30 ng/mL). No statistically significant relationship between the observed symptoms (dizziness, nausea, vomiting, weakness, deterioration, angina) and the blood grayanotoxins levels in the study group was seen. Similar results were reported by Aygun et al. (2018) from 25 cases of 'mad honey' poisoning with mean concentrations in blood of 4.8 ng GTX I/mL and 6.6 ng GTX III/mL. No association was found between grayanotoxin levels in blood and clinical symptoms which included decreases of systolic blood pressure and heart rate.

[21] https://www.efsa.europa.eu/en/partnersnetworks/eumembers#focal-points-eu-food-safety-interfaces

In addition to the well-known cardiovascular symptoms of 'mad honey' poisoning as reported in Table 10, a more recent retrospective study on 76 patients with 'mad honey' intoxication showed that an interatrial block was detected in 28 (36%) of them (Ösken et al., 2021).

In the study of Okuyan et al. (2010), the patients' histories of cardiovascular diseases and medications were considered when evaluating the effects of the 'mad honey' intoxication. From the 42 patients included in the trial, 13 had hypertension and five had coronary artery disease. Before admission to hospital, eight patients were on beta-blocker medication and 10 received an antihypertensive therapy,[22] including administration of angiotensin-converting enzyme (ACE) inhibitors and of the calcium channel blocker (CCB) amlodipine. The mean heart rate and mean systolic blood pressure level of patients on beta-blocker therapy at admission were 35 ± 6 bpm (25–54 bpm) and 68 ± 14 mmHg (50–90 mmHg), respectively, and significantly lower than of patients without known beta-blocker administration (mean heart rate bpm: 43 ± 8 bpm (28–59 bpm), mean systolic blood pressure: 78 ± 11 mm Hg (50–100 mmHg)). No significant difference in heart rate and blood pressure were observed in patients receiving antihypertensive therapy compared to patients without this medication. The authors conclude that patients using beta-blockers and antihypertensive therapies seem to be more vulnerable to the cardiac effects of grayanotoxins than others. They recommend that physicians in the Turkish Black Sea area should warn their patients about adverse effects of 'mad honey' and antihypertensive drug combinations.

Repeated daily exposure to 'mad honey'

A prospective study was conducted in the department of cardiology of the Istanbul University (Aliyev et al., 2009b). In all patients presented with slow heart rate or atrioventricular conduction abnormalities between April 2008 and December 2008, the history of non-commercial honey intake was investigated. One hundred and seventy-three patients were referred to the institution with sinus bradycardia and atrioventricular block (various degrees) and/or for permanent pacemaker implantation. The subjects were asked for information on their honey intake. In five of the patients, a history of daily honey intake for a long period of time was revealed as either three to five teaspoons daily for 3 months, or 3–10 teaspoons daily for > 12 months. The honeys consumed originated from non-commercial beekeepers in the Eastern Black Sea region of Turkey. No further information with respect to the botanical origin or the chemical composition of the honeys was reported. All five patients experienced dizziness and presyncope. Discontinuation of the honey consumption resulted in rapid improvement of symptoms and of the electrocardiographic outcome. The Panel noted that data on the presence of grayananes in the honeys repeatedly consumed by the five patients are missing and that, therefore, the study is of limited relevance for the present assessment.

3.1.3.3. Case reports on acute intoxications due to ingestion of certain plants, their parts or preparations

The occurrence of grayananes in foods other than honey derived from certain Ericaceae plants has also led to human cases of intoxications. The same characteristic symptoms as for *Rhododendron* honey intoxications have been described from the consumption of *Rhododendron* liqueur (Choi et al., 2017), of herbal teas prepared from *Pieris japonica* (Aleguas et al., 2008) or the leaves of *Agarista salicifolia* (Martinet et al., 2005) or from the consumption of blossoms of *R. japonicum* (Koda et al., 2016) or *R. mucronulatum* (Lee et al., 2007) due to misinformation on their edibility. However, none of these publications allows an estimate of the dose of grayananes ingested. The publication of Choi et al. (2017) is not considered further due to inconsistencies in the reporting of the GTX I and GTX III concentrations in the liqueur and the subsequent calculated doses.

The large genus *Rhododendron* includes many species for which traditional medicinal uses, including those in Traditional Chinese Medicine and the Ayurvedic medical system have been described (e.g. Popescu and Kopp, 2013). However, acute intoxications may occur as a consequence of the misuse of *Rhododendron* species for medicinal purposes as observed by Poon et al. (2008). A 57-day-old infant was presented after vomiting in a cyanotic and unresponsive state with convulsions (twitching in all four limbs), sinus bradycardia, hypotension and shock to an emergency department in Hong Kong. Symptoms had started 20 min after administration of a bottle of milk mixed with a homemade decoction of *R. simsii* to treat the infant's bronchiolitis. The grandmother had collected the plant in an area nearby believing in the antitussive effect of the plant described in folk medicine. The infant was intubated and put on mechanical ventilation. Seizure activity ceased due to benzodiazepam

[22] Number of patients receiving a combined medication of beta-blockers with ACE inhibitors or CCB is not indicated.

infusion. Discharge from hospital took place on day 8 after admission with full recovery confirmed 4 months later. GTX I was identified in residues of the plant and the decoction and in the patient's urine, without quantitative data being presented.

Accidental ingestion of parts (e.g. buds) of *Pieris japonica*, which is grown as a garden plant, is another cause for intoxications of young children with grayananes (Tschekunow et al., 1989; Aleguas et al., 2008; Masom et al., 2008). In a 21-month-old girl presented with pallor, main symptoms of the intoxication with *P. japonica* (unknown which plant parts were ingested) were vomiting and a decrease of the heart rate to 40 bpm (Aleguas et al., 2008). Similar symptoms, burning in the mouth, drooling, lethargy and a slight hypotension were also reported from a 2-year-old boy after ingestion of a 'cluster of *Pieris japonica* buds' (Tschekunow et al., 1989). Apart from bradycardia (71 bpm), lethargia and oral secretions, main complaints observed in a 2-year-old girl after intake of several leaves of *P. japonica* were nausea and vomiting (Masom et al., 2008). All three patients recovered after receiving atropine intravenously. The grayananes levels in the plant material ingested and in the children's blood or urine were not analysed.

3.1.3.4. Clinical studies

Clinical studies in humans investigating the pharmacological effects of grayananes after oral administration were not identified. Results of trials in Chinese hypertensive patients (Mao et al., 1981; Li and Mao, 1982; Chen et al., 1985), showing that intravenous infusion of rhodojaponin III (synonym: rhomotoxin) was effective in lowering systolic and diastolic blood pressures and influenced other cardiovascular parameters (e.g. reduction of left ventricular afterload, of heart rate and of myocardial oxygen consumption), did not allow conclusions to be drawn on dose–effect relationships of grayananes orally ingested with food. Therefore, these reports were not considered relevant for this risk assessment and are not further described.

3.1.3.5. Summary of observations in humans

Overall, it can be concluded that in regions where consumption of preparations originating from grayanane-containing *Rhododendron* species, such as 'mad honey', is popular, acute intoxications typical from the ingestion of grayanotoxins are consistently seen in emergency departments. Acute symptoms observed affect the muscles and the nervous and cardiovascular systems with bradycardia and hypotension being most prominent and without fatal cases being reported in the last decades. In acute poisoning cases observed with *Rhododendron* honeys from the Turkish Black Sea region, the estimated intakes on a single occasion of GTX I or GTX III as part of the mixture of grayananes present in the honey range from about 0.6 to 17 μg/kg bw or 3 to 24 μg/kg bw, respectively, and from 4.8 to 40 μg/kg bw for the sum of GTX I and GTX III. A possible contribution of other grayananes present in *Rhododendron* honeys from the Turkish Black Sea region to the development of adverse effects cannot be quantitatively assessed due to insufficient information on their occurrence in these honeys and on their toxicological profile. From cases of acute intoxications following the ingestion of a Nepalese *Rhododendron* honey, in which GTX I and III could not be detected, it can be concluded that also grayananes other than GTX I and III present in *Rhododendron* species cause adverse effects on the muscles and the nervous and cardiovascular systems, when ingested in a single dose as low as 4.3 μg/kg bw. An NOAEL for GTX I, GTX III, for one of their analogues or for total grayananes could not be identified from the data available for human acute exposure to these compounds.

From the human case reports on poisonings, there are no indications that 'mad honey' or grayanane containing plants or their preparations or components induce liver toxicity as has been observed in experimental animal studies.

For human chronic exposure, reported symptoms do not differ from those of acute exposure. However, information on dose–effect relationships for long-term intake of grayananes could not be identified.

Available data are too limited to draw conclusions if children or patients with certain underlying diseases, are more vulnerable towards the toxicity of grayanotoxins than healthy adults. However, results from Okuyan et al. (2010) suggest that patients with cardiovascular diseases, when receiving anti-ischaemic or antihypertensive therapy (e.g. beta-blockers), are at higher risk of developing adverse cardiovascular effects when exposed to grayanotoxins compared to healthy subjects.

3.1.4. Mode of action

Regarding acute toxicity, the Panel decided to focus on the endpoint of most relevance for the hazard characterisation, i.e. the action of grayananes on the voltage-gated sodium (Nav) channels. The most relevant papers are addressed in this section.

Grayananes are so far considered the effectors of *Rhododendron* honey toxicity in humans, being able to reproduce similar symptoms, both in terms of quality and duration, in animal models. *Ex vivo* and *in vitro* studies on brain, muscle and cardiac preparation identify the voltage-gated sodium (Nav) channels as molecular target of grayanotoxins (Seyama and Narahashi, 1973; Narahashi and Seyama, 1974; Creveling et al., 1980; Matthews et al., 1981; Frelin et al., 1982; Kim et al., 2010). Nav involvement also comes from indirect evidence showing that Na^+ channel blocker, procaine, counteracts reduction of blood pressure and heart rate induced by rhodojaponin III in animal models (Mao et al., 1982).

Nav channels are multimeric complexes consisting of the pore-forming α-subunit associated with auxiliary β-subunits (See Figure 3) that modulate the kinetics and voltage dependence of channel gating and are involved in channel localisation (Catterall, 2000). β-Subunits display tissue specificity, heart contain a mixture of β_1-β_4 subunits, while skeletal muscles have only the β_1 subunit (Isom, 2001; Brackenbury and Isom, 2011). The pore-forming α-subunit is organised in four homologous domains (Iuphar: I-IV, literature: D1-D4), each one with six transmembrane hydrophobic domains (S1-S6) (Figure 3). S5 and S6 segments form the inner cavity of the pore, while S4 segments contain positive residues (usually arginine) which confer sensitivity of the channels to depolarisation initiating their activation (Catterall, 2000). Inactivation of the channel is ruled by the short intracellular loop in between domain III and IV (Catterall, 2000). Nine mammalian Nav channel isoforms (Nav1.1-Nav1.9) have been identified, which share greater that 50% identical amino acid sequence in transmembrane and extracellular domains but show different tissue presence and pharmacological features (Catterall, 2000).

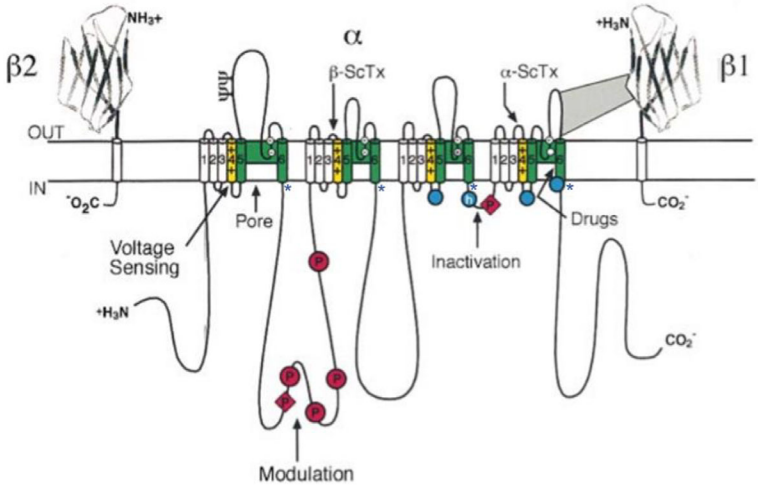


Figure 3: Subunit structure of the voltage-gated sodium channels, based on Catterall, 2000. The binding site is indicated with a blue * in the figure

Agents that act on Nav bind to receptor sites on the pore-forming α-subunit (Cestèle and Catterall, 2000). Mutagenesis studies showed that critical replacement of amino acids located within S6 segment on all four domains abolishes GTX I effects on rat skeletal muscle and heart Nav (Ishii et al., 1999; Kimura et al., 2001). In addition, relative conductance results show that skeletal muscle channels are more sensitive to GTX I than cardiac channels due to the specific characteristics of the intracellular loop of domain I S4-S5 and Ile-433 in the transmembrane segment of domain I S6 (Kimura et al., 2001). GTX I binds only to the open state, driven by membrane depolarisation, and dissociates from the closed state (Ito et al., 1985; Maejima et al., 2002). Due to this mechanism, excitable cells (neurons, muscle, myocytes) appear to be sensitive to GTX (Hotta et al., 1994; Ishii et al., 1999; Kimura et al., 2001). GTX I binding to the four domains of the pore-forming α-subunit prolongs the open time of Na^+ channels and shifts the sodium–conductance–voltage curve in the

direction of hyperpolarisation, thus inhibiting inactivation of voltage-gated Na channels (Maejima et al., 2002).

Since Nav channels activation and deactivation in excitable cells are responsible for action potential initiation and propagation, these changes in gating kinetics cause long-lasting depolarisation of excitable cells and increase the frequency of post-synaptic currents in primary neurons facilitated by pre-synaptic neurotransmitters release (Seyama, 1978, Hong and Chang, 1983; Ito et al., 1985; Kim et al., 2010). GTX I and rhodojaponin III-induced decreased blood pressure, contractile force and heart rate, are strongly reduced after vagotomy in animal models (Moran et al., 1954; Cotten et al., 1956; Mao et al., 1982). It is thus possible to hypothesise that these two grayananes also induce long-lasting depolarisation of the vagus nerve through Nav channels facilitating acetylcholine release. This might also explain the efficacy of atropine, a non-specific anti-muscarinic agent, to counteract the effects of rhodojaponin III on blood pressure and heart rate (Mao et al., 1982). In accordance with the role of grayananes in *Rhododendron* honey toxicity, both atropine and AF-DX 116, a selective M2-muscarinic receptor antagonist, restored bradycardia and atropine also respiratory rate depression induced by toxic *Rhododendron* honey extract in the rat (Onat et al., 1991a,b). Indeed, atropine is effective in the treatment of human intoxication with *Rhododendron* honey.

Nav channel subtypes display different kinetic, voltage-dependent and pharmacological properties (Catterall, 2000). The different distribution among cells thus contributes to distinct functional roles and sensitivity to targeting substances. Information on how different Nav isoforms respond to grayananes are sparse. GTX I exerts a greater effect on Nav 1.4 compared to Nav 1.5 (Kimura et al., 2001), although activity on other Nav channel subtypes remains to be determined.

No mechanistic data on genotoxicity and repeated dose toxicity were identified.

3.1.5. Relative potencies of grayananes for acute toxicity

The following discussion on differences in the potencies of grayananes relates only to acute toxicity. There is no data on the differences in potency related to the genotoxicity of grayananes.

Single-dose studies used to estimate LD_{50} values in experimental animals (see Section 3.1.2.1) describe similar symptoms to those reported in humans after consumption of 'mad honey'. Thus, the possibility of using the LD_{50} values in animals to rank the potency of grayananes and derive RPFs for acute toxicity was investigated.

It was noted that the studies deriving these LD_{50} values in rodents use mainly the i.p. or i.v. route of exposure instead of the oral route. Because of their structural similarity, it was assumed that the relative potency of substances was not markedly affected by the route of exposure.

Thus, the Panel derived RPFs for groups of grayananes and ranked them in Table 11 based on the LD_{50} values derived from i.p. studies in mice, as shown in Table 6 (when data were available). When data were not available, grayananes were ranked based on their structural alerts, as described in Tables A.1–A.3 in Appendix A and shown in Figure 4. In the absence of LD_{50} values, grayananes are allocated to groups 1–3 as shown in Table 11, based on the number of structural alerts. In case of grayananes with three structural alerts (group 1), the default allocation would be to group 1A.

GTX III Rhodojaponin III

Figure 4: Structural alerts of grayananes (circled)

The Panel noted that all grayananes with high potency share three structural features circled in Figure 4: They all contain (i) a 3β-hydroxy or a 2β,3β-epoxy group, (ii) a 6β-hydroxy and (iii) a 10β-methyl group (Hikino et al., 1976; Li et al., 2013; Masutani et al., 1981). When one of these structural features is modified or missing, toxicity is greatly reduced, as in the case of GTX II and GTX IV where the 10β-methyl group is replaced by a C10 exocyclic double bond.

Table 11: Relative potency factors for acute toxicity: grouping of grayananes

Group	Indicative LD_{50} range, *i.p.* (mg/kg bw)		RPF	Grayanane
Group 1, most potent (3 structural alerts)	Subgroup 1A	0.3[(a)]–0.6	2	e.g. rhodojaponin III
	Subgroup 1B	0.6–2.0	1	e.g. GTX I and GTX III
Group 2, medium potency (2 structural alerts)	10–20		0.1	e.g. GTX II and GTX IV
Group 3, no observed toxicity (1 or no structural alert)	> 100		< 0.01[(b)]	e.g. GTX XVI and GTX XVII

(a): A lower LD_{50} value is estimated for asebotoxin III (0.1 mg/kg bw), but because the substance is not known to occur in 'certain honey', it was not included in the table.
(b): Assumed negligible contribution to risk assessment calculations (see Section 3.1.7).

Rhodojaponin III has been identified in a wide range of species, including *Rhododendron* spp. Its presence has been implicated in Grouse Mountain honey from British Columbia (Canada) (Scott et al., 1971), but not in 'mad honey' from the Black Sea region (These et al., 2015). Recent results from Nepalese honey samples suggest that rhodojaponin III may be present in these honeys (Mulder and de Vries, 2022 under Documentation as provided to EFSA).

Note was taken that asebotoxin III is approximately 10 times more potent than GTX I and III. However, asebotoxin III has not been identified in or associated with 'mad honey'. So far, asebotoxin III has only been isolated from *P. japonica and P. formosa*, which are not among the sources of 'mad honey' produced in Nepal or the Black Sea region. Based on the above, the Panel did not consider it relevant to derive a separate RPF for asebotoxin III. If new evidence suggests that asebotoxin III occurs in 'mad honey', the Panel will consider the possibility of deriving a separate RPF for the substance.

Table 12 reports the dose levels of GTXs I, II and III affecting locomotive activity and salivation. The results are consistent with those based on LD_{50} and support the derived RPFs.

Table 12: Relative potencies of GTX I, GTX II and GTX III

Grayanotoxin	Locomotion, i.p.	Salivation (EC_{50}), i.p.	LD_{50}, i.p.	RPF
GTX III	0.1 mg/kg	0.15 mg/kg	0.77–1.03 mg/kg	**1**
GTX I	0.25 mg/kg	0.22 mg/kg	1.1–1.5 mg/kg	**1**
GTX II	> 0.25 mg/kg	10–15 mg/kg	10–28.7 mg/kg	**0.1**

3.1.6. Considerations of critical effects and dose–response analysis

3.1.6.1. RP based on acute/single-dose exposure to grayananes

RP based on animal data

The derivation of an RP from experimental animal studies was considered.

Some i.p. and i.v. studies with grayananes or 'mad honey' in experimental animals describe acute effects on heart rate and blood pressure which are consistent with those observed in humans (see Section 3.1.2.1). The Panel noted that the parameters in these studies are altered in a dose-dependent manner (Onat et al., 1991a,b; Turkmen et al., 2013; Yilmaz et al., 2017).

A BMD analysis was performed based on the reduction of heart rate after i.p. injection of GTX III in rats (Turkmen et al., 2013) (see Table 13). Instead of selecting the default benchmark response (BMR) of 5% for continuous effects, the Panel decided to select a BMR of 10% for this endpoint, i.e. a 10% decrease in the mean response compared to the controls, also considering the transient nature of this effect. Using model averaging, a $BMDL_{10}$ of 15.3 μg/kg bw was estimated for GTX III. A detailed description of the BMD analysis is presented in Appendix C.

Table 13: $BMDL_{10}$ and $BMDU_{10}$ calculated from the reduction of heart rate observed after i.p. injection of GTX III in rats (based on dose–response data by Turkmen et al., 2013)

	$BMDL_{10}$(μg/kg bw)	$BMDU_{10}$ (μg/kg bw)	Lowest dose in the study (μg/kg bw)
Results at 60 min	15.3	261	179

The estimated $BMDL_{10}$ of 15.3 μg/kg bw for GTX III (*i.p.*) based on Turkmen et al. (2013) was used as reference point (RP) for acute toxicity for the sum of GTX I and III considering that the two substances have similar potencies (see Tables 11 and 12).

Although data from an oral study might derive a higher RP than data from an i.p. study, the Panel decided to not extrapolate results from i.p. to oral exposure for the following reasons:

i) Although the available toxicokinetic studies do not allow comparison of oral vs. i.p. bioavailability, there are studies in the scientific literature which compare i.p. to oral toxicokinetic values for different molecules. In a study by Turner et al. (2011), the authors report that because the primary route of absorption under i.p. is into the mesenteric vessels and guides the substance through the liver, substances administered i.p. may have similar or same order pharmacokinetics with those seen after oral administration. In addition, Al Shoyaib et al. (2020) reported that substances with low lipid-water partition coefficient (GTX III has a logP of -0.237 ± 0.514 based on SciFinder) are less easily released into the systemic circulation after i.p. injection than substances with higher lipid-water partition coefficient.
ii) The data allowing comparison between i.p. and oral routes do not come from studies on toxicokinetics but studies on the potency of GTX III, following i.p. and oral administration in male mice (Hikino et al., 1976 and 1979). Both studies by Hikino et al. are subject to uncertainties mainly associated with testing male mice only and the gross endpoint used (mortality).

Although derived for the sum of GTX I and III, this group RP for acute toxicity may apply to other grayananes, such as GTX II, by making use of their RPFs (e.g. for GTX II with RPF = 0.1, concentrations in food samples would have to be converted by using the factor 0.1, see Table 11).

Uncertainties associated with the use of i.p. instead of oral studies, the oral bioavailability of GTX I and III, the effect of the matrix (honey), the higher and/or lower potency of other co-occurring grayananes in honey, etc., were considered in the uncertainty analysis (see Section 3.6).

Other effects observed in single-dose studies in experimental animals are small alterations in clinical parameters which are inconsistent and not supported by histopathological findings.

RP based on human data on acute intoxications

Consumption of dietary sources rich in grayananes is associated with acute effects on the nervous and cardiovascular systems, the most prominent ones being bradycardia and hypotension. As dose-response data in humans relate to cases of poisoning from consumption of 'mad honey', an NOAEL for grayanotoxins or other grayananes could not be identified. In the absence of an NOAEL from human studies, the Panel referred to dose–response data derived from intoxication cases.

In two poisoning cases with 'mad honey' from Turkey reported by Aygun et al. (2015), the combined intake of GTX I and GTX III resulted in total doses of 4.84 and 4.90 μg/kg bw (GTX I: 0.94 and GTX III: 3.90 μg/kg bw or GTX I: 0.64 and GTX III: 4.25 μg/kg bw, respectively) and led to presentation in an emergency department with dizziness, nausea, hypotension and bradycardia (see Table 9).

In a separate publication, acute intoxications with symptoms of headache and feeling 'high' have been associated with intake of Nepalese honey containing two grayananes that were different from GTX I, II, III and their structures were not identified. The doses were estimated to be 4.3 μg/kg bw on the first day and < 4.3 μg/kg bw on the third day of 'mad honey' consumption (Kerkvliet, 1981). It was noted, however, that the results from this publication were semi-quantitative and therefore can only be used as supportive data.

From the data by Aygun et al. (2015), the lowest observed acute intoxication level identified is 4.8 μg/kg bw for the sum of GTX I and GTX III following single exposure in humans. In the absence of an NOAEL, the Panel estimated an RP of 0.48 μg/kg bw for the sum of GTX I and GTX III, at which no adverse effects would be expected, by applying an uncertainty factor of 10 to the lowest observed poisoning dose. This approach is prone to a number of additional uncertainties, including the unknown contribution of other grayananes to the poisoning effect of the 'mad honey' sample (see Section 3.6).

3.1.6.2. RP based on repeated dose exposure to grayananes

In an *in vivo* micronucleus (MN) assay in male mice, both, grayanane-containing *Rhododendron* honey and the isolated GTX III, showed a clear genotoxic effect in the presence of bone marrow exposure (Rasgele et al., 2021).

No studies in experimental animals on chronic toxicity/carcinogenicity are available.

Effects observed in subacute and subchronic studies in experimental animals were small alterations in clinical parameters which were inconsistent and not supported by histopathological findings.

Histological findings in the testes from repeated dose i.p. injection of GTX III in rats at doses from 0.1 to 0.8 μg/kg bw per day for 3 weeks suggest effects on the testes. Other critical effects could not be confirmed because of the lack of repeated-dose toxicity data in both animals and humans.

Therefore, no RP could be identified from repeated dose toxicity studies on grayananes.

3.1.7. Consideration of a Health-Based Guidance Value (HBGV) and a margin of exposure

Due to the indicated *in vivo* genotoxicity for the pure GTX III and for one honey sample containing GTX I and III, a health-based guidance value (HBGV) cannot be derived for grayananes (EFSA Scientific Committee, 2017b).

However, considering that single exposure to 'certain honey' may lead to intoxication in humans, the Panel decided to carry out an acute risk assessment.

Regarding the risks for acute toxicity, the Panel considered the overall data situation to be limited for the following reasons:

i) No adequate animal studies with oral administration of grayanotoxins are available,
ii) The few quantitatively evaluable cases of poisoning in humans do not allow conclusions to be drawn about possible adverse effects of GTX I and III in the low-dose range,
iii) Animal and human data on the toxicological characterisation of grayananes other than GTX I and III are sparse or missing,
iv) No adequate data to perform an acute exposure assessment are available.

Although exposure data on acute consumption were limited, the Panel performed a margin of exposure (MOE) calculation for acute toxicity based on exposure estimated from selected concentrations for the sum of GTX I and III reflecting the concentrations measured in 'certain honeys' and the RP of 15.3 μg/kg bw derived from one study in rats (Turkmen et al., 2013), in order to assess the health concern (See Section 3.4.1.1).

Based on the above considerations, the Panel calculated *highest concentration(s) of* grayanane below which no acute effects on heart rate and blood pressure would be expected following acute consumption of honey containing grayananes by applying a factor of 100 for inter- and intraspecies variability (WHO/IPCS, 2005; EFSA Scientific Committee, 2012) (see Section 3.5). This assumes that the two substances have equivalent potency (see Hikino et al., 1976 and 1979) and considers that GTX I and III are the only two grayananes consistently identified and quantified in 'certain honey' (see Section 3.2).

3.2. Occurrence data

3.2.1. Previously reported occurrence data in the open literature

A limited number of surveys on GTXs in honey and other food products has been published in the open literature. Table 14 reports results from these surveys, where analyses were performed for GTX I, and/or GTX III, and/or the sum of GTX I and III in honeys and other foods. In some of the surveys, (semi-)quantitative results for grayananes are also presented. The analytical methodologies supporting the results for GTX I and III were conducted using LC-MS-based techniques and were based on fit-for-purpose protocols with regard to the availability of standards, accuracy and reproducibility. The sensitivity of some methods, however, is not low enough to quantify GTX I and III in honeys at concentrations as low as 0.05 mg/kg (see Section 3.5).

3.2.1.1. Grayanane occurrence in honeys from the EU

Recently, Lucatello et al. (2022) analysed 125 samples of *Rhododendron* honey collected in the Italian alpine regions of Valle d'Aosta, Piemonte, Lombardia, Trentino-Alto Adige and Veneto, for the content of GTX I (LOQ: 0.01 mg/kg) as well as for pollen and different sensory parameters. The samples were collected over a 3-year period (2017–2019). Concentrations for GTX I ranged from < LOQ to 0.10 mg/kg honey and, in total, 38 out of 125 (30%) of the samples contained measurable amounts of GTX I (see Table 14). The samples were also screened for the presence of GTX III, but only semi-quantitative information is reported in the paper suggesting that GTX III is present in 74% of samples with quantifiable levels of GTX I, at levels similar to or lower than GTX I.

These et al. (2015) analysed 49 samples from the German retail market, including beekeeper honeys, from various sources, but no GTXs could be detected in any of the samples (LOQ $\leq$ 0.17 mg/kg). The origin of these samples is reported in Table 14. The Panel noted that only one sample of 'Heather honey' was of Ericaceae origin but is aware that polyfloral honeys may also contain a contribution from *Rhododendron* species. As the LOQ for the method used is not specified in the study, the Panel cannot assess whether it could identify and quantify GTX I and/or GTX III at concentrations lower than 0.17 mg/kg.

Mulder and de Vries (2022 under Documentation as provided to EFSA) also screened honeys from the EU for grayanane occurrence (LOQ: 0.1 mg/kg). These included European 'Heather honey' from the Netherlands and France, one *Rhododendron* honey from Spain and one Arbutus honey from Portugal. No grayananes were identified in any of these honeys, but the method could not quantify grayananes at concentrations below 0.1 mg/kg.

3.2.1.2. Grayanane occurrence in non-European honeys

Rhododendron honey samples collected from the Turkish Black Sea region were found to contain grayananes. In 2017, Dönmez and Kaya conducted a survey in the Ducze area, where honey is often sold as 'mad honey' by local people (Dönmez and Kaya, 2020). Eighty-one samples were analysed for their GTX III content, which ranged from < LOQ (0.001 mg/kg) to 0.07 mg/kg. Kurtoglu et al. (2014) collected the largest number of samples (187) over the course of 3 years (LOQ: 0.1 mg/kg). The samples were pooled per year and only average concentrations were published. GTX I was present in higher concentrations than GTX III (Table 14) and there was some yearly variation with higher levels present in honey in 2011 and 2012 compared with 2010. In his Ph.D. thesis, Sahin (2014) reported results for GTX III in six 'suspect' honey samples, *Rhododendron* honey (16 samples) and royal jelly (bee milk) (8 samples) collected from different locations in the Black Sea region (Sahin, 2014, see also Sahin et al., 2015). All samples contained GTX III, the highest levels being found in the 'suspect' honeys, while the royal jelly contained the lowest amounts (LOQ: 0.01 mg/kg). Smaller surveys were reported by Kaplan et al. (2014) (10 samples; LOQ: 0.01 mg/kg) and Silici et al. (2016) (6 samples; LOQ not reported); the latter survey only included GTX III. Kaplan et al. (2014) reported GTX I to be present in higher concentrations than GTX III. From these surveys, it can be concluded that there is a wide variation in total GTX concentration in the samples originating from the Black Sea region, ranging from < 0.001 mg/kg to 74 mg/kg.

Lee et al. (2008) analysed a set of wild honeys and comb honeys produced in the Republic of Korea as well as imported products. Most honey types from domestic and foreign origin did not contain measurable amounts of GTX I, II and III (LOD: 0.15 mg/kg). However, four out of eight wild honeys imported from Nepal contained substantial amounts of GTXs, ranging from 3.4 to 17 mg/kg. GTX I was the dominant compound in the samples (3.1–13 mg/kg). GTX II (< LOD-1.1 mg/kg) and GTX III (0.25–3.3 mg/kg) were present at lower levels in the four positive samples.

Recently, Ahn et al. (2022) reported results for GTX I and III in 60 samples of 'mad honey' from Nepal confiscated at airports in the Republic of Korea. Thirty-three samples contained GTX I and III (LOQ: 0.25 mg/kg) in concentrations ranging from 0.8 to 65 mg/kg for GTX I and from 0.25 to 64 mg/kg for GTX III. The sum of GTX I and III ranged from 1.0 to 100 mg/kg. In the positive samples, the average concentration of GTX I (25 mg/kg) was somewhat higher than the average concentration of GTX III (17 mg/kg).

Mulder and de Vries (2022 under Documentation as provided to EFSA) recently analysed two *Rhododendron* honey samples from Turkey (including one 'mad honey' sample purchased on the internet) and nine *Rhododendron* honey samples from Nepal (Table 14). In two samples from Nepal and one sample from Turkey, GTX I, II, III and rhodojaponin III were (semi-)quantified. In the absence of an analytical standard of rhodojaponin III, the substance was prepared from commercially available rhodojaponin II. This allowed confirmation of the presence of rhodojaponin III in the 'mad honey' samples. However, only semi-quantitative concentrations were obtained for rhodojaponin III since the purity of the compound was not checked.

Semi-quantitative results from the studies by Mulder and de Vries (2022 under Documentation as provided to EFSA) and These et al. (2015) suggest that grayananes other than GTX I, II and III co-occur in *Rhododendron* honeys from the Turkey Black Sea Region and Nepal. The total grayanane concentrations in Turkish honeys may be up to three times higher than the sum of GTX I and III (These et al., 2015). In Nepalese honeys, seeming to be richer in other grayananes, notably rhodojaponin III, the total grayanane concentrations may be up to seven times higher than the sum of GTX I and III (Mulder and de Vries, 2022 under Documentation as provided to EFSA) (see Table 15). It

was noted that the levels of rhodojaponin III in Nepalese honey could not be validated in the absence of a certified analytical standard.

Table 15 reports quantitative and semi-quantitative results on GTXs in honey from intoxication cases.

3.2.1.3. Grayanane occurrence in other foods

For other food categories, only limited data are available. A single study was reported on the presence of GTXs I and III in dietary supplements and homemade wine from local markets in the Republic of Korea (LOQ: 0.008 mg/kg). Six out of 39 supplements were found to contain relatively low levels of GTXs (Table 14). This was also the case for eight out of 12 homemade wine samples: total concentrations were below 0.5 mg/L. However, four wine samples contained high levels of GTX I and III, in the range of 64 and 154 mg/L for the sum (Hwang, 2018). The authors correlated the high levels of GTXs in wine to the use of *R. brachycarpum*, which is collected locally.

Table 14: Summary results of surveys conducted on GTXs in honey and other foodstuffs

Country of sampling	Year of survey	Matrix	N	No of positives	LOD or LOQ (mg/kg)	GTX I (mg/kg)	GTX II (mg/kg)	GTX III (mg/kg)	GTX total[(a)] (mg/kg)	GTX average[(b)] (mg/kg)	Remarks	Reference
Grayanane occurrence in honeys of EU origin												
Italy	2017	Italian *Rhododendron* honey	45	16	0.01	< LOQ-0.07	n.a.	n.a.	< LOQ-0.07 (< LOQ-0.15)[(c)]	0.04 (pos) 0.01 (all)	Samples from alpine region, where *R. ferrugineum* is abundant	Lucatello et al., 2022
	2018	Italian *Rhododendron* honey	45	13	0.01	< LOQ-0.07	n.a.	n.a.	< LOQ-0.07 (< LOQ-0.14)[(c)]	0.03 (pos) 0.01 (all)		
	2019	Italian *Rhododendron* honey	35	9	0.01	< LOQ-0.10	n.a.	n.a.	< LOQ-0.10 (< LOQ-0.21)[(c)]	0.05 (pos) 0.01 (all)		
Germany	n.i	Retail honey (18), beekeeper honey (6)	24	0	< 0.17	< LOD	< LOD	< LOD	< LOD	< LOD	Samples from Germany (6), Hungary (2), Spain (2), Austria (1), Italy (1), Romania (1), other EU countries (12)	These et al., 2015
Nether-lands	2021	Ericaceae honey	4	0	0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	Samples (4) from the Netherlands and France, Spain and Portugal	Mulder and de Vries, 2022 under Documentation as provided to EFSA
Grayanane occurrence in honeys of non-EU origin												
Germany	n.i	Retail honey (25)	25	0	< 0.17	< LOD	< LOD	< LOD	< LOD	< LOD	Samples from South America (7), China (7), Turkey (4), not specified (7)	These et al., 2015

Country of sampling	Year of survey	Matrix	N	No of positives	LOD or LOQ (mg/kg)	GTX I (mg/kg)	GTX II (mg/kg)	GTX III (mg/kg)	GTX total[a] (mg/kg)	GTX average[b] (mg/kg)	Remarks	Reference
Korea	n.i.	Korean honey	25	0	0.15	< LOD	< LOD	< LOD	< LOD	< LOD		Lee et al., 2008
		Korean wild honey	21	0	0.15	< LOD	< LOD	< LOD	< LOD	< LOD		
		Korean comb honey	13	0	0.15	< LOD	< LOD	< LOD	< LOD	< LOD		
		Imported honey	44	0	0.15	< LOD	< LOD	< LOD	< LOD	< LOD	Samples from New Zealand (11), Italy (9), Canada (8), USA (7), France (4), Australia (3), others (2)	
		Imported wild honey	8	4	0.15	3.1-13	< LOD-1.1	0.25-3.3	3.4-17	11 (pos) 5.5 (all)	All samples imported from Nepal	
Korea	n.i.	'Mad honey' from Nepal	60	33	0.25	< LOQ-65	n.a.	< LOQ-64	< LOQ-100	42 (pos) 23 (all)	Samples were confiscated by customs at Korean airports	Ahn et al., 2022
Nether-lands	n.i.	Honey from Nepal	9	2	0.1	< LOQ-8.5	< LOQ-0.50[d]	< LOQ-2.4	< LOQ-11 (14)[e]	7.2 (pos) (9.9)[e] 1.7 (all) (2.2 (all))[e]	Several other GTXs tentatively identified and quantified in the 2 honeys, including rhodojaponin III (up to 1.2 mg/kg)	Mulder and de Vries, 2022 under Documentation as provided to EFSA
	n.i.	Honey from Turkey	2	2	0.1	0.27–0.94	< LOQ-0.22[d]	< LOQ-1.53	0.27–2.7 (0.27–3.3)[e]	1.5 (1.8)[e]	Rhodojaponin III (0.34 mg/kg) was detected in one sample	

Country of sampling	Year of survey	Matrix	N	No of positives	LOD or LOQ (mg/kg)	GTX I (mg/kg)	GTX II (mg/kg)	GTX III (mg/kg)	GTX total[a] (mg/kg)	GTX average[b] (mg/kg)	Remarks	Reference
Turkey	2010-2012	*R. ponticum* honey	187 (3)[f]	–	0.1	16–26	n.a.	2.5–12	19–38	30	Samples from western Black Sea region Only aggregated data are presented	Kurtoglu et al., 2014
Turkey	2012	*Rhododendron* honey	6	6	n.i.	n.a.	n.a.	2.1–11	2.1–11	6.2	Monofloral *Rhododendron* honeys from Black Sea region	Sibel et al., 2014; Silici et al., 2016
Turkey	n.i.	Honey, local production	10	10	0.01	< LOD-39	n.a.	< LOD-35	0.1–74	22	Samples from Black Sea region	Kaplan et al., 2014
Turkey	n.i.	'Suspect' honey	6	6	0.01	n.a.	n.a.	11–69	11–69	25	Samples from Black Sea region	Sahin, 2014[g]
Turkey	2009-2012	*Rhododendron* honey	16	16	0.01	n.a.	n.a.	0.63–39	0.63–39	7.5	Samples from Black Sea region	Sahin, 2014[g]
Turkey	2013	Royal jelly/ bee milk	8	8	0.01	n.a.	n.a.	0.19–7.3	0.19–7.3	2.4	Samples from Artvin area, Turkey	Sahin, 2014
Turkey	2017	Honey, local production	81	52	0.001	n.a.	n.a.	< LOQ–0.067	< LOQ–0.067	0.014 (pos) 0.009 (all)	Samples from Duzce area, Turkey	Donmez and Kaya, 2020
Grayanane occurrence in other foods												
Korea	n.i.	Dietary supplements	39	6	0.008	0.087–0.347[h]	n.a.	0.037–0.127[h]	0.124–0.474[h]	0.301 (pos)[h] 0.046 (all)[h]	Obtained from local Korean markets. All positive samples were liquid supplements	Hwang et al., 2018
		Homemade wine	12	12	0.008	< LOQ–101[h]	n.a.	0.015–56[h]	0.034–154[h]	36.8[h]	Obtained from local Korean markets	

n.i: not indicated; n.a: not analysed.
(a): Calculated concentration range for the sum of GTX concentrations per sample; the calculations do not take into account that GTX II has a lower RPF than GTX I and III.
(b): Average calculated from the GTX I, II and III total concentration divided by the number of samples.
(c): Semi-quantitative sum of GTX I and GTX III; no analytical standard was available for GTX III in the study by Lucatello et al. (2022). Assuming an equal response between the isoforms of GTX I and GTX III, the authors estimated that 73.7% of samples with GTX I contain equal or lower levels of GTX III but never higher.
(d): Semi-quantitative result.
(e): Semi-quantitative sum of all (tentatively) identified grayananes.
(f): Only aggregated results per year are reported.
(g): Data shown are from Sahin (2014). The results for a selection of 10 samples are also reported in Sahin et al. (2015).
(h): Concentration in mg/L.

Table 15: Summary results of analytical reports on GTXs in honey from intoxication cases

Country of sampling	Year of survey	Matrix	N	No of positives	GTX I (mg/kg)	GTX II (mg/kg)	GTX III (mg/kg)	GTX total [(a)] (mg/kg)	GTX average[(b)] (mg/kg)	Remarks	Reference
Netherlands	2010	Nepalese wild honey	1	1	n.a.	n.a.	30	30	n.a.		Mol et al., 2011
		(c)			2.7	< LOQ	13	16 (111)[(d)]	n.a.	Several other GTXs tentatively identified and quantified, including rhodojaponin III (60 mg/kg)	Mulder and de Vries, 2022 under Documentation as provided to EFSA
Germany	2010	Black Sea region honey	1	1	58[(d)]	2.0[(e)]	54	114 (358)[(d)]	n.a.	12 other GTXs tentatively identified and quantified	These et al., 2015
Turkey	n.i.	Turkish 'mad honey'	4	4	0.45–9.9	n.a.	3.0–17	3.1–27	13		Aygun et al., 2015
Turkey	2013–2014	Turkish 'mad honey'	25	25	0.17–20	n.a.	< LOD–76	n.a.	36		Aygun et al., 2018
France	n.i.	Nepalese wild honey	2	2	26–28	n.a.	n.a.	26–28	27		Nassibou et al., 2020

n.i.: not indicated; n.a.: not analysed/not applicable.
(a): Calculated concentration range for the sum of GTX I, II and III concentrations per sample.
(b): Average calculated from the GTX total concentration divided by the number of samples.
(c): The original sample from 2010 was reanalysed in 2022.
(d): Semi-quantitative sum of all (tentatively) identified grayananes.
(e): Semi-quantitative result.

3.2.2. Processing of honey

No studies were identified that have investigated the effect of processing (e.g. heat) of raw honey on GTX levels.

Storage of honey for up to 6 months at room temperature had only limited effects on the concentration of GTX analogues. The concentration of GTX I and III increased slightly in one sample but remained constant in two other samples that were analysed (Kurtoglu et al., 2014). Kaplan et al. (2014) reported GTX I and III to be stable in honey when stored at room temperature for 12 months.

Grayanotoxins were found to be relatively resistant to biodegradation when *R. ponticum* leaves were subjected to pilot-scale composting for up to 3 months (Hough et al., 2010). The concentration of GTX III remained constant during the duration of the study, but that of GTX I declined by approximately a factor 2.

3.3. Dietary exposure assessment for humans

In the absence of representative occurrence data for the sum of the GTX I and III and consumption data from Ericaceae honey, dietary exposure was estimated based on specific concentrations for GTX I and III reflecting the concentrations measured in 'certain honeys' (see Section 3.4).

In relation to other products based on *Rhododendron* spp. (e.g. dietary supplements, herbal teas originating from *Rhododendron* species and processed foods (wine) from third countries), no information was available regarding GTX occurrence in similar foods in the EU, nor on their consumption by European consumers. A search on the GNPD database[23] did not produce any results for food products having *Rhododendron* as ingredient in the EU. Such products seem to be niche products in the EU with unknown relevance for the internal market and were not considered in the current assessment.

3.4. Risk characterisation

In order to provide information that might be useful to all stakeholders including risk managers, two approaches were followed by the Panel to characterise the risk from consumption of 'certain honey': one assessing the acute effects of grayananes, and a second one addressing the risks of the chronic effects.

As no chronic toxicity or carcinogenicity studies could be identified for GTXs, no RP for chronic toxicity could be identified.

3.4.1. Risk characterisation of acute effects

The risk characterisation was based on acute consumption of honey for toddlers (the age group with the highest P95 acute consumption) and for adults. The Panel decided to not include other populations in the risk characterisation for the following reasons: Children and adolescents had acute consumption values within the range of those of toddlers; elderly and very elderly age groups had acute consumption values within the range of adults. For infants and the specific population groups such as pregnant and lactating women and vegetarians due to the limited number of surveys (less than 3) available with at least 60 subject days of honey consumption to ensure reliable statistics for the 95th percentile of the consumption (EFSA 2011), the risk could not be characterised separately.

3.4.1.1. Margin of exposure approach

The Panel considered it relevant to proceed with a margin of exposure (MOE) estimate for the sum of GTX I and III in order to characterise the risk from acute consumption of 'certain honey'. In the absence of representative occurrence data for the sum of GTX I and III and consumption data from Ericaceae honey, the Panel estimated MOEs based on the following data and assumptions:

[23] https://www.gnpd.com/sinatra/gnpd/search/#

- The RP for acute effects of 15.3 μg/kg bw for the sum of GTX I and III;
- three concentrations of 0.1 mg/kg,[24] 1.0 mg/kg[25] and 10 mg/kg,[25] selected for illustrative purposes for the sum of GTX I and III;
- the maximum P95 for acute consumption of honey per kg bw among EU surveys and population subgroups.

The results from the MOE calculations are reported in Table 16.

Table 16: MOE estimates (rounded) for acute effects based on the selected concentrations and the RP of 15.3 μg/kg bw for the sum of GTX I and III

	Maximum of P95 consumption[(a)] (g/kg bw)	Exposure and MOE using a selected concentration of 10 mg/kg		Exposure and MOE using a selected concentration of 1.0 mg/kg		Exposure and MOE using a selected concentration of 0.1 mg/kg	
		Exposure (μg/kg bw)	MOE	Exposure (μg/kg bw)	MOE	Exposure (μg/kg bw)	MOE
Toddlers	3.00	30	**0.5**	3.0	**5**	0.3	**50**
Adults	1.22	12	**1.3**	1.2	**13**	0.12	130

(a): For the estimation of the 95th percentile, surveys with less than 60 observations were excluded since results may not be statistically robust (EFSA 2011).

These MOE calculations suggest that consumption of 'certain honeys' containing GTX I and/or III within the range of concentrations indicated in Table 16 raises a health concern for acute toxicity. The Panel is aware that 'mad' honeys in Tables 9 and 15 are very unlikely to be consumed by toddlers or other children. Furthermore, consumption by adults of 1.22 g honey/kg bw is at the top of the range of advised portion sizes for 'mad' honey.

3.4.2. Risk characterisation for genotoxic/chronic effects

Positive evidence of induction of chromosomal damage in an *in vivo* micronucleus (MN) test in male mice indicates genotoxicity of GTX III and honey containing GTX I and III (see Section 3.1.2.3). In the same honey sample, the analysis of chromosomal aberrations confirmed this result. There is no indication whether this is via a direct or indirect mode of action.

Due to the lack of information on the underlying mode of action of genotoxicity and the lack of data on chronic toxicity and carcinogenicity, the Panel is unable to assess the risk related to chronic/ repeated exposure.

3.4.3. Non-dietary sources of exposure

No non-dietary sources of exposure were identified.

3.5. Estimation of the highest concentrations of the sum of GTX I and III in 'certain honey' below which no acute effects are expected

The Panel estimated the *highest grayanane concentration(s)* below which no acute effects in heart rate and blood pressure would be expected following consumption of 'certain honey' (See Section 3.1.7). These estimates for the sum of GTX I and III in the honey are based on the RP of 15.3 μg/kg bw and an MOE higher than 100.

The Panel is aware that this methodology does not consider the impact of other co-occurring grayananes in 'certain honey' which cannot be analysed presently. When analytical standards for other grayananes become available, it may be possible to refine the assessment and include other substances.

The backward calculation for the *highest concentration* of the sum of GTX I and III in 'certain honey' was performed based on the following consumption scenarios:

[24] Based on the highest value for only GTX I for a honey sample from the EU (Lucatello et al., 2022). This result does not include the contribution of GTX III and other grayananes in the honey sample.
[25] Levels of GTX I and/or III in 'certain honeys' (see Tables 9, 14 and 15).

3.5.1. Scenario A: Consumption of *Rhododendron* honey as 'normal' honey

Since acute intoxications with *Rhododendron* honeys have been reported from consumption of 'certain honey' as 'normal honey' (Kerkvliet, 1981; Nassibou et al., 2020), the Panel considered it appropriate to build a scenario where 'normal honey' consumption from the EFSA Comprehensive database was used (details in Section 2). In this scenario, it is assumed that *Rhododendron* honey is intentionally or accidentally consumed as normal food and not because of its use as traditional folk medicine.

Acute consumption statistics of honey per country and age class (consumers only), including specific population groups, are reported in Annex C.2 in g/kg bw per day and in g per day. Annex C.3 shows the range of consumption statistics across surveys per each population group.

Results for toddlers and adults are presented in Table 17. Under this scenario, the concentration for toddlers was estimated to be 0.05 mg GTX I and III/kg honey and that for adults to be 0.12 mg GTX I and III/kg honey.

Table 17: *Calculated highest concentrations* of GTX I and III in honey below which no acute effects are expected at mean and P95 consumption of honey

	No of surveys	Honey consumption[(a)] (g/kg bw per day)	Highest concentration in honey (mg/kg)
Results for mean consumption			
Toddlers	16	1.35	0.11
Adults	24	0.44	0.34
Results for consumption at P95			
Toddlers	5	3.00	**0.05**
Adults	22	1.22	0.12

(a): Maximum mean and P95 acute consumption across dietary surveys (consumers only).

3.5.2. Scenario B: Consumption of *Rhododendron* honey as traditional folk medicine in adults

In certain regions (e.g. Turkey and Nepal), *Rhododendron* honey rich in grayananes can be used as a traditional folk medicinal product. Human intoxications due to consumption of such honey are often associated with honeys originating from these two regions (see Section 3.1.3.1). The consumption of 'mad honey' in Europe is not recorded in the EFSA Consumption Database and, as suggested by acute intoxication statistics in the EU, it is likely a sporadic practice in Europe (see Section 3.1.3.1).

Portions of *Rhododendron* honey were derived from labels of 'mad honey' products sold on the internet and originating from Nepal or Turkey (as described in Section 2.3). The recommended portions on the labels ranged from a minimum portion of 8 g (1 teaspoon) up to 66 g (equal to 3 tablespoons) of honey. Results are reported in Table 18. Considering the highest recommended portion of 66 g of 'mad honey', this would result in a *highest concentration* of 0.16 mg GTX I and III/kg honey for the adult population.

Table 18: *Calculated highest concentration* of GTX I and III in honey below which no acute effects are expected based on recommended portions[(a)] on product labelling

Portion[(a)]	Honey consumption[(b)] (g/kg bw per day)	***Highest concentration*** in honey (mg/kg)
8 g (1 teaspoon)	0.11	1.31
16 g (2 teaspoons)	0.23	0.65
66 g (3 tablespoons)	0.94	0.16

(a): Portion values are indicative and subject to uncertainty, see Section 2.5.
(b): estimated for a standard bw of 70 kg (EFSA, 2012).

3.6. Uncertainty analysis

The purpose of the uncertainty analysis was to identify and quantify sources of uncertainty affecting the risk assessment and combine them to assess the overall certainty of the final conclusions, as recommended in EFSA's guidance on uncertainty analysis (EFSA, 2018a).

In a first step, sources of uncertainties related to hazard identification and characterisation of grayananes and consumption of honey containing grayananes in the EU were listed and discussed. A complete list is presented in Annex D. The most important uncertainties are listed in order of importance in Appendix E, together with 'low' and 'high' scenarios describing the Panel's qualitative evaluation of their potential impact on the assessment.

Hazard identification for genotoxic effects followed EFSA's guidance for genotoxicity assessment (EFSA Scientific Committee, 2011, 2017b), which can be considered as a standardised procedure for the purposes of uncertainty analysis (Section 3 in EFSA Scientific Committee, 2018a). As there was a positive result from a valid *in vivo* MN study with GTX III and *Rhododendron* honey, the Panel concluded that GTX III and honey containing GTX I and III should be considered as *in vivo* genotoxins and, therefore, an HBGV cannot be established. The Panel did not identify any non-standard uncertainties affecting these conclusions, so no further uncertainty analysis was required for them (Section 3.1 in EFSA Scientific Committee, 2018a).

The assessment of acute toxicity and risk were affected by major uncertainties. The Panel's analysis of these is described in detail in Appendix D. In summary, the Panel quantified by expert judgement the impact of the identified uncertainties on the RP for acute toxicity for the sum of GTX I and III, the RPFs of other grayananes and the P95 of honey consumption for EU toddlers and adults. These judgements were elicited by semi-formal structured methods of Expert Knowledge Elicitation (semi-formal EKE, Annex B.8 of EFSA (2018b)). The quantified uncertainties affecting the RP for acute toxicity and P95 consumption were then combined by probability bounds analysis (section 14.1 of EFSA, 2018a) to quantify the uncertainty of the MOEs calculated by the Panel for the exposure at selected concentrations for the sum of GTX I and GTX III and acute consumption from the EFSA database. The Panel also calculated the combined uncertainty associated with the *calculated highest concentrations*, based on the RP for acute toxicity and data on acute consumption (i) from the EFSA database, and (ii) from consumption of 'certain honey' as folk medicine.

Uncertainties affecting the RP for acute toxicity for the sum of GTX I and GTX III were quantified by eliciting expert judgements of the probability that this RP would be higher than the assessed value of 15.3 μg/kg bw if the uncertainties were resolved. This provides a measure of the conservativism of the assessed value. The judgements were made by four members of the WG with specialist expertise on toxicology and for the interpretation of reported cases of human intoxication. The judgements of the four experts were all in the range 70–90% probability. After discussion, the experts agreed on a consensus of 85% probability, considering the balance between the most important sources of uncertainty: the potential for a lower value of the RP for acute toxicity in females than males, higher values for oral compared to i.p. exposure, and the bioavailability of GTX I and III when administered via honey instead of as pure substances. On this basis, the Panel is 85% certain[26] that the RP for acute toxicity of 15 μg/kg bw for the sum of GTX I and III is protective (i.e. would not be lower if the uncertainties were resolved).

RPFs for the acute toxicity of grayananes classified into four groups are presented in Table 11 (Section 3.1.5). The combined impact of uncertainties affecting these was quantified by eliciting expert judgements of the probability that the RPF for each group is conservative (i.e. would not be higher if the uncertainties were resolved) for all grayananes in Table 1 of the Opinion that would fall in that group. Expert judgements were made by four experts: three toxicologists in the WG and a fourth WG member with expertise on assessment of relative potencies. Considering the range of judgements of the four experts, the Panel is 70–80% certain for Group 1A, 90–95% for Group 1B and 80–90% certain for Groups 2 and 3 (Table 11) that the RPFs are conservative. The difference in certainty between groups reflects the different amounts of data on which their RPFs are based.

Maximum values for the P95 of acute consumption of honey for toddlers and adults who are consumers of honey are presented in Table 17. The value for each age group is the maximum of P95 values from surveys in the EFSA Comprehensive Database from a subset of EU countries. Taking the maximum of the available values is intended to provide a conservative estimate of the P95 for each age group in the whole of the EU. The combined impact of uncertainties affecting estimation of the P95 for each age group in the whole of the EU was quantified by eliciting expert judgements from three members of the WG with expertise in exposure assessment. Considering the range of

[26] The impact of uncertainties on the conclusion of each part of the risk assessment was quantified using % probabilities assessed by expert judgement, following EFSA's guidance on uncertainty analysis (EFSA, 2018a). The resulting probabilities are reported as % certainty for the more probable outcome for each conclusion, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

judgements of the four experts for each of the two age groups, the Panel is at least 90% certain that the 95th percentile of acute consumption of honey by EU consumers of honey, is not higher than the assessed value, i.e. 3 g/kg bw per day for toddlers and 1.22 g/kg bw per day for adults. The Panel did not include estimates of P95 consumption for other age groups in Table 17. However, based on the data that were available for other age groups (Annex C), the experts judged with 90–95% probability that the P95 of acute honey consumption for infants, adolescents and other children in the EU would be lower than 3 g/kg bw (the value for toddlers), and 95–99% probability for the elderly and very elderly. These judgements took account of all identified sources of uncertainty but excluded consumption of honey as folk medicine.

The MOEs in Table 16 were calculated as the ratio of the RP of 15.3 μg/kg bw to the estimated exposure, based on the selected concentrations and the P95 of acute honey consumption for each age group from dietary surveys (see Section 3.4.1.1). Therefore, the uncertainty of the RP must be combined with the uncertainty of the P95 consumption for the EU population in each age group to assess the uncertainty of each MOE. The selected concentrations used by the Panel when calculating the MOEs are fixed values (not subject to uncertainty), so the calculated MOEs are conditional on them. The combination of the uncertainties relating to the RP and acute consumption was performed by probability bounds analysis (Section 14.1 and Annex B.13 of EFSA (2018b)) and is described in Appendix D.

Based on this assessment of the combined uncertainties, the Panel is 75% or more certain for toddlers and for adults that the MOEs shown in Table 16 are conservative, i.e. would not be lower if the uncertainties affecting the RP and P95 consumption estimates were resolved. The Panel noted that this is a minimum probability (see Appendix D).

The Panel did not calculate MOEs for age groups other than toddlers and adults. However, substituting the experts' judgements about P95 consumption for other age groups into the probability bounds analysis conducted for toddlers and adults, the Panel was 75% or more certain that the MOE for toddlers is conservative (i.e. would not be lower) for infants, adolescents and other children, and 80% or more certain that it is also conservative for the elderly and very elderly.

The Panel calculated *highest concentrations* of GTX I and III in 'certain honey' at which there is no concern *for acute toxicity* based on the P95 of acute honey consumption (Table 17). These were calculated as the ratio of the RP for acute toxicity to the estimated P95 of acute honey consumption for each age group, based on dietary surveys and taking into account that an MOE above 100 implies a low health concern (see Section 3.5.1). Therefore, the uncertainty of the RP for acute toxicity must be combined with the uncertainty of the P95 consumption to assess the uncertainty of these *highest concentrations*.

The combination was performed by probability bounds analysis, as was done for the MOE. Based on the result, the Panel is 75% or more certain for toddlers and for adults that the *highest concentrations* shown in Table 17 are protective, i.e. would not lead to a concern for *acute toxicity* at the P95 of normal honey consumption by that age group in the EU. The Panel noted again that the result of the probability bounds analysis is a minimum probability.

The Panel did not calculate *highest concentrations* for age groups other than toddlers and adults. However, substituting the experts' judgements about P95 consumption for other age groups into the probability bounds analysis conducted for toddlers and adults, the Panel was 75% or more certain that the *highest concentration* of 0.05 mg/kg for toddlers is also protective for infants, adolescents and other children, and at least 80% certain that it is also protective for the elderly and very elderly.

The Panel also calculated *highest concentrations* for indicative portion sizes of 'certain honey' used as folk medicine (Table 18). There were insufficient data for the Panel to quantify uncertainties regarding variation of recommended portion sizes on product labels or the actual portion sizes consumed by users of those products. Instead, the Panel quantified the uncertainty of the *highest concentrations* for folk medicine use conditional on the specific portion sizes considered. Therefore, the specified portion size was considered as fixed and the Panel's certainty that the corresponding *highest concentration* is protective is determined solely by the Panel's certainty of 85% that the RP for acute toxicity is conservative. Consequently, the same level of certainty applies to each of the indicative portion sizes shown in Table 18. For example, for a portion size of 66 g, the Panel is 85% certain that concentrations up to the calculated *highest concentration* of 0.16 mg/kg would not lead to a concern *for acute toxicity*, taking into account the uncertainty of that level. For portion sizes larger than 66 g, proportionately lower *highest concentrations* would be needed.

The Panel noted that the calculated MOEs and *highest concentrations* are based on acute toxicity of GTX I and III and do not consider the presence of other grayananes in 'certain honey'.

4. Conclusions

4.1. General

Grayananes are polyhydroxylated diterpenes. There are approximately 180 natural grayananes that have been isolated from Ericaceae plants and identified of which approximately 20 grayananes have been found in or associated with 'certain honeys'.[27] The majority of the available analytical and toxicological data concern GTX I and III.

Certain Ericaceae honeys, mostly from *Rhododendron* species from the Turkish Black Sea region and Nepal are often described as 'mad honeys' or 'toxic honeys' and are associated with the presence of grayananes and acute intoxication in humans. Non-*Rhododendron* honey from *Agarista salicifolia* is associated with grayanane intoxications in humans on the French island of La Réunion.

For this Opinion, the most important *Rhododendron* species used for honey production are *R. ponticum*, *R. luteum* and *R. ferrugineum*.

The first two species are particularly abundant in Turkey and are associated with intoxication in humans due to honey consumption. The latter species occurs in the Alps and Pyrenees and the honey produced is not reported to induce intoxication.

The yearly production volume of Ericaceae honey including *Rhododendron* honey in Europe is unknown but estimated to be small. The import volumes of these honeys from Turkey and Nepal in the EU are also limited.

4.2. Hazard identification and characterisation

4.2.1. Toxicokinetics

- Few toxicokinetic studies are available and data are limited.
- Oral toxicokinetic studies with GTX I and III in experimental animals do not allow conclusions on the bioavailability of GTX I and III following exposure to 'certain honey'.
- In case reports on intoxications related to consumption of 'mad honey', GTX I and GTX III were detected in human blood and urine, showing that they are absorbed from the GI-tract.
- No information on the biotransformation of grayananes in mammals is available to EFSA.

4.2.2. Single-dose toxicity studies in experimental animals

- Few oral studies with grayananes or preparations containing grayananes (e.g. 'mad honey') in experimental animals have been undertaken. Most toxicodynamic studies with grayananes use i.p. or i.v. injection and are LD_{50} studies, mainly in male rodents. Cardiovascular effects and depression of respiration are the main causes of death.
- The acute effects arising from the animal models most relevant for this opinion are impairment of the nervous system, bradycardia and reduction of blood pressure.
- Studies indicating differences in blood chemistry parameters were inconsistent and not supported by histological changes.
- Characterisation of the acute toxicity of grayananes other than GTX I and GTX III is limited.
- The lack of appropriate studies did not allow to extrapolate data from i.p. injection to an oral dose.
- Based on their structure–activity relationships, grayananes can be divided into four groups. The two most potent groups showed three structural alerts, and the number of alerts was inversely proportional to the LD_{50} values. Relative potency factors (RPFs) were derived for each group: 2 for Group 1A, 1 for Group 1B, 0.1 for Group 2 and < 0.01 (negligible potency) for Group 3. An RPF of 1 was assigned to GTX I and III which have similar toxicity.
- Grayananes with high potency share three structural features: They all contain a 3β-hydroxy or a 2β,3β-epoxy group, a 6β-hydroxy and a 10β-methyl group. When one of these structural features is modified or missing, toxicity is greatly reduced.

[27] The term 'certain honey' shall refer to honey and honey products originating from Ericaceae family plants and that contain grayananes.

4.2.3. Repeated-dose toxicity studies in experimental animals

- Only subacute and subchronic studies in rodents with grayananes or 'mad honey' were available. Changes were mostly observed in biochemical parameters and were considered not toxicologically relevant.
- One study suggests potential effects on the testes but the available data are limited.
- No chronic/carcinogenicity studies have been carried out.

4.2.4. Genotoxicity

- There is evidence of chromosomal damage for GTX III and honey containing GTX I and III from an *in vivo* MN test in mice. In the same honey sample, the analysis of chromosomal aberrations confirmed this result. There is no indication whether this is via a direct or indirect mode of action.

4.2.5. Observations in humans

- Acute symptoms from human intoxications with honey containing grayananes affect the muscles and the nervous and cardiovascular systems with bradycardia and hypotension being most prominent. Other common symptoms are dizziness, nausea, paraesthesia, blurred vision, weakness and change of consciousness. Higher doses may lead to complete atrioventricular block, convulsions, mental confusion, agitation, syncope and respiratory depression.
- Severe cases of poisoning are in general effectively treated in emergency medical care with atropine administered intravenously, fatal cases not having been reported in the last decades.
- The doses associated with acute poisoning cases on a single occasion observed with *Rhododendron* honeys from the Turkish Black Sea region range from 0.6 to 17 μg/kg bw or 3.9 to 24 μg/kg bw for GTX I and GTX III, respectively, and from 4.8 to 40 μg/kg bw for the sum of GTX I and III.
- From cases of acute intoxications following the ingestion of a Nepalese *Rhododendron* honey, where GTX I and III were not detected, it can be concluded that other grayananes present in *Rhododendron* species cause adverse effects on the muscles and the nervous and cardiovascular systems.

4.2.6. Mode of action

- Grayananes bind specifically to the voltage operated sodium channels preventing their inactivation and resulting in long-lasting depolarisation of excitable cells like neurons, muscle cells and cardiomyocytes.
- Reversal with atropine, a muscarinic antagonist, in human intoxication is consistent with a facilitated release of acetylcholine due to overactivation of sodium channels on cholinergic neurons (e.g. the vagus nerve).

No mechanistic data on genotoxicity and repeated dose toxicity were identified.

4.2.7. Critical effects and dose–response analysis

- The acute critical effects in experimental animals are on heart rate and blood pressure which are consistent with those observed in humans.
- An RP for GTX I, GTX III or other grayananes could not be identified from the data available for acute intoxications in humans.
- The RP associated with the acute critical effects was derived from BMD analysis of the reduction of heart rate after i.p. injection of GTX III in rats. A $BMDL_{10}$ of 15.3 μg/kg bw was determined and considered as the RP for the sum of GTX I and III, considering equal potency of both GTXs.
- The RP for the sum of GTX I and III may be applied to other grayananes, such as GTX II, by making use of their RPFs.
- It was not possible to take into account potential differences in grayanane bioavailability after oral and i.p. exposure to 'certain honey' or the pure substance(s) because of the limited toxicokinetic studies.
- No RP could be identified from repeated dose toxicity studies on grayananes.
- A health-based guidance value (HBGV) could not be derived for grayananes due to the indicated genotoxicity *in vivo*.

4.2.8. Occurrence

- Occurrence data on grayananes are only available from literature and limited to GTX I and/or GTX III. Most of the data are on *Rhododendron* honey from Turkish or Nepalese origin. Levels of GTX I and/or GTX III in these honeys range from 0.1 to 54 mg/kg.
- One study is available with quantifiable levels of GTX I in *Rhododendron* honey of EU origin (Italian Alps), showing a highest level for GTX I of 0.1 mg/kg. GTX III was also detected in some samples but not quantified.
- Grayananes other than GTX I and III have been detected in 'certain honey' originating from the Ericaceae family using modern methods of analysis.
- The lack of reference standards and the lack of analytical methods with sensitivity as low as 0.01 mg GTX per kg honey were identified as important factors hampering the collection of representative occurrence data.
- For other food categories, only limited data are available.
- No conclusions on the effect of processing can be drawn as no studies were identified. The limited available information suggests that GTX I and III are stable in honey when stored at room temperature for 12 months.

4.2.9. Dietary exposure assessment

In the absence of representative occurrence data for the sum of the GTX I and III and consumption data from Ericaceae honey, dietary exposure was estimated based on specific concentrations for GTX I and III in 'certain honey'. These reflect the highest measured concentration for GTX I in a honey sample of EU origin (0.1 mg/kg) and concentrations measured for the sum of GTX I and III in 'certain honeys' (1 and 10 mg/kg).

4.2.10. Risk characterisation

Risk characterisation for acute effects

- In the absence of an HBGV, the Panel decided to apply an MOE approach.
- MOEs were estimated from the RP of 15.3 μg/kg bw for GTX I and III, and three exposure estimates based on selected concentrations for the sum of GTX I and III reflecting the concentrations measured in 'certain honey' and the maximum P95 for acute consumption of honey per kg bw among EU surveys and population subgroups.
- The estimated MOEs raised health concerns for acute toxicity.

Risk characterisation for genotoxic/chronic effects

- Induction of chromosomal damage as shown in an *in vivo* micronucleus (MN) test in male mice indicates genotoxicity for GTX III and honey containing GTX I and III. Due to the lack of information on the underlying mode of action of genotoxicity and the lack of data on chronic toxicity and carcinogenicity, the Panel is unable to assess the risk related to chronic/repeated exposure.

4.2.11. Derivation of the highest levels in honey below which no acute effects are expected

- *Highest concentrations* for the sum of GTX I and III in honey below which no acute effects are expected were calculated based on the RP and an MOE of at least 100.
- When *Rhododendron* honey is consumed as 'normal' honey, a *highest concentration* for the sum of GTX I and III was estimated for toddlers being 0.05 mg GTX I and III/kg honey, based on the P95 acute consumption of honey from the EFSA database. For other age groups, the *highest concentrations* in 'normal' honey were higher.
- When *Rhododendron* honey is consumed by adults as traditional folk medicine, the *highest concentration* was estimated to be 0.16 mg GTX I and III/kg honey for consumption of 3 tablespoons (66 g) of honey. For larger portion sizes, the *highest concentration* would be lower.
- The estimated MOE and *highest concentrations* do not consider the impact of other co-occurring grayananes in 'certain honey'.

4.2.12. Uncertainty analysis

- The risk assessment on genotoxicity was not subject to any non-standard uncertainties, so no further uncertainty analysis was required for this.
- In the assessment of acute toxicity, taking account of all identified sources of uncertainty:
  - The Panel is 85% certain[28] that the RP for the sum of GTX I and III is protective (i.e. would not be lower if the uncertainties were resolved).
  - The Panel's certainty that the RPFs presented in the opinion are conservative is 70–80% for Group 1A, 90–95% for Group 1B and 80–90% for Groups 2 and 3.

When *Rhododendron* honey is consumed as 'normal' honey,

  - The Panel is at least 90% certain that the 95th percentile of acute consumption of honey by EU consumers of honey, is not higher than 3 g/kg bw per day for toddlers and 1.22 g/kg bw per day for adults (excluding consumption as folk medicine).
  - The Panel is 75% or more certain for toddlers and adults that the MOEs presented in the opinion are conservative, i.e. would not be lower if the uncertainties affecting the RP and P95 consumption estimates were resolved. The same conclusion applies to all other age groups.
  - The Panel is 75% or more certain that a highest concentration of 0.05 mg/kg below which no acute effects are expected is protective for toddlers, i.e. will not lead to a concern for acute toxicity at the 95th percentile of acute consumption. The same conclusion applies to all other age groups.

When *Rhododendron* honey is consumed as folk medicine,

  - there were insufficient data for the Panel to quantify uncertainties regarding the portion sizes consumed. Instead, uncertainty was quantified for a selection of indicative portion sizes. For a portion size of 66 g, the Panel is 85% certain that a *concentration* of 0.16 mg/kg or lower would not lead to a *concern for acute toxicity*. For larger portion sizes, proportionally lower concentrations would apply.

- The Panel noted that the calculated MOEs and *highest concentrations* are based on acute toxicity of GTX I and III and do not consider the presence of other grayananes in 'certain honey'. In addition, they may not be protective for genotoxic effects.

5. Recommendations

The following needs have been identified to improve the risk assessment for humans and reduce the uncertainties:

- Analytical standards are required for grayananes other than GTX I and III to enable accurate measurement of these substances in 'certain honey' and other foods, in particular for rhodojaponin III.
- Validated analytical methods should be developed for the quantification of total grayananes in 'certain honey' with LOQs per grayanane at 0.01 mg/kg or lower.
- There is a need for occurrence data on grayananes in honey and commodities thereof from Ericaceae species. Quantitative data should be collected for at least GTX I and GTX III.
- Integration of the monitoring of grayananes in *Rhododendron* honeys on the EU market in the national control activities is recommended, especially from specific regions of production (e.g. Alps and Pyrenees).
- Investigations of the market share of *Rhododendron* honeys in the EU market are needed.
- Mechanistic studies are required to understand the mode of action of genotoxicity.
- The short- and long-term effects of orally administered grayananes on the reproductive system need to be investigated with single and repeated-dose toxicity studies across a broader dose range (> 0.8 μg/kg bw).
- Experimental studies via the oral route on the effects of acute and chronic exposure/carcinogenicity to grayananes are required.
- Observations/surveys in humans on the acute effects of grayananes in the low-dose range and on the NOAEL are desirable.

[28] i.e. judges with 85% probability (EFSA, 2019).

Documentation as provided to EFSA

1) France, 2022. Description of poisoning cases related to consumption of honey contaminated with grayanatoxins registered by the French Poison Control Centres. Information submitted from ANSES to EFSA on 1 March 2022.
2) Germany, 2022. Case reports on Poisonings assigned to grayanotoxins from 1990 to 2021, retrieved for BfR National Case Database of Poisonings. Information submitted from BfR to EFSA on 2 February 2022.
3) Mulder P.P.J. and de Vries V.E, 2022. Preliminary results on the presence of grayanotoxins in 16 Ericaceae honey samples by LC-Orbitrap-MS analysis. Information provided to EFSA, 26 August 2022.
4) Stakeholder and focal points inputs on grayanotoxins, 2022. Information submitted to EFSA in reply to the call for stakeholder inputs in relation to the EFSA mandate on grayanotoxins in 'certain honey', from 17 January-28 February 2022.
5) Stout J. March 2022. Data submitted by Dr Jane Stout about the characteristics of *Rhododendron* species and honey and used in Section 1.2.2 Sources.
6) Turkey, 2022. Grayanane-containing Ericaceae and grayanane-containing honey. Information submitted to EFSA from the Turkish Ministry of Agriculture and Forestry on 11 February 2022.

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Abbreviations

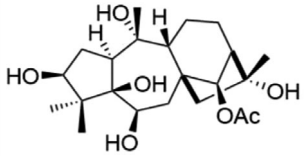
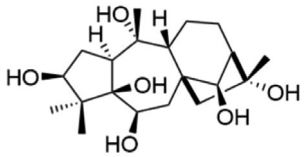
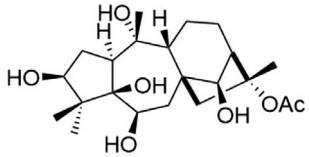
ADME	Absorption, Distribution, Metabolism, Elimination
BfR	German Federal Institute for Risk Assessment
BMD	Benchmark dose modelling
$BMDL_{10}$	benchmark dose lower bound at 10% BMR
$BMDU_{10}$	benchmark dose upper bound at 10% BMR
BMI	body mass index
BMR	benchmark response value
CONTAM	The EFSA Panel on Contaminants and in the Food Chain
ECHA	European Chemicals Agency
EMA	European Medicines Agency
EKE	expert knowledge elicitation
ESI	electrospray ionisation mode
GC	gas chromatography
GTX	grayanotoxin
HBGV	health-based guidance value
LC	liquid chromatography
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MS	mass spectroscopy
NOAEL	no-observed adverse effect level
RP	reference point

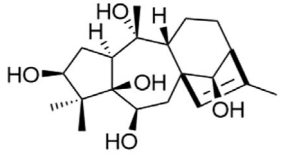
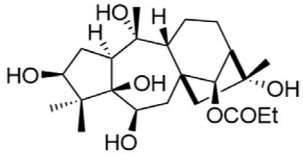
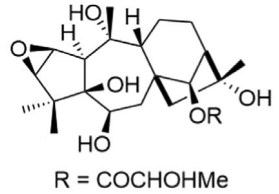
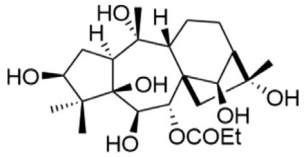
RPF	relative potency factor
SPE	solid phase extraction
TK	toxicokinetic
UB	upper bound
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

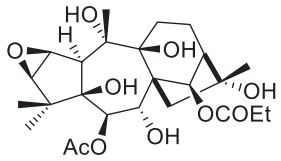
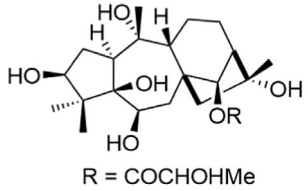
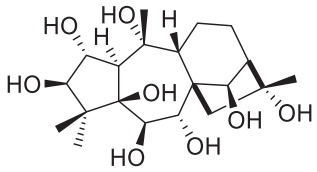
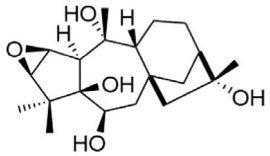
Appendix A – Grayananes considered in the opinion

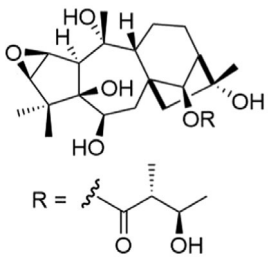
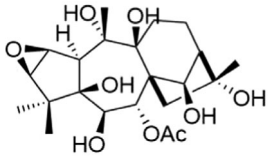
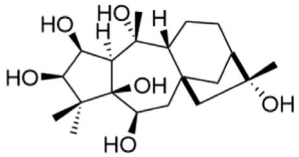
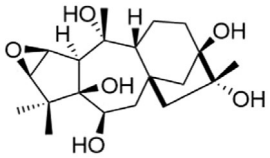
Grayananes considered in this opinion

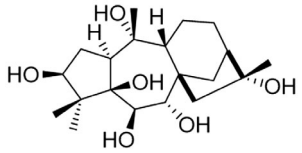
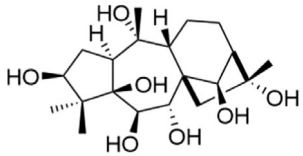
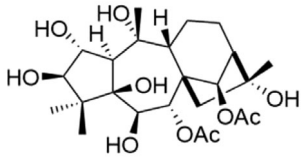
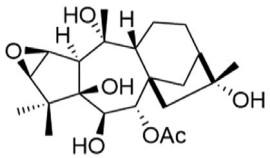
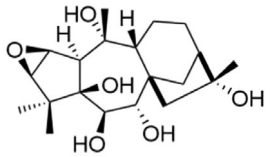
Table A.1: STRUCTURAL ALERTS for relevant biological activity: position 10β-CH_3 and position 6β-OH and position 3β-OH or 2β,3β-epoxide (Li et al., 2013)

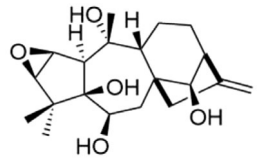
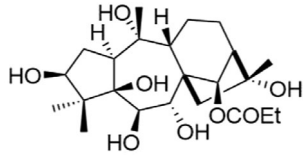
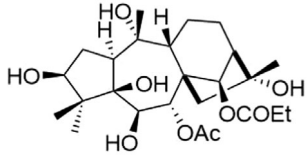
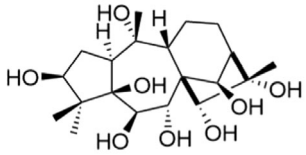
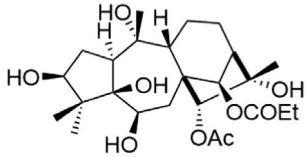
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[a]
1	**1. Grayanotoxin I** 2. [(1S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-3,4,6,9,14-pentahydroxy-5,5,9,14-tetramethyl-16-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] acetate 3. Andromedotoxin; acetylandromedol; andromedol acetate; rhodotoxin; asebotoxin	$C_{22}H_{36}O_7$ 4720-09-6 412.52 0.785 ± 0.508 258–260°C	5		14-acetate	*L. grayana*[b], *K. angustifolia, R. decorum* (Li et al., 2013), *P. formosa* (Li et al., 2017), *R. molle, R. decorum, R. yedoense, R. brachycarpum, R. przewalskii, R. oveodoxa* (Qjang et al., 2011); *R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al., 2020)
2	**1. Grayanotoxin III** 2. (1S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,9,14,16-hexol 3. Andromedol; deacetylandromedotoxin; deacetylasebotoxin I	$C_{20}H_{34}O_6$ 4678-45-9 370.48 −0.237 ± 0.514 218°C (decomp)	6			*L. grayana*[b], *K. latifolia, R. molle* (Li et al., 2013), *P. formosa* (Li et al., 2017), *R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al, 2020)
	1. 16-O-Acetylgrayanotoxin III 2. 3.	$C_{22}H_{36}O_7$ 412.52 No exp. value	5		16-acetate	*R. micranthum* (Chai et al, 2020)

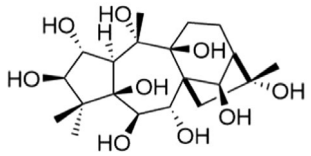
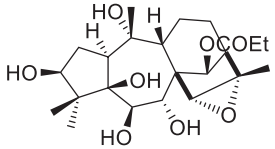
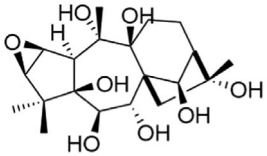
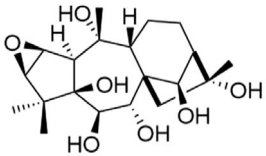
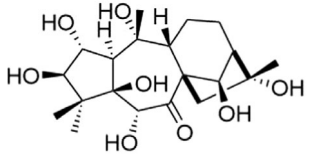
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Grayanotoxin VI 2. (3R,4R,6S,8S,9R,10R,16S)-5,5,9,14-tetramethyltetracyclo[11.2.1.01,10.04,8]hexadec-14-ene-3,4,6,9,16-pentol 3.	$C_{20}H_{32}O_5$ 30460-36-7 352.47 0.717 ± 0.493 No exp. value	5		15,16-dehydro	*L. grayana*[(b)] (Li et al., 2013), *R. dauricum, R. micranthum* (Chai et al., 2020; Peng et al., 2020, Zhang et al., 2015)
	1. Asebotoxin I 2. [(1S,3R,4R,6S,8S,9R,10R,13R,14R)-3,4,6,9,14-pentahydroxy-5,5,9,14-tetramethyl-16-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] propanoate 3. Pierisformosin B	$C_{23}H_{38}O_7$ 23984-17-0 426.54 1.295 ± 0.508 196–198°C	5		14-propionate	*P. japonica* (Li et al., 2013), *P. formosa* (Li et al., 2017), *Rhododendron spp*. (Qjang et al., 2011)
	1. **Asebotoxin III** 2. (3,4,10,15-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8] heptadecan-17-yl) 2-hydroxypropanoate 3.	$C_{23}H_{36}O_8$ 28894-73-7 440.53 0.877 ± 0.594 258–260°C	4 (5)	 R = COCHOHMe	2,3-epoxide, 14-hydroxy-proprionate	*P. japonica* (Li et al., 2013)
	1. Asebotoxin VI 2. [(1R,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-3,4,6,9,14,16-hexahydroxy-5,5,9,14-tetramethyl-2-tetracyclo[11.2.1.01,10.04,8]- hexadecanyl] propanoate 3.	$C_{23}H_{38}O_8$ 63529-01-1 442.54 0.920 ± 0.540 No exp. value	6		7-propionate	*P. japonica* (Li et al., 2013)

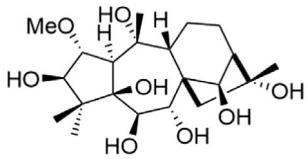
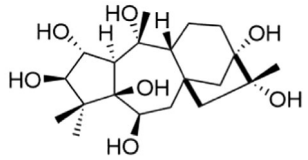
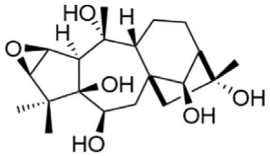
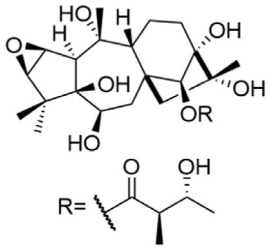
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Asebotoxin VII 2. [(1R,2S,3R,4R,6R,8S,9R,10S,11S,14R,15R,17R)-2-acetyloxy-3,4,10,11,15-pentahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecan-17-yl] propanoate 3.	$C_{25}H_{38}O_{10}$ 34183-45-4 498.6 −0.7 No exp. value	5		2,3-epoxide, 7-acetate, 14-proprionate	*P. japonica* (Li et al., 2013)
	1. Asebotoxin X 2. 3. Pieristoxin I	$C_{23}H_{38}O_8$75796-14-4 442.54 −0.039 ± 0.539 No exp. value	5 (6)	 R = COCHOHMe	14-hydroxy-propionate	*P. japonica* (Li et al., 2013)
	1. Bisdeacetylkalmitoxin VI 2. 3.	$C_{20}H_{34}O_8$ 75909-24-9 402.47 −2.551 ± 0.604 No exp. value	8			*L. ovalifolia* (Zhang et al., 2020)
	1. Craiobiotoxin II 2. 3.	$C_{20}H_{32}O_5$ 864679-90-3 352.47 1.822 ± 0.551 115–117°C	4		2,3-epoxide	*C. yunnanense* (Li et al., 2013)

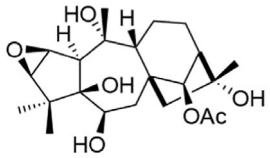
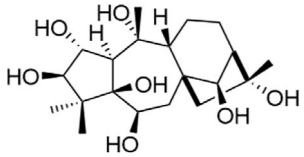
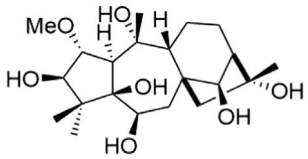
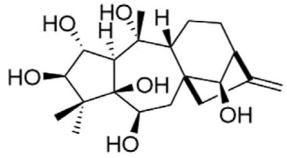
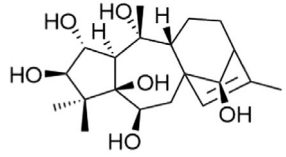
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Craiobiotoxin III 2. 3.	$C_{25}H_{40}O_8$ 864679-91-4 468.58 1.367 ± 0.580 135–137°C	4 (5)		2,3-epoxide, 14-hydroxymethyl-butanoate	*C. henryi; C. yunnanense* (Li et al., 2013; Zhang et al., 2005)
	1. Craiobiotoxin IV 2. 3.	$C_{22}H_{34}O_8$ 864679-92-5 426.50 1.502 ± 0.624 146–147°C	6		2,3-epoxide, 7-acetate	*C. yunnanense* (Li et al., 2013)
	1. Craiobiotoxin VI 2. Grayanotoxin-2β,3β,5β,6β,10α,16α-hexol 3.	$C_{20}H_{34}O_6$ 864679-94-7 370.48 −0.237 ± 0.514 162–163°C	6			*C. henryi; C. yunnanense* (Li et al., 2013; Zhang et al., 2005)
	1. **Craiobiotoxin IX** 2. (1aS,1bS,2R,2aR,5R,6R,7aR,9R,9aR,10aR)-Dodecahydro-2,6,10,10-tetramethyl-5,7a-methano-7aH-cyclohept[6,7]azuleno[1,2-b] oxirene-2,5,6,9,9a-pentol 3.	$C_{20}H_{32}O_6$ 1166135-98-3 368.46 −0.103 ± 0.588 No exp. value	5		2,3-epoxide	*R. decorum* (Li et al., 2013; Qjang et al., 2011)

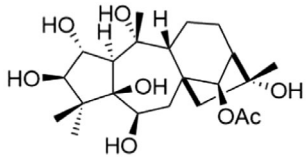
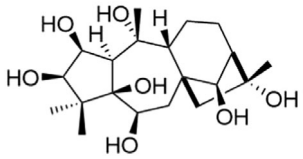
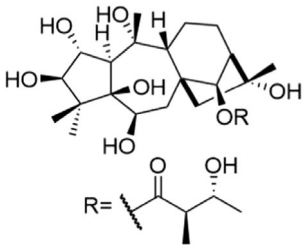
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. **Dihydrolyoniol B** 2. (2S,3R,4R,6S,8S,9R,10R,13S,14R)-5,5,9,14-tetramethyltetracyclo[11.2.1.01,10.04,8] hexadecane-2,3,4,6,9,14-hexol 3. Dihydrodeacetylyoniatoxin	$C_{20}H_{34}O_6$ 29708-82-5 370.48 −0.128 ± 0.533 No exp. value	6			
	1. Kalmitoxin I 2. (1R,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-2,3,4,6,9,14,16-heptol 3. Desacetylpieristoxin C; desacetylpieristoxin F; bisdesacetylasebotoxin V	$C_{20}H_{34}O_7$ 56663-60-6 386.48 −1.237 ± 0.561 255–257°C	7			*K. latifolia* (Li et al., 2013), *P. formosa* (Li et al., 2017)
	1. Kalmitoxin VI 2. [(1R,2S,3R,4R,6R,7R,8S,9R,10R,13R,14R,16R)-2-acetyloxy-3,4,6,7,9,14-hexahydroxy-5,5,9,14-tetramethyl-16-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{24}H_{38}O_{10}$ 72514-67-1 486.55 0.401 ± 0.579 No exp. value	6		7,14-diacetate	*K. latifolia* (Li et al., 2013)
	1**. Lyoniol A** 2. [(1R,2S,3R,4R,6R,8S,9S,10R,11R,14R,15R)-2,4,10,15-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo[12.2.1.01,11.04,9.06,8] heptadecan-3-yl] acetate 3. Lyoniatoxin	$C_{22}H_{34}O_7$ 31136-61-5 410.50 2.098 ± 0.576 No exp value	4		2,3-epoxide, 7-acetate	*L. ovalifolia, C. yunnanense, K. latifolia* (Li et al., 2013)
	1. **Lyoniol B** 2. (2S,3R,4R,6R,8S,9S,10R,11R,14S,15R)-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecane-2,3,4,10,15-pentol 3. Desacetyllyoniatoxin; desacetyllyoniol A	$C_{20}H_{32}O_6$ 28894-74-8 368.46 −0.3 279–281°C	5		2,3-epoxide	*L. ovalifolia, C. yunnanense* (Li et al., 2013)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Mollfoliagein B 2. 2β,3β-epoxy-5β,6β,10α,14β-tetrahydroxygrayan-16(17)-ene 3.	$C_{20}H_{30}O_5$ 350.45 209–211°C	4		2,3-epoxide, 16-exomethylene	*R. molle* (Zhou et al., 2018)
	1. Pierisformosin C 2. Grayanotoxane-3,5,6,7,10,14,16-heptol, 14-propanoate, (3β,6β,7α,14R) 3.	$C_{23}H_{38}O_8$ 220796-29-2 442.54 0.728 ± 0.549 No exp. value	6		14-propionate	*P. formosa* (Li et al., 2013)
	1. Pierisformosin D 2. Grayanotoxane-3,5,6,7,10,14,16-heptol, 7-acetate 14-propanoate, (3β,6β,7α,14S) 3.	$C_{25}H_{40}O_9$ 217652-87-4 484.58 1.645 ± 0.534 No exp. value	5		7-acetate, 14-propionate	*R. japonicum, R. molle* (Li et al., 2013), *P. formosa* (Li et al., 2017)
	1. Pierisformosoid D 2. 3.	$C_{20}H_{34}O_8$ 402.48 No exp. value	8			*P. formosa* (Niu et al., 2018)
	1. Pierisformosoid F 2. 3.	$C_{25}H_{40}O_9$ 484.58 No exp. value	5		14-propionate, 15-acetate	*P. formosa* (Niu et al., 2018)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Pierisformosoid G 2. 3.	$C_{20}H_{34}O_9$ 418.46 No exp. value	9			*P. formosa* (Niu et al., 2018)
	1. Pierisoid D	$C_{23}H_{36}O_8$ 440.52 No exp. value	5		14-propionate, 15,16-epoxide	*P. formosa* (Li et al, 2017
	1. **Pieristoxin G** 2. (2S,3R,4R,6R,8S,9R,10S,11S,14S,15R,17S)-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecane-2,3,4,10,11,15,17-heptol 3. Bisdesacylasebotoxin VII	$C_{20}H_{32}O_8$ 34206-60-5 400.46 −0.570 ± 0.669 No exp. value	7		2,3-epoxide	*P. japonica* (Li et al., 2013)
	1. **Pieristoxin H** 2. 3. Desacetylkalmitoxin IV	$C_{20}H_{32}O_7$ 75899-53-5 384.46 0.465 ± 0.615 No exp. value	6		2,3-epoxide	*P. formosa* (Li et al., 2017), *P. japonica*, *C. yunnanense* (Li et al., 2013)
	1. **Pieristoxin O** 2. (1R,2S,3R,4R,6R,7R,8S,9R,10R,13R,14R, 16R)-7-methoxy-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-2,3,4,6,9,14,16-heptol 3.	$C_{20}H_{32}O_8$ 1836210-49-1 400.46 −4.080 ± 0.633 No exp. value	7		7-ketone	*P. formosa* (Niu et al., 2016)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. **Pieristoxin Q** 2. (1R,2S,3R,4R,6R,7R,8S,9R,10R,13R,14R, 16R)-7-methoxy-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8] hexadecane-2,3,4,6,9,14,16-heptol 3.	$C_{21}H_{36}O_8$ 1836210-53-7 416.46 −0.710 ± 0.635 No exp. value	7		2-methoxy	*Lyonia ovalifolia* (Zhang et al., 2020), *P. formosa* (Niu et al., 2016)
	1. **Rhododecorumin XII** 2. 3.	$C_{20}H_{34}O_7$ 2230479-43-1 386.48 −1.766 ± 0.577 No exp. value	7			*R. decorum* (Zhu et al, 2018)
	1. **Rhodojaponin III** 2. (1S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17R)-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecane-3,4,10,15,17-pentol 3. Rhomotoxin, desacetylpieristoxin B	$C_{20}H_{32}O_6$ 26342-66-5 368.46 0.949 ± 0.572 285–287°C	5		2,3-epoxide	*Lyonia ovalifolia* (Zhang et al., 2020), *R. decorum, R. japonicum, R. molle, R. przewalski* (Li et al., 2013; Qjang et al., 2011; Wang et al., 2014; Zou et al., 2014)
	1. 14β-O-(2S,3R-nilyl)Rhodojaponin III 2. [(1S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17R)-3,4,10,15-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo[12.2.1.01,11.04,9.06,8] heptadecan-17-yl] (2S,3R)-3-hydroxy-2-methylbutanoate 3.	$C_{25}H_{40}O_8$ 1118548-06-3 468.58 1.367 ± 0.580 No exp. value	4(5)		14-nilylate	*C. henryi* (Li et al., 2013)

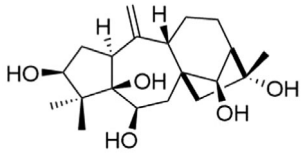
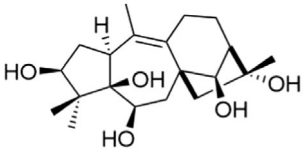
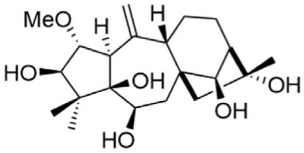
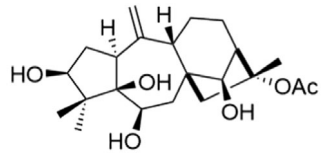
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. **Rhodojaponin V** 2. (3,4,10,15-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8] heptadecan-17-yl) acetate 3.	$C_{22}H_{34}O_7$ 37720-86-8 410.50 1.701 ± 0.566 No exp. value	4		2,3-epoxide, 14-acetate	*R. japonicum* (Li et al., 2013), *R. molle* (Zhou et al., 2018)
	1. Rhodojaponin VI 2. (1S,3R,4R,6R,7R,8S,9R,10R,13R,14R,16R)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,7,9,14,16-heptol 3.	$C_{20}H_{34}O_7$ 37720-87-9 386.48 −1.237 ± 0.561 No exp. value	7			*Lyonia ovalifolia* (Zhang et al., 2020), *P. japonica* (Zheng et al., 2019), *R. molle* (Li et al., 2013; Qjang et al., 2011; Zou et al., 2014)
	1. 2-Methoxyrhodojaponin VI 2. 3.	$C_{21}H_{36}O_7$ 400.5 No exp. value	6		2-methoxy	*P. japonica* (Zheng et al., 2019), *R. molle* (Li et al., 2020)
	1. **Rhodomollein IX** 2. (1R,3R,4R,6R,7R,8S,9R,10R,13S,16R)-5,5,9-trimethyl-14-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,7,9,16-hexol 3.	$C_{20}H_{32}O_6$ 798558-80-2 368.46 −0.133 ± 0.542 133–135°C	6		16-exomethylene	*R. molle* (Li et al., 2013; Qjang et al., 2011)
	1. **Rhodomollein X** 2. (1S,3R,4R,6R,7R,8S,9R,10R,13S,16R)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8] hexadec-14-ene-3,4,6,7,9,16-hexol 3.	$C_{20}H_{32}O_6$ 798558-81-3 368.46 −0.133 ± 0.542 223–224°C	6		15,16-dehydro	*P. japonica* (Zheng et al., 2019), *R. molle* (Li et al., 2013; Qjang et al., 2011)

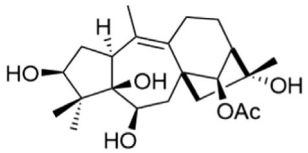
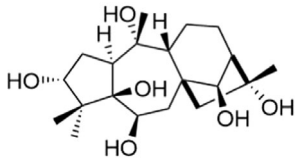
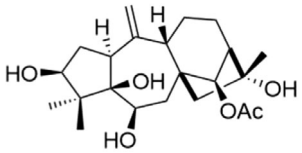
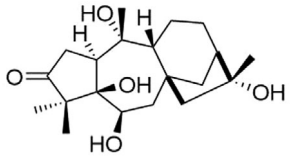
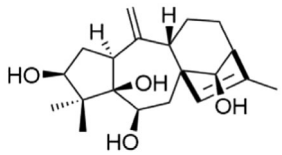
No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. **Rhodomollein XVI** 2. [(1S,3R,4R,6R,7R,8S,9R,10R,13R,14R,16S) -3,4,6,7,9,14-hexahydroxy-5,5,9,14-tetramethyl-16-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{36}O_8$ 292164-18-2 428.5 0.051 ± 0.555 No exp. value	6		14-acetate	*R. molle* (Li et al., 2013; Zou et al., 2014)
	1. Rhodomollein XVIII 2. (1S,3R,4R,6R,7S,8S,9R,10R,13R,14R,16S)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,7,9,14,16-heptol 3.	$C_{20}H_{34}O_7$ 292164-20-6 386.48 −1.237 ± 0.561 No exp. value	7			*R. molle* (Li et al., 2013; Qjang et al., 2011)
	1. 14β-O-(2S,3R-nilyl)Rhodomollein XVIII 2. [(1S,3R,4R,6R,7S,8S,9R,10R,13R,14R,16R) -3,4,6,7,9,14-hexahydroxy-5,5,9,14-tetramethyl-16-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] (2S,3R)-3-hydroxy-2-methylbutanoate 3.	$C_{25}H_{42}O_9$ 870785-29-8 486.55 −0.283 ± 0.569 103–105°C	6(7)		14-nilylate	*C. henryi* (Li et al., 2013)

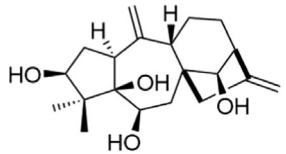
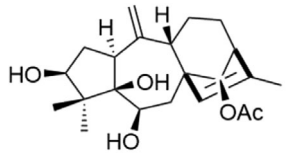
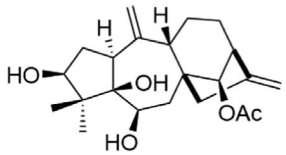
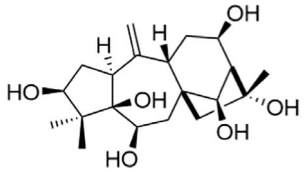
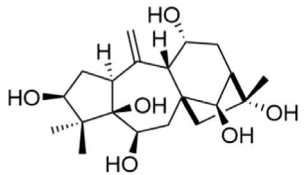
(a): In this table all references are from Li et al., 2013, unless otherwise stated.
(b): *Leucothoe grayana* and not *Lyonia grayana*. *Lyonia grayana* is not an accepted name according to World Flora Online and probably a synonym of *Leucothoe grayana* (see http://www.worldfloraonline.org/search?query=Leucothoe+grayana and http://www.theplantlist.org/tpl1.1/record/kew-2349974).

Grayananes considered in this opinion

Table A.2: STRUCTURE ALERTS for relevant biological activity: (i) position 6β-OH and position 3β-OH or 2β,3β-epoxide; or (ii) position 10β-CH_3 and position 3β-OH or 2β,3β-epoxide; or (iii) position 6β-OH and position 10β-CH_3

No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[a]
	1. Grayanotoxin II 2. (1S,3R,4R,6S,8S,10S,13R,14R,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,14,16-pentol 3. $\Delta^{10(18)}$-Andromedenol	$C_{20}H_{32}O_5$ 4678-44-8 352.47 0.532 ± 0.484 No exp. value	5		10-exomethylene	*L. grayana*[b] (Iwasa et al., 1961; Kumazawa et al., 1961*), Lyonia ovalifolia* (Zhang et al., 2020), *P. formosa* (Li et al., 2017); *P. japonica* (Zheng et al., 2019), *R. molle, R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al, 2020)
	1. Isograyanotoxin II 2. (1S,3R,4R,6S,8S,13R,14R,16R)-5,5,9,14-tetramethyltetracyclo[11.2.1.01,10.04,8] hexadec-9-ene-3,4,6,14,16-pentol 3.	$C_{20}H_{32}O_5$ 104459-10-1 352.47 0.426 ± 0.488 183–185°C	5		9,10-dehydro	*L. grayana*[b] (Terai et al., 2000); *R. micranthum, R. dauricum* (Chai et al., 2020; Peng et al., 2020), *R. molle* (Li et al., 2019)
	1. 2α-O-methylgrayanotoxin II 2. 3.	$C_{21}H_{34}O_6$ 2171096-78-7 382.5 0.594 ± 0.547 No exp. value	5		2-methoxy, 10-exomethylene	*P. japonica* (Zheng et al., 2019)
	1. 16-O-Acetylgrayanotoxin II 2. [(1S,3R,4R,6S,8S,10S,13R,14R,16R)-3,4,6,16-tetrahydroxy-5,5,14-trimethyl-9-methylidene-14-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{22}H_{34}O_6$ 394.50 0.9 No exp. value	4		10-exomethylene, 16-acetate	*R. micranthum* (Zhang et al., 2015)

No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. 14-O-Acetylisograyanotoxin II 2. 3.	$C_{22}H_{34}O_6$ 394.50 No exp. value	4		9,10-dehydro, 14-acetate	*R. micranthum, R. dauricum* (Chai et al., 2020, Peng et al., 2020)
	1. 3-Epigrayanotoxin III 2. (3R,4R,6R,8S,9R,10R,14R,16S)-5,5,9,14-tetramethyltetracyclo[11.2.1.01,10.04,8] hexadecane-3,4,6,9,14,16-hexol 3.	$C_{20}H_{34}O_6$ 30460-38-9 370.48 −0.237 ± 0.514 No exp. value	6		3-epi-hydroxy	*L. grayana*[(b)] (Hikino et al. 1976)
	1. Grayanotoxin IV 2. [(1S,3R,4R,6S,10R,13R,14R,16R)-3,4,6,14-tetrahydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] acetate 3. $\Delta^{10(18)}$-Andromedenol, acetate; Acetylandromed-10(18)-enol; $\Delta^{10(18)}$-acetylandromedenol	$C_{22}H_{34}O_6$ 30272-17-4 394.50 1.379 ± 0.481 177–179°C	4		10-exomethylene, 14-acetate	*L. grayana*[(b)] (Li et al., 2013), *R. molle, R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al, 2020)
	1. Grayanotoxin V 2. (3R,4R,8S,9R,10R,14R,16S)-3,4,9,14,16-pentahydroxy-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecan-6-one 3.	$C_{20}H_{32}O_6$ 30272-18-5 368.46 −2.189 ± 0.539 230–232°C	4		3-ketone	*L. grayana*[(b)] (Li et al., 2013)
	1. Grayanotoxin VII 2. (1S,3R,4R,6S,8S,10S,13S,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadec-14-ene-3,4,6,16-tetrol 3. Principinol E	$C_{20}H_{30}O_4$ 30460-59-4 334.45 1.237 ± 0.462 185–187°C	4		10-exomethylene, 15,16-dehydro	*L. grayana*[(b)] (Li et al., 2013), *P. formosa* (Niu et al., 2018), *P. japonica* (Li et al., 2017), *R. molle* (Li et al., 2019), *R. micranthum* (Chai et al., 2020, Zhang et al., 2015)

No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[a]
	1. Grayanotoxin VIII 2. (3R,4R,6S,8S,10S,16S)-5,5-dimethyl-9,14-dimethylidenetetracyclo[11.2.1.01,10.04,8]hexadecane-3,4,6,16-tetrol 3.	$C_{20}H_{30}O_4$ 30460-60-7 334.45 1.237 ± 0.462 193–196°C	4		10-exomethylene, 16-exomethylene	*L. grayana*[b] (Li et al., 2013), *R. molle, R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al, 2020, Zhang et al., 2015)
	1. Grayanotoxin IX 2. [(1S,3R,4R,6S,8S,10S,13S,16R)-3,4,6-trihydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo[11.2.1.01,10.04,8]hexadec-14-enyl] acetate 3. Andromedienol acetate	$C_{22}H_{32}O_5$ 30460-58-3 376.49 2.129 ± 0.467 No exp. value	3		10-exomethylene, 14-acetate, 15,16-dehydro	*L. grayana*[b] (Li et al., 2013); *R. micranthum* (Zhang et al., 2015; Chai et al., 2020)
	1. Grayanotoxin X 2. [(1R,3R,4R,6S,8S,10S,13S,16R)-3,4,6-trihydroxy-5,5-dimethyl-9,14-dimethylidene-16-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{32}O_5$ 36660-75-0 376.49 2.173 ± 0.467 No exp. value	3		10-exomethyene, 14-acetate, 16-exomethylene	*L. grayana*[b] (Li et al., 2013), *R. micranthum* (Chai et al., 2020, Zhang et al., 2015)
	1. Grayanotoxin XI 2. (1S,3R,4R,6S,8S,10S,12R,13R,14R,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,12,14,16-hexol 3.	$C_{20}H_{32}O_6$ 33880-98-7 368.46 −0.735 ± 0.503 No exp. value	6		10-exomethylene	*L. grayana*[b] (Li et al., 2013)
	1. Grayanotoxin XII 2. 1,1,8-trimethyl-4-methylidenedodecahydro-4a,7-methanocyclopenta[b]heptalene-2,5,8,11,11a,12(1h)-hexol 3.	$C_{20}H_{32}O_6$ 35928-08-6 368.46 −1.083 ± 0.504 No exp. value	6		10-exomethylene	*L. grayana*[b] (Li et al., 2013)

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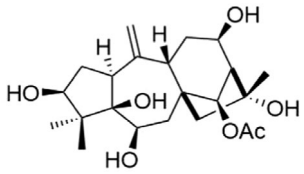
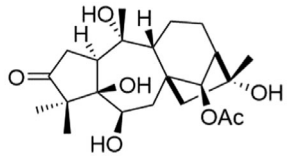
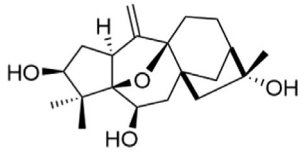
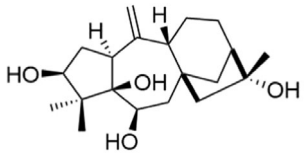
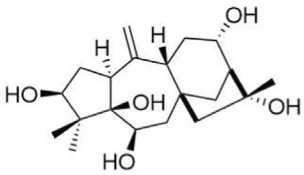
	1. Grayanotoxin XIII 2. [(1S,3R,4R,6S,8S,10S,12R,13R,14R,16R)-3,4,6,12,14-pentahydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo [11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{22}H_{34}O_7$ 35928-07-5 410.50 0.303 ± 0.494 No exp. value	5		10-exomethylene, 14-acetate	*L. grayana*[b] (Li et al., 2013)
	1. Grayanotoxin XIV 2. [(3R,4R,8S,9R,13S,14R)-3,4,9,14-tetrahydroxy-5,5,9,14-tetramethyl-6-oxo-16-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{34}O_7$ 39012-12-9 410.50 −1.437 ± 0.533 No exp. value	4		3-ketone, 14-acetate	*R. molle, R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020, Peng et al, 2020)
	1. Grayanotoxin XV 2. (1R,2R,4S,6R,7R,10S,12S,14S)-6,15,15-trimethyl-11-methylidene-16-oxapentacyclo [8.5.1.14,7.01,12.04,10]heptadecane-2,6,14-triol 3.	$C_{20}H_{30}O_4$ 39012-13-0 334.45 1.290 ± 0.590 No exp. value	3		5,9-epoxide, 10-exomethylene	*L. grayana*[b] (Li et al., 2013), *P. formosa* (Li et al., 2017)
	1. Grayanotoxin XVIII 2. (1R,3R,4R,6S,8S,10R,13R,14R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,14-tetrol 3.	$C_{20}H_{32}O_4$ 70474-76-9 336.47 1.669 ± 0.463 No exp. value	4		10-exomethylene	*L. grayana*[b] (Li et al., 2013), *P. formosa* (Li et al., 2017); *P. japonica* (Zheng et al., 2019), *R. molle, R. micranthum, R. auriculatum, R. dauricum* (Chai et al., 2020; Peng et al., 2020)
	1. 12β-Hydroxygrayanotoxin XVIII 2. 3.	$C_{20}H_{32}O_5$ 2402790-40-1 352.47 0.126 ± 0.476 180–181	5		10-exomethylene	*P. japonica* (Zheng et al., 2019)

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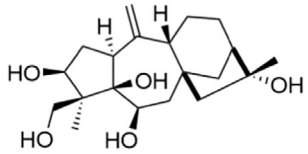
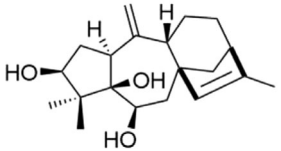
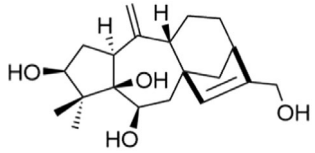
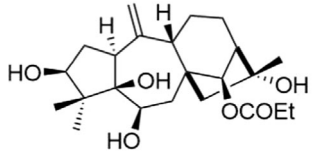
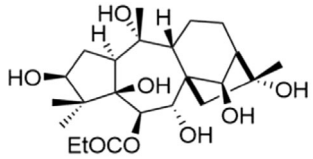
	1. 18-Hydroxygrayanotoxin XVIII 2. 3.	$C_{20}H_{32}O_5$ 352.47 No exp. value	5		10-exomethylene	*R. molle* (Zhou et al., 2018)
	1. Grayanotoxin XIX 2. (1R,3R,4R,6S,8S,10S,13R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8] hexadec-14-ene-3,4,6-triol 3.	$C_{20}H_{30}O_3$ 75829-08-2 318.45 3.184 ± 0.448 No exp. value	3		10-exomethylene, 15,16-dehydro	*L. grayana*[(b)] (Li et al., 2013)
	1. 17-Hydroxygrayanotoxin XIX 2. 3.	$C_{20}H_{30}O_4$ 2402790-35-4 334.45 1.708 ± 0.558 154–155°C	4		10-exomethylene, 15,16-dehydro	*P. japonica* (Zheng et al., 2019)
	1. Asebotoxin II 2. [(3R,4R,6S,8S,10S,14R,16S)-3,4,6,14-tetrahydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] propanoate 3.	$C_{23}H_{36}O_6$ 23984-18-1 408.53 1.889 ± 0.481 No exp. value	4		10-exomethylene, 14-propionate	*P. japonica* (Li et al., 2013)
	1. Asebotoxin IV 2. [(1R,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-2,4,6,9,14,16-hexahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] propanoate 3.	$C_{23}H_{38}O_8$ 33476-73-2 442.54 0.691 ± 0.534 No exp. value	6		6-propionate	*P. japonica* (Li et al., 2013)

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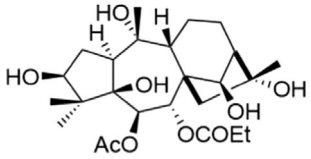
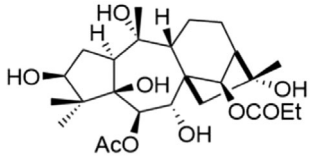
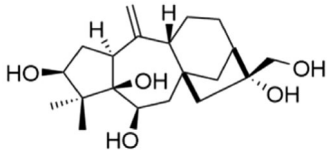
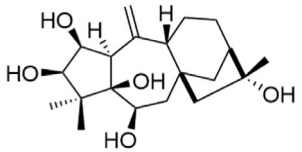
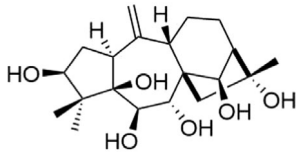
	1. Asebotoxin V 2. [(1R,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-3-acetyloxy-4,6,9,14,16-pentahydroxy-5,5,9,14-tetramethyl-2-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] propanoate 3.	$C_{25}H_{40}O_9$ 33476-74-3 484.58 1.873 ± 0.523 No exp. value	5		6-acetate, 7-propionate	*P. japonica* (Li et al., 2013)
	1. Asebotoxin VIII 2. [(1S,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-3-acetyloxy-2,4,6,9,14-pentahydroxy-5,5,9,14-tetramethyl-16-tetracyclo [11.2.1.01,10.04,8] hexadecanyl] propanoate 3.	$C_{25}H_{40}O_9$ 63529-89-5 484.58 No value No exp. value	5		6-acetate, 14-propionate	*P. japonica* (Li et al., 2013)
	1. Craiobiotoxin I 2. 3.	$C_{20}H_{32}O_5$ 864679-90-3 352.47 1.822 ± 0.551 No exp. value	5		10-exomethylene	*C. yunnanense* (Zhang et al., 2005); *R. auriculatum* (Sun et al., 2019)
	1. Craiobiotoxin V 2. 3.	$C_{20}H_{32}O_5$ 864679-93-6 352.47 0.649 ± 0.509 137–139°C	5		10-exomethylene	*C. yunnanense* (Zhang et al., 2005), *P. japonica* (Zheng et al., 2019)
	1. Kalmitoxin II 2. (1R,2S,3R,4R,6S,8S,10S,13R,14R,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-2,3,4,6,14,16-hexol 3.	$C_{20}H_{32}O_6$ 63529-90-8 368.46 −0.319 ± 0.530 No exp. value	6		10-exomethylene	*K. latifolia* (Li et al., 2013), *P. formosa* (Niu et al., 2018)

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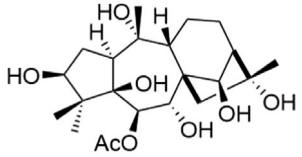
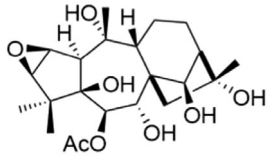
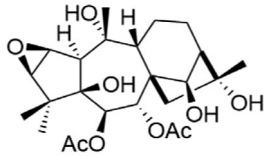
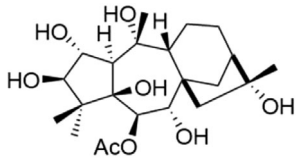
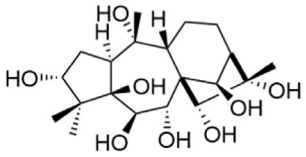
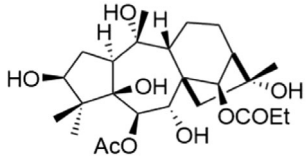
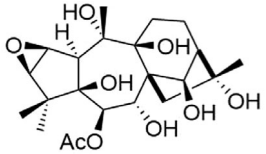
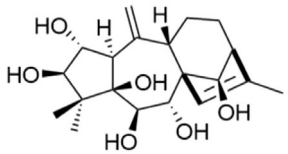
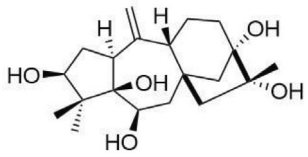
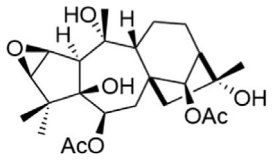
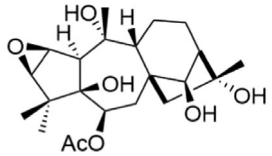
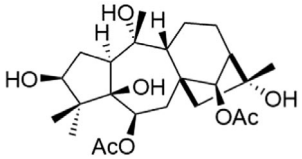
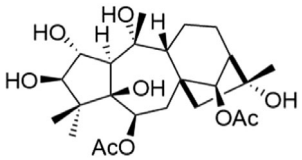
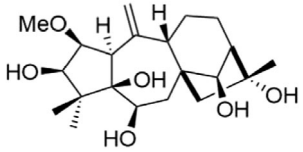
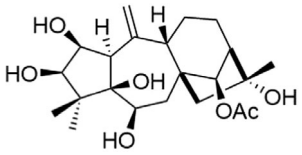
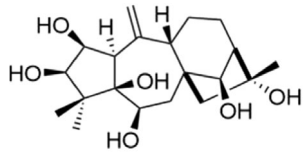
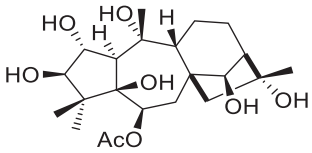
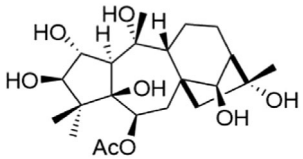
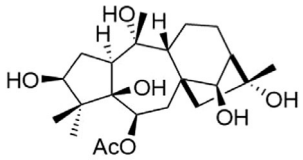
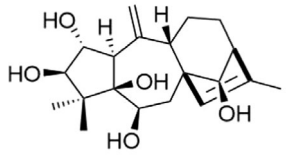
	1. Kalmitoxin III 2. [(1R,2S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-2,4,6,9,14,16-hexahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{36}O_8$ 72514-64-8 428.51 0.181 ± 0.534 No exp. value	6		6-acetate	*K. latifolia* (Li et al., 2013)
	1. Kalmitoxin IV 2. [(1R,2S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17R)-2,4,10,15,17-pentahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo[12.2.1.01,11.04,9.06,8]heptadecan-3-yl] acetate 3.	$C_{22}H_{34}O_8$ 72514-65-9 426.50 1.425 ± 0.591 No exp. value	5		2,3-epoxide, 6-acetate	*K. latifolia* (Li et al., 2013)
	1. Kalmitoxin V 2. [(1R,2S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17R)-3-acetyloxy-4,10,15,17-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo[12.2.1.01,11.04,9.06,8]heptadecan-2-yl] acetate 3.	$C_{24}H_{36}O_9$ 72514-66-0 468.5 2.246 ± 0.582 No exp. value	4		2,3-epoxide, 6,7-diacetate	*K. latifolia* (Li et al., 2013)
	1. Lyoniol D 2. [(1R,2S,3R,4R,6R,7R,8S,9R,10R,13R,14R)-2,4,6,7,9,14-hexahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{36}O_8$ 38011-92-6 428.5 0.313 ± 0.552 No exp. value	6		6-acetate	*Lyonia ovalifolia* (Li et al., 2013)
	1. Pierisformosoid E 2. 3.	$C_{20}H_{34}O_8$ 402.48 No exp. value	8		3-epi-hydroxy	*P. formosa* (Niu et al., 2018)

No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Pieristoxin F 2. [(2S,3R,4R,6S,8S,9R,14R,16R)-3-acetyloxy-2,4,6,9,14-pentahydroxy-5,5,9,14-tetramethyl-16-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] propanoate 3.	$C_{25}H_{40}O_9$ 56662-60-3 484.58 1.948 ± 0.525 190–195°C	5		6-acetate, 14-propionate	*P. japonica* (Li et al., 2013)
	1. Pieristoxin J 2. [(1R,2S,3R,4R,6R,8S,9R,10S,11S,14R,15R,17R)-2,4,10,11,15,17-hexahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecan-3-yl] acetate 3.	$C_{22}H_{34}O_9$ 34183-49-8 442.5 0.636 ± 0.645 No exp. value	6		2,3-epoxide, 6-acetate	*P. japonica* (Li et al., 2013)
	1. Pieristoxin R 2. (1R,2S,3R,4R,6R,7R,8R,10S,13S,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadec-14-ene-2,3,4,6,7,16-hexol 3.	$C_{20}H_{30}O_6$ 1836210-55-9 366.4 5−0.944 ± 0.549 No exp. value	6		10-exomethylene, 15,16-dehydro	*P. formosa* (Niu et al., 2016)
	1. Pieristoxin S 2. (1R,3R,4R,6S,8S,10R,13S,14R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,13,14-pentol 3.	$C_{20}H_{32}O_5$ 1836210-57-1 352.47 0.479 ± 0.483 No exp. value	5		10-exomethylene	*P. formosa* (Niu et al., 2016), *P. japonica* (Zheng et al., 2019)
	1. Rhodojaponin I 2. [(1S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17S)-17-acetyloxy-4,10,15-trihydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo [12.2.1.01,11.04,9.06,8]heptadecan-3-yl] acetate 3.	$C_{24}H_{36}O_8$ 26116-88-1 452.5 2.337 ± 0.557 248.5–250°C	3		2,3-epoxide, 6,14-diacetate	*R. japonicum* (Li et al., 2013), *R. molle* (Zhou et al., 2018; Zou et al., 2014)

No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Rhodojaponin II 2. [(1S,3R,4R,6R,8S,9S,10R,11R,14R,15R,17R)-4,10,15,17-tetrahydroxy-5,5,10,15-tetramethyl-7-oxapentacyclo[12.2.1.01,11.04,9.06,8]heptadecan-3-yl] acetate 3.	$C_{22}H_{34}O_7$ 26116-89-2 410.50 1.595 ± 0.560 294.5–296°C	4		2,3-epoxide, 6-acetate	*R. japonicum* (Li et al., 2013); *R. molle* (Zhou et al., 2018)
	1. Rhodojaponin IV 2. [(1S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-16-acetyloxy-4,6,9,14-tetrahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3. 6-O-Acetylgrayanotoxin I	$C_{24}H_{38}O_8$ 30460-34-5 454.6 1.691 ± 0.496 No exp. value	4		6,14-diacetate	*R. formosum, R. japonicum* (Li et al., 2013)
	1. Rhodojaponin VII 2. [(1S,3R,4R,6R,7R,8S,9R,10R,13R,14R,16R)-16-acetyloxy-4,6,7,9,14-pentahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{24}H_{38}O_9$ 38290-01-6 470.6 1.045 ± 0.540 No exp. value	5		6,14-diacetate	*R. formosum, R. japonicum* (Li et al., 2013)
	1. Rhodomolin A 2. (1S,3R,4R,6R,7S,8R,10S,13R,14R,16R)-7-methoxy-5,5,14-trimethyl-9-methylidene-tetracyclo[11.2.1.01,10.04,8]hexadecane-3,4,6,14,16-pentol 3.	$C_{21}H_{34}O_6$ 858940-89-3 382.5 0.594 ± 0.547 No exp. value	5		2-methoxy, 10-exomethylene	*R. molle* (Li et al., 2013)
	1. Rhodomolin B 2. [(1S,3R,4R,6R,7S,8R,10S,13R,14R,16R)-3,4,6,7,14-pentahydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{22}H_{34}O_7$ 858940-90-6 410.50 0.358 ± 0.526 282-284°C	5		10-exomethylene, 14-acetate	*R. molle* (Li et al., 2013)

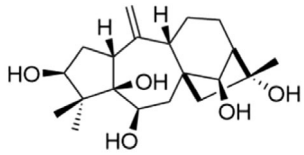
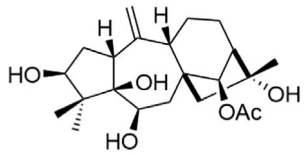
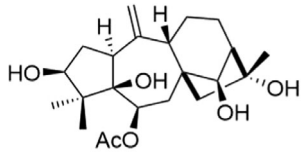
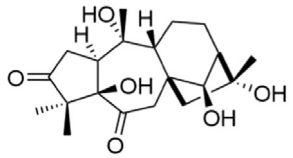
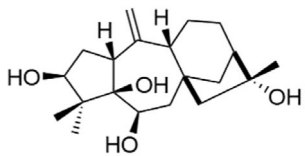
No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. **Rhodomollein I** 2. (3R,4R,6R,7S,8R,10S,14R,16S)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,7,14,16-hexol 3.	$C_{20}H_{32}O_6$ 126223-68-5 368.46 −0.628 ± 0.529 No exp. value	6		10-exomethylene	*P. japonica* (Zheng et al., 2019), *R. molle* (Li et al., 2013; Zou et al., 2014)
	1. Rhodomollein III 2. Grayanotoxane-2,3,5,6,10,14,16-heptol,6-acetate, (2b,3b,6b,14R)- 3.	$C_{22}H_{36}O_8$ 142060-05-7 428.5 0.013 ± 0.543 No exp. value	7		6-acetate	*R. molle* (Li et al., 2013, Zou et al., 2014)
	1. Rhodomollein XI 2. [(1S,3R,4R,6R,7R,8S,9R,10R,13R,14R,16R)-4,6,7,9,14,16-hexahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{22}H_{36}O_8$ 798558-82-4 428.5 0.013 ± 0.543 170-172°C	6		6-acetate	*R. molle* (Zhou et al., 2018)
	1. Rhodomollein XIII 2. [(1S,3R,4R,6S,8S,9R,10R,13R,14R,16R)-4,6,9,14,16-pentahydroxy-5,5,9,14-tetramethyl-3-tetracyclo[11.2.1.01,10.04,8] hexadecanyl] acetate 3. 6-O-Acetylgrayanotoxin III	$C_{22}H_{36}O_7$ 54781-72-5 412.5 0.809 ± 0.499 > 255°C	5		6-acetate	*R. molle* (Li et al., 2013)

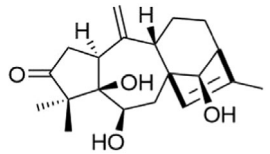
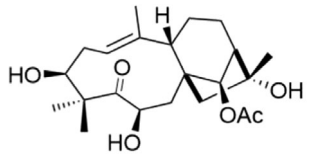
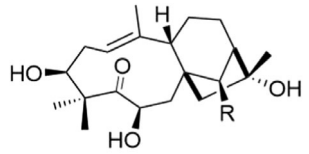
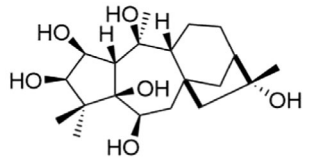
No	Name: 1. Trivial2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Rhodomollein XIX 2. (1S,3R,4R,6R,7R,8R,10S,13S,16R)-5,5,14-trimethyl-9-methylidenetetracyclo[11.2.1.01,10.04,8]hexadec-14-ene-3,4,6,7,16-pentol 3.	$C_{20}H_{30}O_5$ 629615-33-4 350.45 No exp value 0.158 ± 0.508	5		10-exomethylene, 15,16-dehydro	*P. formosa* (Niu et al., 2018), *P. japonica* (Zheng et al., 2019), *R. molle* (Li et al., 2013)

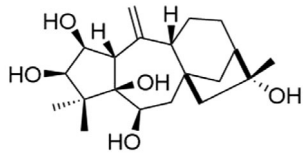
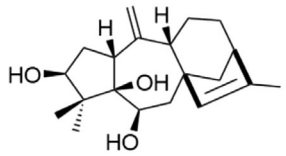
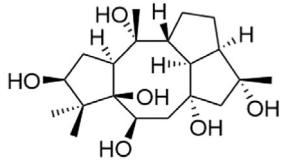
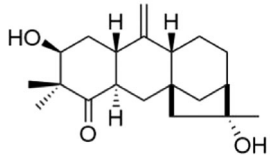
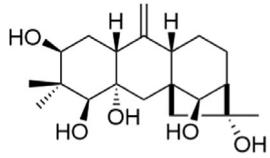
(a): In this table all references are from Li et al., 2013, unless otherwise stated.
(b): Leucothoe grayana and not Lyonia grayana for which the name is unresolved. Some sources state that Lyonia grayanane may be a synonym of Leucothoe grayanane (see http://www.theplantlist.org/tpl1.1/record/kew-2349974).

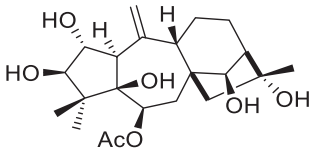
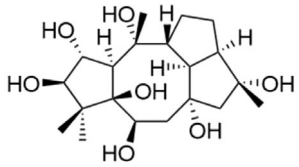
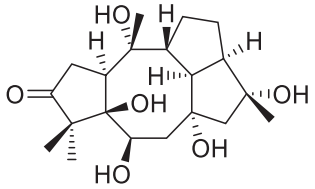
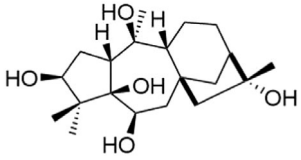
Grayananes considered in this opinion

Table A.3: STRUCTURE ALERTS: (i) Position 10β-CH_3 or (ii) position 6β-OH or (iii) position 3β-OH or (iv) still to be classified

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[a]
	1. 1-Epigrayanotoxin II 2. (1S,3R,4R,6S,8R,10S,13R,14R,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,14,16-pentol 3. Principinol D	$C_{20}H_{32}O_5$ 352.47 239–240°C	5		epi-grayanane structure, 10-exomethylene	*R. principis*, *R. micranthum* (Li et al., 2019)
	1. 1-Epigrayanotoxin IV 2. [(1S,3R,4R,6S,8R,10S,13R,14R,16R)-3,4,6,14-tetrahydroxy-5,5,14-trimethyl-9-methylidene-16-tetracyclo [11.2.1.01,10.04,8]hexadecanyl] acetate 3.	$C_{22}H_{34}O_6$ 394.50 0.9 239–240°C	4		epi-grayanane structure, 10-exomethylene, 14-acetate	*R. micranthum* (Zhang et al., 2015)
	1. Grayanotoxin XVI2. [(3R,4R,6S,8S,10S,14R,16S)-4,6,14,16-tetrahydroxy-5,5,14-trimethyl-9-methylidene-3-tetracyclo[11.2.1.01,10.04,8]hexadecanyl] acetate 3. 6-O-Acetylgrayanotoxin II	$C_{22}H_{34}O_6$ 59236-87-2 394.50 1.353 ± 0.472 No exp. value	4		6-acetate, 10-exomethylene	*R. molle*, *R. micranthum*, *R. auriculatum*, *R. dauricum* (Peng et al, 2020)
	1. Grayanotoxin XVII2. (1R,4R,8S,9R,10R,13R,14R,16R)-4,9,14,16-tetrahydroxy-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-3,6-dione 3.	$C_{20}H_{30}O_6$ 59740-27-1 366.45 –2.558 ± 0.584 No exp. value	4		3,6-diketone	*L. grayana*[b] (Li et al., 2013)
	1. 1-Epigrayanotoxin XVIII 2. 3. Pierisformosin A	$C_{20}H_{32}O_4$ 336.47 No exp. value	4		epi-grayanane structure 10-exomethylene	*P. formosa* (Li et al., 2013) *P. japonica* (Zheng et al., 2019)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Grayanotoxin XX 2. 3.	$C_{20}H_{28}O_4$ 78534-58-4 332.43 −0.967 ± 0.544 No exp. value	3		3-ketone, 10-methylene, 15,16-dehydro	
	1. Grayanotoxin XXI 2. 3.	$C_{22}H_{34}O_6$ 850494-13-2 394.50 1.247 ± 0.502 150–152 °C	3		1,5-seco-grayanane structure	*R. micranthum* (Zhang et al., 2015)
	1. Grayanotoxin XXII 2. 3.	$C_{23}H_{36}O_6$ 1252585-86-6 408.53 1.757 ± 0.502 No exp. value	3	 R = $OCOCH_2CH_3$	1,5-seco-grayanane structure	*P. formosa* (Li et. al., 2017)
	1. Craiobiotoxin VII 2. 3.	$C_{20}H_{34}O_6$ 864679-95-8 370.48 0.172 ± 0.539 165–167 °C	6		epi-grayanane structure	C. yunnanense (Li et al., 2013)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Craiobiotoxin VIII 2. 3.	$C_{20}H_{32}O_5$ 864680-12-6 352.47 0.649 ± 0.509 No exp. value	5		epi-grayanane structure, 10-exomethylene	*C. yunnanense* (Zhang et al., 2005), *P. japonica* (Zheng et al., 2019), *R. micranthum* (Zhang et al., 2015), *R. molle* (Li et al., 2019)
	1. Grayathol A 2. (1R,3R,4R,6S,8R,10S,13R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.04,8]hexadec-14-ene-3,4,6-triol 3.	$C_{20}H_{30}O_3$ 71493-02-2 318.45 3.184 ± 0.448 No exp. value	3		epi-grayanane structure, 10-exomethylene, 15,16-dehydro	*L. grayana*[(b)] (Li et al., 2013)
	1. Kalmanol 2. (1R,2R,3S,5S,7R,8R,10S,12R,13R)-2,6,6,12-tetramethyltetracyclo [8.5.1.03,7.013,16]hexadecane-2,5,7,8,10,12-hexol 3.	$C_{20}H_{34}O_6$ 118997-92-5 370.48 0.010 ± 0.461 No exp. value	6		kalmane structure	*K. angustifolia, R. molle* (Li et al., 2013; Qjang et al., 2011); *R. micranthum* (Zhang et al., 2015)
	1. Leucothol A 2. (1R,3R,6S,8S,10R,13R,14R)-6,14-dihydroxy-5,5,14-trimethyl-9-methylidene-tetracyclo[11.2.1.01,10.03,8] hexadecan-4-one 3.	$C_{20}H_{30}O_3$ 39012-11-8 318.4 1.150 ± 0.506 No exp. value	2		leucothane structure, 4-ketone	*L. grayana*[(b)], *C. yannanense* (Li et al., 2013, Wang et al., 2014)
	1. Leucothol B 2. (1R,3S,4S,6S,8R,10S,13R,14R,16R)-5,5,14-trimethyl-9-methylidenetetracyclo [11.2.1.01,10.03,8]hexadecane-3,4,6,14,16-pentol 3.	$C_{20}H_{32}O_5$ 38302-26-0 352.4 0.485 ± 0.484 No exp. value	5		leucothane structure	*L. grayana*[(b)] (Li et al., 2013)

No	Name: 1. Trivial 2. IUPAC 3. Synonyms	Elem. Comp CAS number MW LogP (25°C) mp (°C)	No of OH	Chemical structure	Remark	Examples of occurrence in Ericaceae[(a)]
	1. Rhodomollein XII 2. [(1S,3R,4R,6R,7R,8R,10S,13R,14R,16R)-4,6,7,14,16-pentahydroxy-5,5,14-trimethyl-9-methylidene-3-tetracyclo [11.2.1.01,10.04,8] hexadecanyl] acetate 3.	$C_{22}H_{34}O_7$ 798558-83-5 410.50.281 ± 0.514 75–77°C	5		6-acetate, 10-exomethylene	*R. molle* (Li et al., 2013; Chen et al., 2004)
	1. Rhodomollein XIV 2. (1R,2R,3S,4R,5R,7R,8R,10S,12R,13R,16R)-2,6,6,12-tetramethyltetracyclo [8.5.1.03,7.013,16]hexadecane-2,4,5,7,8,10,12-heptol 3.	$C_{20}H_{34}O_7$ 798558-84-6 386.48 −1.083 ± 0.512 133–135°C	7		kalmane structure	*R. molle* (Li et al., 2013)
	1. Rhodomollein XV 2. (1R,2R,3S,7R,8R,10S,12R,13R,16R)-2,7,8,10,12-pentahydroxy-2,6,6,12-tetramethyltetracyclo [8.5.1.03,7.013,16]hexadecan-5-one 3.	$C_{20}H_{32}O_6$ 292164-17-1 368.5 −1.897 ± 0.489 No exp. value	5		kalmane structure, 2-keto	*R. molle* (Zhong et al., 2005)
	1. Rhodomollein XVII 2. (1R,3R,4R,6S,8R,9S,10R,13R,14R)-5,5,9,14-tetramethyltetracyclo [11.2.1.01,10.04,8]hexadecane-3,4,6,9,14-pentol 3.	$C_{20}H_{34}O_5$ 292164-19-3 354.48 0.906 ± 0.491 No exp. value	5		epi-grayanane structure	*R. molle* (Li et al., 2013; Qjang et al., 2011)

(a): In this table all references are from Li et al., 2013, unless otherwise stated.
(b): Leucothoe grayana and not Lyonia grayana for which the name is unresolved. Some sources state that Lyonia grayanane may be a synonym of Leucothoe grayanane (see http://www.theplantlist.org/tpl1.1/record/kew-2349974).

Appendix B – Other toxicological studies in experimental animals

Table B.1: Summary of acute toxicity (LD_{50}) of naturally occurring grayananes of potential concern (Route of exposure other than oral or *i.p.*). *Compounds are ranked on the table based on their potency*

Compound		Species/ strain/sex	Route	LD_{50}	Reference
Trivial name	***Origin***			***(mg/kg bw)***	
GTX I	*R. hymenanthes*	Mice, M&F	Subcutaneous	0.15 ± 0.01	Kohanawa[(a)] (1956)[§]
Rhodojaponin III	*P. japonica*	Mice, M	Subcutaneous	0.65	Meguri (1959), Scott (1971)
GTX I	*R. hymenanthes*	Frog	Into lymphsack	3.9 ± 0.12	Kohanawa (1956)[§]
Asebotoxin III	*P. japonica*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.02	Hotta et al. (1980)[(b),§]
Asebotoxin I	*P. japonica*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.04	Hotta et al. (1980)[(b),§]
Lyoniol B	*L. ovalifolia*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.09	Hotta et al. (1980)[(b),§]
Rhodojaponin III	*R. japonicum*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.14	Hotta et al. (1980)[(b),§]
GTX III	*L. grayana*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.4	Hotta et al. (1980)[(b),§]
Lyoniol A	*L. ovalifolia*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	0.4	Hotta et al. (1980)[(b),§]
Pieristoxin G	*P. japonica*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	1.0	Hotta et al. (1980)[(b),§]
GTX I	*L. grayana*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	1.3	Hotta et al. (1980)[(b),§]
Rhodojaponin IV[(c)]	*L. grayana*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	2.5	Hotta et al. (1980)[(b),§]
GTX IV	*L. grayana*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	3.1	Hotta et al. (1980)[(b),§]
Rhodojaponin I	*R. japonicum*	Guinea Pig, Hartley, M	*i.v.* (dorsalis penis)	3.1	Hotta et al. (1980)[(b),§]

[§]: Isolated from plant material or semi-synthesised, purity not provided.

(a): To derive an LD50, Kohanawa (1956) exposed mice subcutaneously to rhodotoxin isolated from *R. hymenanthes* and andromedotoxin from *P. japonica* (synonym: *Andromeda japonica*). The author used the names rhodotoxin and andromedotoxin, both synonyms of GTX I, to identify two different substances that actually provide two different LD50s. Based on the melting point of rhodotoxin and andromedotoxin, as reported by the authors, it is possible to identify rhodotoxin as GTX I (data reported in the table) while the nature of andromedotoxin remains unidentified.

(b): 1 h.

(c): Synonym of 6-acetyl-GTX I.

Table B.2: Reports on single dose toxicity studies with Ericaceae honey and other matrices allowing to estimate the intake of grayananes

Animals	Type of honey as described (geographical and botanical origin when reported)	Concentration of grayananes in honey or other matrices	Exposure and doses of honey or other matrices administered	Estimated single doses of grayananes	Findings	Reference
Rat, Sprague Dawley[a] Female (250–300 g) 6-8 Mo n = 12 *per* experimental group	*Rhododendron* honey from beekeepers, mixture of 6 samples (≥ 45% *Rhododendron* pollen, contains also pollen from Apiaceae, Asteraceae, Fabaceae, Fagaceae (*Castanea sativa*), Rosaceae collected in June-July 2012 from different parts of Black Sea region of Turkey	GTX III: between 2.11 and 11.4 mg/kg (average 6.225 mg/kg; n = 6 different *Rhododendron* honey samples)	Gavage 0.1; 0.5; 2.5 g/kg bw 1h	Average GTX-III: 0.62; 3.11; 15.6 µg/kg bw	*Oxidative stress* Dose dependent decrease in activity of CAT, GSH-Px, SOD coupled to an increase of malondialdehyde (MDA) in erythrocytes, liver, kidney, lung, heart, spleen, testicle, epididymis and brain. The number of affected enzymes is dependent on *Rhododendron* honey dose and tissue type. 2.5 g/kg bw *Rhododendron* honey was able to affect all the tested enzymes in all analysed tissues. 0.1 g/kg bw *Rhododendron* honey decreased CAT, GSH-Px and SOD only in erythrocytes and kidney No histopathology is reported	Sibel[b] et al. (2014)
Wistar rats Male (150–200 g) n = 10 control, 12 treated group	'Mad honey', Black Sea region, from a beekeeper	GTX I: 34.05 mg/kg GTX III: 6.5 mg/kg	Gavage 12.5 g/kg bw 24 h	GTX I: 429 µg/kg bw GTX III: 81.9 µg/kg bw	Increased MDA, NO and decreased CAT in liver, kidney, brain, testes, heart, and plasma or erythrocytes. Decreased SOD in liver, kidney, brain (increased in testes, no effect in heart and erythrocytes) Increased GSH-Px in liver and decrease in kidney, brain, testes, heart (no effect in erythrocytes) Increased serum 4-hydroxynonenal No histopathology is reported	Eraslan et al. (2018)

Animals	Type of honey as described (geographical and botanical origin when reported)	Concentration of grayananes in honey or other matrices	Exposure and doses of honey or other matrices administered	Estimated single doses of grayananes	Findings	Reference
Rat, Sprague Dawley Female (250–300 g) 6-8 Mo n = 12 *per* experimental group	*Rhododendron* honey from beekeepers, (58.3% *Rhododendron* pollen, contains also pollen from Apiaceae, Asteraceae, Fabaceae, Fagaceae (*Castanea sativa*), Rosaceae collected in June-July 2012 from different parts of Black Sea region of Turkey	GTX III: 5.9 mg/kg	Gavage 0.1; 0.5; 2.5 g/kg bw 1 h	GTX III: 0.59; 2.95; 14.7 µg/kg bw	*Clinical biochemistry* 0.1 and 0.5 g/kg bw: significant reduction in blood phosphorous (no effect. at 2.5 g/kg bw) 2.5 g/kg bw and 0.5 g/kg bw: significant increase of blood urea nitrogen (BUN) and T-bilirubin, respectively No effect on blood levels of glucose, triglyceride, cholesterol, tot. protein, ALT, AST, ALP, LDH, uric acid, Ca, Na, Cl, mg, K, creatinine, HDL cholesterol, LDL cholesterol, CPK, pseudocholinesterase Kidney histology: normal structure with minimal changes in proximal and distal tubules (no control sections are provided) No liver histopathology Summary: suggested kidney toxicity due to BUN increase at the highest dose, T-bilirubin may represent liver toxicity still is the only parameter altered among liver function tests (e.g. ALT, AST, ALP, tot. protein)	Silici[(b)] et al. (2016)

Animals	Type of honey as described (geographical and botanical origin when reported)	Concentration of grayananes in honey or other matrices	Exposure and doses of honey or other matrices administered	Estimated single doses of grayananes	Findings	Reference
Mice Male (20–25 g) 8-12 weeks n = 12 *per* experimental group	*Rhododendron* honey, Duzce, western Black Sea region of Turkey	Analysed by Kaplan et al. (2014) GTX I: 18.4 mg/kg GTX III: 7.4 mg/kg	Gavage 25; 50; 75 mg/kg bw 24 h	GTX I: 0.46-0.92; 1.38 μg/kg bw GTX III: 0.185; 0.37; 0.555 μg/kg bw	*Liver:* Analysed different parameters of liver injury 25 mg/kg bw: no effect (congestion and steatosis occurs at 48 h after repeated exposure) 50 mg/kg bw: significant congestion and steatosis (5-33%) no effect on necrosis, sinusoidal dilatation, and inflammation (sinusoidal dilatation, and inflammation occurs at 48 h after repeated exposure) 75 mg/kg bw: significant congestion, steatosis (5-33%), sinusoidal dilatation and scattered presence of inflammatory cells	Kukner et al. (2016)
Mice Male (20–25 g) 8-12 weeks n = 6 *per* experimental group	*Rhododendron* honey from beekeepers (96% *R. ponticum* pollen), Duzce, western Black Sea region of Turkey	GTX I: 32 mg/kg GTX III: 8 mg/kg	Gavage 25; 50; 75 mg/kg bw 24 h	GTX I: 0.8; 1.6; 2.4 μg/kg bw GTX III: 0.2; 0.4; 0.6 μg/kg bw	*Liver*: All doses decreased C–O/protein and C–O/lipid ratios significantly in liver. No dose response. No effect on the other parameters affected by 75 mg/kg 75 mg/kg: decrease in CH2/lipid and carbonyl/lipid ratios and increase in CH3/lipid ratio, interpreted as sign of lipid peroxidation. Ratio of Amide I/ Amide II decrease indicate disturbance of conformation and structure of the proteins. Changes in lipid/protein ratio No histopathology is reported Summary: an altered energy metabolism is suggested	Cakmak-Arslan et al. (2020a)

Animals	Type of honey as described (geographical and botanical origin when reported)	Concentration of grayananes in honey or other matrices	Exposure and doses of honey or other matrices administered	Estimated single doses of grayananes	Findings	Reference
Mice Male (20–25 g) 8-12 weeks n = 6 *per* experimental group	*Rhododendron* honey from beekeepers (≥ 45% *R. ponticum* pollen), Duzce, western Black Sea region of Turkey	GTX I: 32 mg/kg GTX III: 8 mg/kg	Gavage 25; 50; 75 mg/kg bw 24 h	GTX I: 0.8; 1.6; 2.4 μg/kg bw GTX III: 0.2; 0.4; 0.6 μg/kg bw	*Heart*: 25 mg/kg: increase in the unsaturated/saturated fatty acid ratio with no significant effect on the amount of unsaturated and saturated lipids 50 mg/kg: increase amount of unsaturated and saturated lipids and unsaturated/saturated fatty acid ratio No histopathology is reported Summary: lipid metabolism disturbance and lipids accumulation	Cakmak-Arslan et al. (2020b)
Sprague Dawley rats Female (200–250 g) n = 4 per experimental group	'Mad honey', Duzce, Turkey from a beekeeper	GTX III: 39.9 ± 0.02 mg/kg	Gavage 0.3; 0.6; 1.2; 2.4 g/kg bw day 8 days	GTX III: 12; 24; 48 and 96 μg/kg bw	Dose dependent increase in AST, ALT, LDH, ALP, CK. CK-MB, in the absence of altered Na^+, K^+, Cl^- No histopathology is reported	Sahin (2016)
Sprague Dawley rats Male (250–300 g) Control group: n = 6 for ventricular function assessment; n = 8 for biochemical analysis Each treated group: n = 8 for ventricular function assessment; n = 12 for biochemical analysis	*Rh. Mollis* Flos (RMF) extract	Mixture containing 0.37% rhodojaponin I, 0.53% rhodojaponin II, 0.11% rhodojaponin III	Oral 21 and 113 mg/kg bw	Rhodojaponin I: 0.08 and 0.42 mg/kg bw Rhodojaponin II: 0.11 and 0.6 mg/kg bw Rhodojaponin III: 0.02 and 0.12 mg/kg bw	High dose: vomiting, muscle rigidity, convulsion and death of 6 out of 20 rats Both doses: heart rate, left ventricular systolic and diastolic pressure decrease; plasma increase in LDH, CK-MB and AST No histopathology is reported	Dong et al. (2014)

(a): Data in the figures in the study refer to Wistar rats. EFSA contacted the study authors who clarified that the study was performed with Sprague Dawley rats.
(b): Sibel and Silici are the same author.

Appendix C – BMD analysis

The text below describes the benchmark dose (BMD) analysis for the variation of heart rate following *i.p.* injection of GTX III in rats (Turkmen et al., 2013), using model averaging. BMD analysis was performed according to the EFSA guidance (EFSA Scientific Committee, 2017a and 2022).

Selection of the BMR

The BMR (benchmark response) used is a 10% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD was estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software Used

Results were obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 70.0.

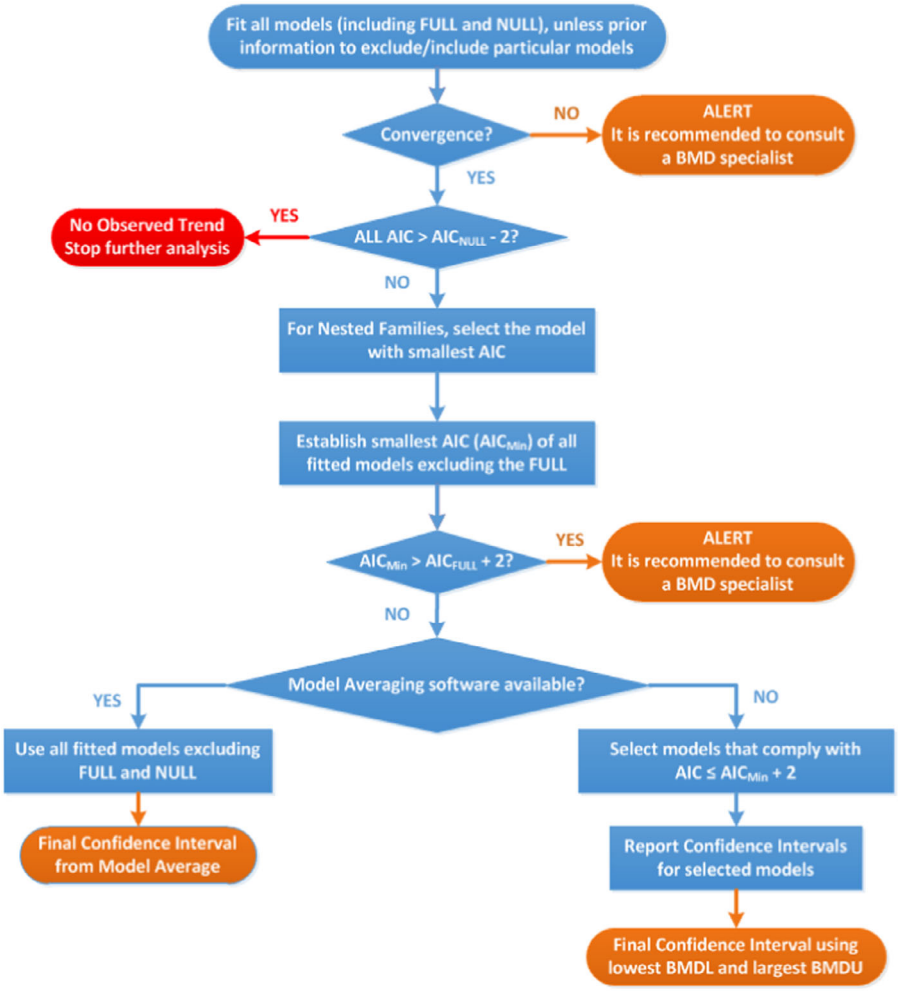
C.1. Specification of Deviations from Default Assumptions

Dose-response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y =$ group mean
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c-(c-1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1-\frac{x^d}{b^d+x^d}\right)$
Hill model 4	4	$y = a \cdot \left(1-\frac{(c-1)\cdot x^d}{b^d+x^d}\right)$
Inverse Exponential	4	$y = a \cdot (1+(c-1)\exp(-bx^{-d}))$
Log-Normal Family	4	$y = a \cdot (1+(c-1)\Phi(\ln b + d\ln x))$

Procedure for selection of BMDL



Flowchart for selection of BMDL

Data description

The endpoint to be analysed is the variation in heart rate (bpm)
Data used for analysis:

GTX III dose (μg/kg bw per day)	Heart rate (bpm)	Std Error	Number of animals
0	363	28	6
179	322	35	6
358	229	4	6
716	191	20	6

C.2. Final BMD values

	BMDL$_{10}$(μg/kg bw)	BMDU$_{10}$ (μg/kg bw)
Results at 60 min of exposure	15.3	261

C.3. Confidence intervals for the BMD are based on 200 bootstrap data sets

C.3.1. Fitted Models

model	converged	loglik	npar	AIC
full model	yes	6.10	5	−2.20
null model	yes	−6.58	2	17.16
Expon. m3-	yes	5.27	4	−2.54
Expon. m5-	yes	6.10	5	−2.20
Hill m3-	yes	5.28	4	−2.56
Hill m5-	yes	6.10	5	−2.20
Inv.Expon. m3-	yes	5.43	4	−2.86
Inv.Expon. m5-	yes	5.68	5	−1.36
LN m3-	yes	5.36	4	−2.72
LN m5-	yes	5.83	5	−1.66

C.3.2. Estimated Model Parameters

EXP

estimate for var-: 0.03773
estimate for a-: 361.3
estimate for CED-: 77.41
estimate for d-: 0.8478

HILL

estimate for var-: 0.03772
estimate for a-: 361.3
estimate for CED-: 77.74
estimate for d-: 0.8527

INVEXP

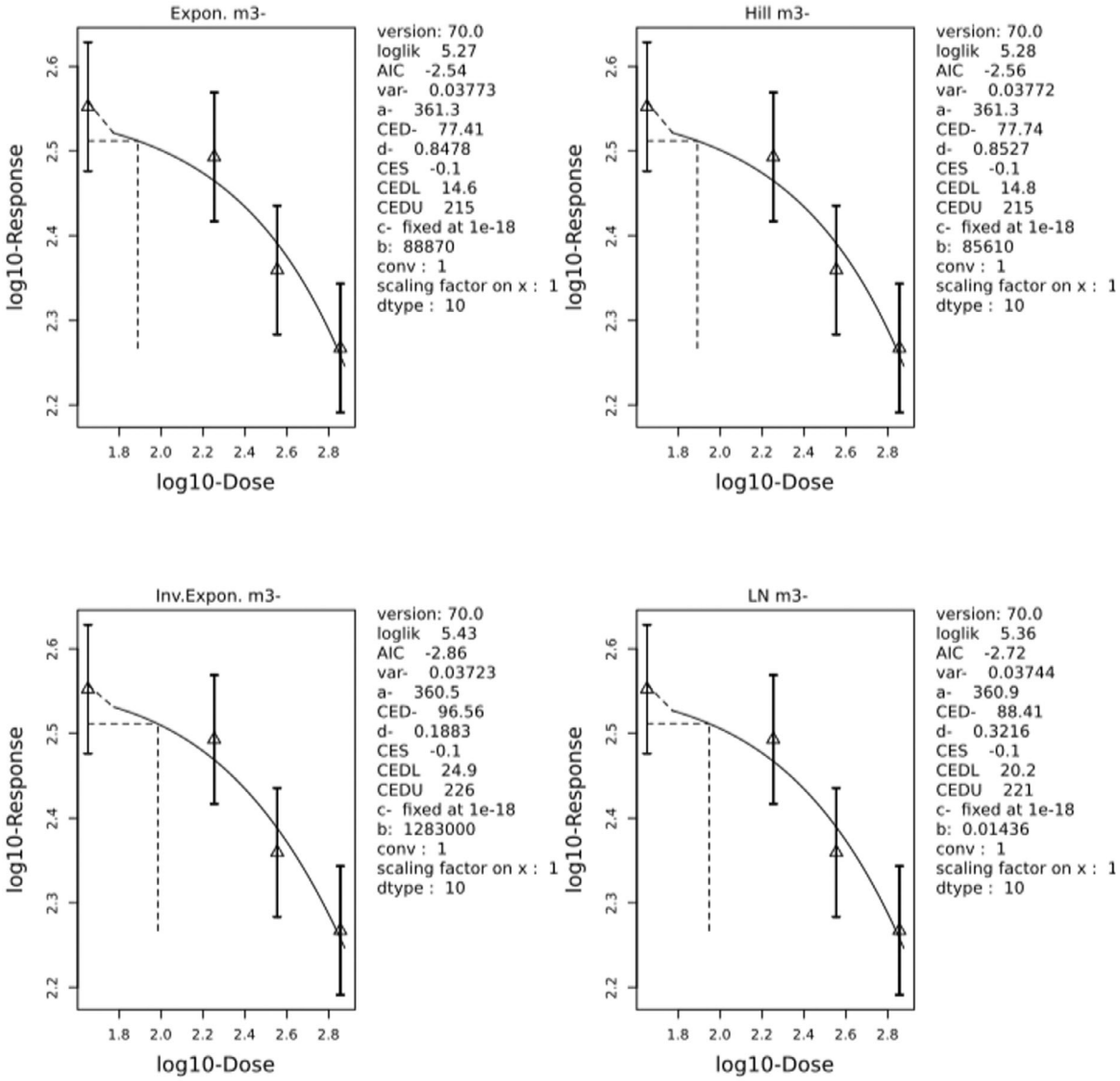
estimate for var-: 0.03723
estimate for a-: 360.5
estimate for CED-: 96.56
estimate for d-: 0.1883

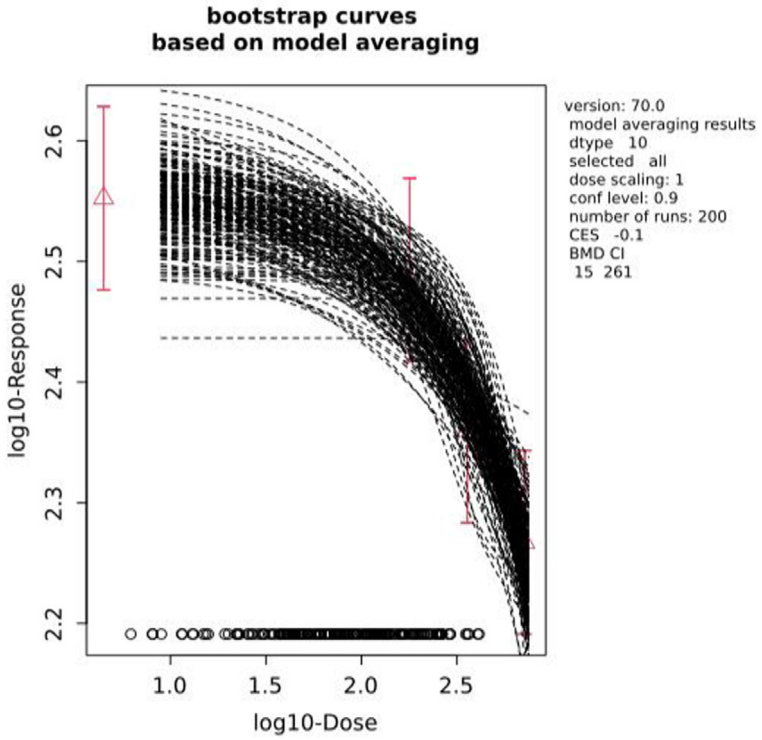
LOGN

estimate for var-: 0.03744
estimate for a-: 360.9
estimate for CED-: 88.41
estimate for d-: 0.3216

C.3.3. Weights for Model Averaging

EXP	HILL	INVEXP	LOGN
0.23	0.24	0.27	0.26


Expon. m3-
version: 70.0
loglik 5.27
AIC -2.54
var- 0.03773
a- 361.3
CED- 77.41
d- 0.8478
CES -0.1
CEDL 14.6
CEDU 215
c- fixed at 1e-18
b: 88870
conv : 1
scaling factor on x : 1
dtype : 10
Hill m3-
version: 70.0
loglik 5.28
AIC -2.56
var- 0.03772
a- 361.3
CED- 77.74
d- 0.8527
CES -0.1
CEDL 14.8
CEDU 215
c- fixed at 1e-18
b: 85610
conv : 1
scaling factor on x : 1
dtype : 10
Inv.Expon. m3-
version: 70.0
loglik 5.43
AIC -2.86
var- 0.03723
a- 360.5
CED- 96.56
d- 0.1883
CES -0.1
CEDL 24.9
CEDU 226
c- fixed at 1e-18
b: 1283000
conv : 1
scaling factor on x : 1
dtype : 10
LN m3-
version: 70.0
loglik 5.36
AIC -2.72
var- 0.03744
a- 360.9
CED- 88.41
d- 0.3216
CES -0.1
CEDL 20.2
CEDU 221
c- fixed at 1e-18
b: 0.01436
conv : 1
scaling factor on x : 1
dtype : 10
log10-Response
log10-Dose
2.2
2.3
2.4
2.5
2.6
1.8
2.0
2.2
2.4
2.6
2.8


bootstrap curves
based on model averaging
version: 70.0
model averaging results
dtype 10
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
CES -0.1
BMD CI
15 261
log10-Response
2.2
2.3
2.4
2.5
2.6
1.0
1.5
2.0
2.5
log10-Dose

Appendix D – Uncertainty analysis

The purpose of the uncertainty analysis was to identify and quantify sources of uncertainty affecting the risk assessment and combine them to assess the overall certainty of the final conclusions, as recommended in EFSA's guidance on uncertainty analysis (EFSA, 2018a).

In a first step, sources of uncertainties related to hazard identification and characterisation of grayananes and consumption of honey containing grayananes in the EU were listed and discussed.

Hazard identification for genotoxic effects followed EFSA's guidance for genotoxicity assessment (EFSA Scientific Committee, 2011, 2017b), which can be considered as a standardised procedure for the purposes of uncertainty analysis (Section 3 in EFSA Scientific Committee, 2018a). As there was a positive result from a valid *in vivo* MN study with GTX III and *Rhododendron* honey, the Panel concluded that these substances should be considered as *in vivo* genotoxins (and potential genotoxic carcinogens) and therefore an HBGV cannot be established. The Panel identified no non-standard uncertainties affecting these conclusions, so no further uncertainty analysis was required for them (Section 3.1 in EFSA Scientific Committee, 2018a).

The assessment of acute toxicity and risk were affected by major uncertainties, which are assessed in detail in the following sections. In summary, the Panel quantified by expert judgement the impact of the identified uncertainties on the RP for acute toxicity for the sum of GTX I and III, the relative potency of other grayananes and the P95 of honey consumption for EU toddlers and adults. These judgements were elicited by semi-formal structured methods of Expert Knowledge Elicitation (semi-formal EKE, Annex B.8 of EFSA (2018b)). The protocol for the EKE including detailed methods and results is presented in Annex D of this opinion.

The quantified uncertainties affecting the RP for acute toxicity and P95 consumption were then combined by probability bounds analysis (section 14.1 of EFSA, 2018a) to quantify the uncertainty of the MOEs calculated by the Panel for the exposure at selected concentrations for the sum of GTX I and GTX III based on acute consumption from the EFSA database. The Panel also *calculated highest concentrations* for honey not resulting in acute effects based on the RP for acute toxicity, an MOE of 100 and data on acute consumption (i) from the EFSA database, and (ii) from consumption of 'certain honey' as folk medicine.

The assessed impact of the uncertainties was quantified using % probability, both when making the expert judgements and in the calculations combining them, as recommended by EFSA's guidance on uncertainty analysis (EFSA Scientific Committee, 2018a). For communication purposes, % probability for the more probable outcome is expressed as % certainty when reporting the conclusions, following advice in EFSA's guidance on communication of uncertainty (EFSA, 2019).

- **Identification of uncertainties**

A complete list of the sources of uncertainty identified by the Panel is presented in Annex D. The most important uncertainties are listed in order of importance in Appendix E, together with 'low' and 'high' scenarios describing the Panel's qualitative evaluation of their potential impact on the assessment. Reference is made in these Tables to the relevant Sections in the Opinion and/or relevant publications which provide the evidence required to support expert judgements quantifying the combined impact of uncertainties affecting different parts of the assessment (see below).

- **Quantification of uncertainties affecting the RP for acute toxicity for the sum of GTX I and GTX III**

Major uncertainties affecting the RP for acute toxicity for the sum of GTX I and GTX III are presented and qualitatively evaluated in Appendix E. The combined impact of these was quantified by eliciting expert judgements of the probability that the RP for acute toxicity would be higher than the assessed value of 15.3 μg/kg bw if the uncertainties were resolved. This provides a measure of the conservativism of the assessed value. The judgements were made by the three toxicologists in the WG, who were provided with training on probability judgements and the semi-formal EKE procedure to be used. When making their judgements, the experts were asked to take into account the evidence and reasoning in the draft Opinion, together with their assessment of the identified uncertainties.

The assessment was conducted twice, first considering only the animal data and then taking into account also the evidence on human intoxications. For the first assessment, the experts were asked to make judgements on the following question: 'The proposed RP for acute toxicity is 15.3 μg/kg bw for the sum of GTX I and III. What is your probability (expressed as %) that the RP for acute toxicity would be higher if the uncertainties affecting it were resolved, considering only animal data?'

The experts were asked to work separately to make a first judgement, considering only the major uncertainties listed in Appendix E, Table E.1A, and to provide a written summary of their reasoning. The judgements and reasoning of all 3 experts were then displayed together for a discussion facilitated by an experienced elicitor. Each expert was then asked to review their judgement and reasoning in the light of the discussion and make any changes that they considered to be appropriate, taking into account also the minor uncertainties affecting this question (listed in Appendix E, Table E.2).

The assessment was then repeated, following the same procedure and addressing the same question with the addition that the evidence on human intoxications was also taken into account (see Appendix E, Table E.1D). As interpretation of the human evidence requires consideration of the occurrence and potency of grayananes other than GTX I and III in honey, a fourth WG member with specialist expertise on these subjects was included in this second assessment. The experts concluded that, due to the large uncertainties affecting interpretation of the human evidence, it did not change the level of certainty based on the animal data alone.

The revised judgements of the four experts were all in the range 70-90% probability. After further discussion, the experts agreed on a consensus of 85% probability, considering the balance between the most important sources of uncertainty: the potential for lower values of the RP for acute toxicity in females than males, higher values for oral compared to *i.p*. exposure, and the lower bioavailability of GTX I and III when administered via honey instead of as pure substances. On this basis, taking account of all the associated sources of uncertainty, the Panel is 85%[28] certain that the RP for acute toxicity of 15.3 μg/kg bw for the sum of GTX I and III is protective (i.e. would not be lower if the uncertainties were resolved).

- **Quantification of uncertainties affecting potency factors for the acute toxicity of grayananes**

Four groups of relative potency factors (RPFs) for the acute toxicity of grayananes are presented in Table 11 (Section 3.1.5). Major uncertainties affecting these RPFs are presented and qualitatively evaluated in Appendix E. The combined impact of these was quantified by eliciting expert judgements of the probability that the RPF for each group is conservative for all grayananes in Table 1 of the Opinion that would fall in that group. Specifically, experts were asked to make judgements on the following question: 'What is your % probability that the actual potency of all grayananes listed in Table 1 that qualify for a specified group in Table 11 would not be higher than implied by the RPF shown for that group in Table 11 of the draft opinion, if the uncertainties affecting it were resolved, not considering human data?' This was assessed separately for each of the 4 groups in Table 11.

Judgements on this question were made by four experts: the three toxicologists in the WG and a fourth WG member with expertise on assessment of relative potency. The judgements were elicited following the same process as that used for the RP for acute toxicity (described in the preceding section): initial judgements and reasoning based on the major uncertainties in Table D.1B, followed by facilitated discussion and revision, taking into account also the minor uncertainties in Appendix E.

The Panel took as its assessment the envelope (overall range) of the final judgements of the four experts. On this basis, the Panel's certainty that the RPFs shown in Table 11 are conservative is 70–80% for Group 1A, 90-95% for Group 1B, and 80-90% for Groups 2 and 3. This takes into account all the associated sources of uncertainty. The difference in certainty between groups reflects the differing amounts of data on which their RPFs are based.

- **Quantification of uncertainties affecting the P95 of honey consumption**

Maximum values for the P95 of acute consumption of honey for toddlers and adults who are consumers of honey are presented in Table 17. The value for each age group is the maximum of P95 values from surveys in the EFSA Comprehensive Database from a subset of EU countries. Taking the maximum of the available values is intended to provide a conservative estimate of the P95 for each age group in the whole of the EU.

Major uncertainties affecting these estimates of P95 consumption are presented and qualitatively evaluated in Appendix E, Table E.1C. The combined impact of these was quantified by eliciting expert judgements on the following question: 'What is your estimate of the 95th percentile of consumption of honey (in the toxicologically relevant period related to grayananes) by healthy people in the specified age group in the EU who are consumers of honey, excluding consumption as folk medicine, such that

[28] i.e. judges with 85% probability (EFSA, 2019).

you judge with 90% probability that the true 95th percentile is lower?' The question refers to the 'toxicologically relevant period' to enable the uncertainty analysis to take into account that, while the maximum P95 values shown in Table 17 relate to consumption over a full day, the period that would ideally be relevant for assessing acute exposure to grayananes may be less.

Judgements on this question were made separately for toddlers and adults, by three exposure experts in the WG. The judgements were elicited following a similar process to that used for the RP for acute toxicity (described above). First, the experts worked separately on their initial judgements and reasoning, considering the major uncertainties in Appendix E. This was followed by facilitated discussion which resulted in consensus on a probability of at least 90% that the P95 consumption of honey consumers in each age group for the whole EU is not higher than the maximum estimate shown in Table 17. The experts subsequently considered the minor uncertainties in Annex D and confirmed that these would not change their judgements.

The Panel took as its assessment the consensus judgements of the three experts. On this basis, for each of the two age groups the Panel is 90% or more certain that the 95th percentile of acute consumption of honey by EU consumers of honey, is not higher than the assessed value, i.e. 3 g/kg bw per day for toddlers and 1.22 g/kg bw per day for adults. This takes account of all identified sources of uncertainty but excludes consumption of honey as folk medicine.

The Panel did not report maximum estimates for the P95 of acute honey consumption for populations other than toddlers and adults for the reasons detailed in Section 3.4. However, based on the data that were available for other age groups (Annex C, worksheet C.2 and C.3), the experts judged with 90-95% probability that the P95 of acute honey consumption for infants, adolescents and other children in the EU who are consumers of honey would be lower than 3 g/kg (the estimated value for toddlers). Similarly, the experts judged with 95-99% probability that the P95 of acute honey consumption for the elderly and very elderly in the EU who are consumers of honey would be lower than 3 g/kg bw, because the available estimates for these age groups are much lower. The Panel did not quantify the uncertainty of the P95 for pregnant or lactating women or vegetarians, because the few surveys available (0-2), did not contain enough consumption events for honey.

- **Quantification of uncertainties affecting the MOEs for acute toxicity**

The limitations described under Section 2.2 did not allow the use of data on the occurrence of grayananes in 'certain honey' for risk characterisation. Regarding the limited data on occurrence and consumption of *Rhododendron* honey, the Panel calculated MOEs for acute exposure of adults and toddlers using selected concentrations for the sum of GTX I and III reflecting concentrations measured in 'certain honey' (Table 16).

These MOEs were calculated as the ratio of the RP of 15.3 μg/kg bw to the estimated exposure, based on selected concentrations and the P95 of acute honey consumption for each age group from dietary surveys (see Section 3.4.1.1). Therefore, the uncertainty of the RP must be combined with the uncertainty of the P95 consumption for the EU population in each age group to assess the uncertainty of each MOE. The selected concentrations are fixed values (not subject to uncertainty), as the calculated MOEs are conditional on them.

The combination of uncertainties relating to the RP and acute consumption was performed by probability bounds analysis (Section 14.1 of EFSA (2018b)). This method for combining uncertain quantities is described in section 14.1 of EFSA (2018a) and in more detail in Annex B.13 of EFSA (2018b). The advantage of this method is that it requires only a probability bound for each quantity: it does not require elicitation of complete probability distributions and allows for any degree of dependence of uncertainty between the quantities. It is conservative in the sense that it overestimates the probability of values outside the resulting bounds for the MOE, compared to a more refined probabilistic assessment, but avoids the need for refined methods when the conservative probabilities are sufficient for decision-making (EFSA Scientific Committee (2018b), page 180).

The MOE is monotonically increasing with respect to the RP and the reciprocal of P95 consumption (1/P95), therefore the probabilities for lower values of each can be combined to obtain a probability for lower values of the MOE using the second of the two simplest methods for probability bounds analysis, described on page 177 of EFSA (2018b). Specifically,

a) The Panel's assessment of 85% probability that the RP is above the assessed value was subtracted from 100%, to obtain the Panel's probability that it is lower: 15%.
b) The Panel's % probability of ≥ 90% for each age group (toddlers and adults) that the P95 consumption is below the assessed value was subtracted from 100%, to obtain the Panel's

probability that it is higher: ≤ 10%. The Panel's probability for higher values of the reciprocal, 1/P95, is therefore also ≤ 10%.

c) Applying the method of probability bounds analysis (EFSA, 2018b), the probabilities from steps (a) and (b) are summed to obtain a maximum probability that the MOE would be lower than the assessed value if the uncertainties affecting the RP for acute toxicity and the estimated P95 consumption were resolved: 15% + ≤ 10% = ≤ 25%.

d) The probability from step c) was subtracted from 100% to obtain a minimum probability, for each age group, that the MOE would be higher than the assessed value: ≥ 75%.

Therefore, taking account of the identified uncertainties, the Panel is 75% or more certain for toddlers and for adults that the MOE shown in Table 16 is conservative, i.e. would not be lower if the uncertainties affecting the RP and P95 consumption estimates were resolved. The Panel noted that the result of the probability bounds analysis is a minimum probability: a higher probability than ≥ 75% would be obtained if full distributions were elicited for the RP and the estimated P95 consumption and combined by Monte Carlo calculations, assuming them to be independent (as is likely in this case). However, this would require additional work, which is unnecessary if the minimum probability is sufficient for decision-making.

The Panel did not calculate MOEs for age groups other than toddlers and adults. However, the Panel judged with 90-95% probability that the P95 of acute honey consumption for infants, other children and adolescents in the EU would be lower than 3 g/kg bw, and 95–99% probability for the elderly and very elderly (see earlier). Substituting the judgement of 90–95% probability for infants, other children and adolescents into the probability bounds calculation described above, in place of the > 90% probability that the P95 for toddlers is below the same value (3 g/kg bw), results in a probability of 75% or more that the MOE for toddlers is also conservative for infants, other children and adolescents. Similarly, substituting the judgement of 95–99% probability for the elderly and very elderly into the probability bounds calculation results in a probability of 80% or more that the MOE for toddlers is also conservative for the elderly and very elderly. The Panel noted that the calculated MOEs are based on acute toxicity of GTX I and III and do not consider the presence of other grayananes in 'certain honey'. In addition, they may not be protective for the genotoxicity endpoint.

- **Quantification of uncertainties affecting the *highest concentrations* in honey below which no acute effects are expected**

The Panel calculated *highest concentrations* of GTX I and III in 'certain honey' below which *no acute effects are expected* based on the P95 of acute honey consumption. For toddlers and adults these were 0.05 mg/kg honey and 0.12 mg/kg honey, respectively. These were calculated using the RP for acute toxicity, an MOE of 100 incorporating intra and inter-species variability (EFSA Scientific Committee, 2012) and the estimated P95 of acute honey consumption for each age group, based on dietary surveys (see Section 3.5.1). Therefore, the uncertainty of the RP for acute toxicity must be combined with the uncertainty of the P95 consumption to assess the uncertainty of the *highest concentration*. This combination was performed by the same probability bounds analysis calculation as was described for the MOEs in the preceding section. (Section 14.1 of EFSA (2018b)). Since the Panel's probabilities for the RP for acute toxicity are the same, the combined probability for the *highest concentrations* is the same.

Consequently, taking account of the identified uncertainties, the Panel is 75% or more certain for both toddlers and adults that the *highest concentrations* shown in Table 17 are protective, i.e. would not lead to a concern for acute effects at the P95 of normal honey consumption by that age group in the EU. The Panel noted again that the result of the probability bounds analysis is a minimum probability.

The Panel did not calculate *highest concentrations* for age groups other than toddlers and adults. However, substituting the experts' judgements about P95 consumption for other age groups into the probability bounds analysis conducted for toddlers and adults, in the same way as was done for MOEs (above), the Panel was 75% or more certain that the *highest concentration* of 0.05 mg/kg for toddlers is also protective for infants, adolescents and other children, and 80% or more certain that it is also protective for the elderly and very elderly.

The Panel also calculated *highest concentrations* for indicative portion sizes of 'certain honey' used as folk medicine (Table 18). These highest concentrations are indicative because they refer to portion sizes recommended on the labels of selected products. There were insufficient data for the Panel to quantify uncertainties regarding variation of recommended portion sizes on product labels or the actual

portion sizes consumed by users of those products. Instead, the Panel quantified the uncertainty of the *highest concentrations* for folk medicine use conditional on the specific portion sizes considered. Therefore, the specified portion size was considered as fixed and the Panel's certainty that the corresponding *highest concentration* is protective is determined solely by the Panel's certainty of 85% that the RP for acute toxicity combined with an MOE of at least 100 is conservative. Consequently, the same level of certainty applies to each of the indicative portion sizes shown in Table 18. For example, for a portion size of 66 g, the Panel is 85% certain that concentrations of up to the calculated *highest concentration* of 0.16 mg/kg would not lead to acute effects, taking into account the uncertainty of that level. For portion sizes larger than 66 g, proportionately lower highest concentrations would be needed.

The Panel noted that the calculated *highest concentrations* are based on acute toxicity of GTX I and III and do not consider the presence of other grayananes in 'certain honey'. In addition, they may not be protective for the genotoxicity endpoint.

Appendix E – Lists of uncertainties

Table E.1: Uncertainties impacting the result from acute exposure to 'certain honey' (ranked per order of priority)

A. Uncertainties relating to the accuracy of the RP for acute toxicity derived for the sum of GTX I and III (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RP for acute toxicity is higher (lower risk)**	**Scenario of maximum probability that the RP for acute toxicity is lower (higher risk)**
ADME: Insufficient information on the absorption of GTX I and III	Bioavailability via oral route might be lower than via the *i.p.* route which would result in lower levels in blood and a higher RP for acute toxicity. Oral bioavailability of GTX III when the exposure to the substance is from honey would be lower than when experimental animals are exposed to the pure substance.	Possibility that the *i.p.* and oral routes are equivalent considering that injection via *i.p.* leads the substance through the liver and that non-lipophilic compounds (such as GTX III) tend to be less absorbed by *i.p.* (see Turner et al., 2011; Al Shoyaib et al., 2020).
Toxicity studies - critical study design: Studies carried out only in one gender See Section 3.1.2, Tables 6 and 7		Experience shows that females can be slightly more sensitive to the acute toxic effect than males (OECD Guidelines 420). This would result in the derivation of a lower RP for acute toxicity.
Toxicity studies - critical endpoints: Extrapolation from *i.p.* to oral administration and uncertainties associated with it, i.e. species difference and mortality Supporting evidence: – Critical studies by Hikino et al. (1976) – *i.p.* injection to mice, and Hikino et al., (1979) – oral administration of GTX III to mice – Table 6	LD_{50} for GTX I and III (oral) is approx. 5 mg/kg bw, or ~5× LD_{50} for GTX I and III (oral) which is approx. 1 mg/kg bw (Hikino et al., 1976, 1979).	Results from *i.p.* and oral exposure to GTX I and III in the study by Hikino et al. (1976 and 1979) suggest that LD_{50}s of GTX I and III are of the same order of magnitude. Therefore, if studies were not based on mortality, but lower doses were used in the study, then the true *i.p.*/oral ED_{50}s for a specific effect might not differ that much (for oral and for *i.p.*, exposures at 0.5 and 0.1 μg/kg bw, respectively), i.e. for exposures corresponding to the derived RP for acute toxicity, the two values are similar.
Dose-response analysis of critical endpoints: Lack of raw data points Supporting evidence: – Critical study by Turkmen et al., 2013 – *i.p.* injection of GTX III in rats	Statistics and confidence intervals (CI) are reported only in graphs and not in Tables in the paper, therefore, data were extracted manually from the graph. If the response values were underestimated, then this would result in a higher RP for acute toxicity.	Statistics and confidence intervals (CI) are reported only in graphs and not in Tables, therefore, data were extracted manually. If response values were overestimated, then they would give a lower RP for acute toxicity.

A. Uncertainties relating to the accuracy of the RP for acute toxicity derived for the sum of GTX I and III (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RP for acute toxicity is higher (lower risk)**	**Scenario of maximum probability that the RP for acute toxicity is lower (higher risk)**
Dose-response analysis of critical endpoints Choice of a BMR of 10% for continuous data instead of 5% and an exposure time of 60 min, considering the transient nature of the effect. The analysis was based the reduction of heart rate after 60 min of *i.p*. exposure to GTX III. Supporting evidence: – Section 3.1.6.1 – Appendix C – Turkmen et al., 2013	If the analysis was performed at 90 min exposure time (instead of 60 min), then the $BMDL_{10}$ would be 65.8 μg/kg bw or around 4× higher than the $BMDL_{10}$ of 15.3 μg/kg bw (the cut-off criteria on the ratios BMDU/BMDL and Lowest dose (LD)/BMD are met for both BMD calculations). This would have resulted in a higher RP for acute toxicity. For 60 and 90 min of *i.p*. exposure to GTX III, the values for heart rate (bpm) are comparable for the two highest doses (400 and 800 μg/kg bw) but for the lowest dose (200 μg/kg bw), the values for bpm±SE* after 90 min are closer to the ones of the control group than after 60 min of exposure. *SE: Standard Error	If the analysis was performed with a BMR of 5% (instead of 10%), then the BMDL would have been 3.89 μg/kg bw or around 4× lower and would result in a lower RP for acute toxicity.

B. Uncertainties relating to the potency of all other grayananes found in 'mad honey', relative to GTX I and III (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RPF per group of grayananes in Table 11 should be higher than the one in the table (lower risk)**	**Scenario of maximum probability that the RPF per group of grayananes in Table 11 should be lower than the one in the table (higher risk)**
ADME: Insufficient information on the absorption of the different grayananes Supporting evidence: Section 3.1.1	If the total grayanane bioavailability is lower than the bioavailability of GTX III, the combined RP for acute toxicity and highest concentration will be higher.	If the total grayanane bioavailability is higher than the bioavailability of GTX III, the combined RP for acute toxicity and highest concentration will be lower.
Mixture group membership and interactions: Lack of information on toxicity of metabolites of grayananes Supporting evidence: Section 3.1.1	The kaurane structure is stable and no cleavage of the grayanane is expected (see Section 3.1.1). Metabolic conversion from grayananes with structural alerts to grayananes with no structural alerts would decrease potency.	The kaurane structure is stable and no cleavage of the grayanane is expected (see Section 3.1.1). Metabolic conversion from grayananes with less or no structural alerts to grayananes with structural alerts would increase potency.

B. Uncertainties relating to the potency of all other grayananes found in 'mad honey', relative to GTX I and III (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RPF per group of grayananes in Table 11 should be higher than the one in the table (lower risk)**	**Scenario of maximum probability that the RPF per group of grayananes in Table 11 should be lower than the one in the table (higher risk)**
Substances used and read across: Calculation/use of potency factors Supporting evidence: – Table 1, Section 1.2.1, – Section 3.1.5, Table 11 – Appendix A and – Section 3.1.2, Table 6	Grayananes identified in honey have been ranked in the following groups: – Subgroup 1A: rhodojaponin III, craiobiotoxin II and rhodomollein XVIII. An LD_{50} is available for rhodojaponin III suggesting that the substance is twice more toxic than GTX I and III. No experimental data are available on craiobiotoxin II and rhodomollein XVIII (ranked to this group based on their structural alerts). If the two substances are less toxic than rhodojaponin III then they should have a lower RPF than the one in Table 11. – Subgroup 1B: GTX I, III, VI, Kalmitoxin I, and rhodojaponin VI. LD_{50} values are available for these grayananes and all of them appear to exhibit lower toxicity than GTX I and III, with the exception of GTX VI. – Subgroup 2: GTX II, IV, VII, VIII, XVII, rhodomollein XIII and XIX, craiobiotoxin I and V. LD_{50} value is available for GTX II only, all other substances were ranked based on their structural alerts. This may overestimate the potency for these substances. – Subgroup 3: GTX XVI, craiobiotoxin VIII, and rhodomollein XI. LD_{50} information is available for GTX XVI but not for the other substances which were ranked based on their structural alerts. This may overestimate the potency for these substances but still, their overall contribution would be negligible.	Grayananes identified in honey have been ranked in the following groups: – Subgroup 1A: rhodojaponin III, craiobiotoxin II and rhodomollein XVIII. An LD_{50} is available for rhodojaponin III suggesting that the substance is twice more toxic than GTX I and III. No experimental data are available on craiobiotoxin II and rhodomollein XVIII (ranked to this group based on their structural alerts). If the two substances are more toxic than rhodojaponin III then they should have a higher RPF than the one in Table 11. – Subgroup 1B: GTX I, III, VI, Kalmitoxin I, and rhodojaponin VI. LD_{50} values are available for these grayananes and all of them appear to exhibit lower toxicity than GTX I and III, with the exception of GTX VI which appears to be as toxic as GTX III. – Subgroup 2: GTX II, IV, VII, VIII, XVII, rhodomollein XIII and XIX, craiobiotoxin I and V. LD_{50} value is available for GTX II only, all other substances were ranked based on their structural alerts. This may underestimate the potency for these substances. – Subgroup 3: GTX XVI, craiobiotoxin VIII, and rhodomollein XI. LD_{50} information is available for GTX XVI but not for the other substances which were ranked based on their structural alerts. This may underestimate the potency for these substances but still, their overall contribution would be negligible.

C. Uncertainties relating to the P95 for acute consumption of normal honey used to calculate *the highest concentration(s)* for the sum of GTX I and III in honey not resulting in a concern for acute effects (ranked per order of priority)

Uncertainty	Scenario of maximum probability that consumption is lower (lower risk)	Scenario of maximum probability that consumption is higher (higher risk)
Representativeness of EU surveys to the EU population	Twenty-two (22) surveys from 22 EU countries are available for adults which would suggest a reliable estimate for the overall adult honey consumption in the EU. The few available national surveys for toddlers (5 surveys) cannot capture the overview of honey consumption in these EU populations and thus it is particularly subjected to uncertainty. If the true highest 95th percentiles for adults and toddlers in the EU are lower than the one estimated in this opinion, then the highest consumptions in Table 17 are overestimated and so it is the risk. Consumption surveys with less than 60 subject days were not included in the assessment since results may not be statistically robust (EFSA, 2011).	Twenty-two (22) surveys from 22 EU countries are available for adults which would derive a reliable estimate for the overall adult honey consumption in the EU. The few available national surveys for toddlers (5 surveys) cannot capture the overview of honey consumption in these EU populations and thus it is particularly subjected to uncertainty. If the true highest 95th percentiles for adults and toddlers in the EU are higher than the one estimated in this opinion, then the highest consumptions in Table 17 are underestimated and so it is the risk. Consumption surveys with less than 60 subject days were not included in the assessment since results may not be statistically robust (EFSA, 2011).
One day consumption vs one eating occasion	EFSA assesses acute consumption based on the total food amount consumed per day rather than each individual eating occasion due to uncertainties in distinguishing between eating occasions/meals. If honey is consumed in smaller portions between wide intervals during the day the risk of intoxication may be lower. Based on data on intoxications, subjects recover after a certain time period which is unknown. When this period is less than 1 day then data from 1-day consumption would overestimate the risk and result in the estimation of lower *highest concentrations*. Consumption per one-eating occasion (which is the one mainly associated with intoxications) is slightly lower than consumption per day.	EFSA assesses acute consumption based on the total food amount consumed per day rather than each individual eating occasion due to uncertainties in distinguishing between eating occasions/meals. If honey is consumed in smaller portions between wide intervals during the day the risk of intoxication may be lower. Based on data on intoxications, subjects recover after a certain time period which is unknown. When this period is more than 1 day then data from 1-day consumption would underestimate the risk and result in the estimation of higher *highest concentrations*.

Sub-population	No of surveys	Max P95 (g/kg bw)	
		/day	/eating occasion
Infant	1	0.83	0.83
Toddler	5	3.00	2.00
Adult	22	1.22	1.18

C. Uncertainties relating to the P95 for acute consumption of normal honey used to calculate *the highest concentration(s)* for the sum of GTX I and III in honey not resulting in a concern for acute effects (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that consumption is lower (lower risk)**	**Scenario of maximum probability that consumption is higher (higher risk)**
Data reporting Uncertainties related to the methodologies of the dietary surveys, over/under-reporting, etc.	In some surveys consumers might have reported honey consumption per teaspoon or tablespoon and not in grams. Conversion to grams is subject to uncertainty due to different conversion factors that could be used (e.g. search on the web regarding standard portions of honey, gave values for 1 teaspoon (7.08 g) and 1 tablespoon (21.25 g) which are different from the values of 8 g and 22 g, respectively, used in this assessment. It is, therefore, possible that the overall honey consumption for the EU population is lower and that the risk is overestimated.	In some surveys consumers might have reported honey consumption per teaspoon or tablespoon and not in grams. Conversion to grams is subject to uncertainty due to different conversion factors that could be used (e.g. search on the web regarding standard portions of honey, gave values for 1 teaspoon (7.08 g) and 1 tablespoon (21.25 g) which are different from the default values of 8 g and 22 g, respectively, used in this assessment. It is, therefore, possible that the overall honey consumption for the EU population is higher and that the risk is underestimated.

D. Uncertainties relating to human observations (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RP for acute toxicity when combined with an MOE of at least 100 will be higher (lower risk)**	**Scenario of maximum probability that the RP for acute toxicity when combined with an MOE of at least 100 will be lower (higher risk)**
Chemical composition: Composition of grayananes in the ingested material is incomplete (no/limited information on the identity/ levels of grayananes other than GTX I and III in honey). Supporting evidence: Section 3.2.1, Table 14	Total grayanane conc. in honey > conc. of the sum of GTX I and III. This suggests that the 'LOAEL' of 4.8 μg/ kg bw for GTX I and III based on data from Aygun et al. (2015) reflects the toxicity of all grayananes in honey and not only the toxicity of GTX I and III. Therefore, the 'LOAEL' for the sum of all GTX present should be the same (if no other grayananes in addition to GTX I and III are present) or higher (if other grayananes are present in addition to GTX I and III). This would result in the same or higher RP for acute toxicity for the sum of GTX I and III. *Based on semi-quantitative determinations, total grayananes in honey may occur at levels up to 3 or 7 times higher than the levels for the sum of GTX I and III* (see Section 3.2.1, Table 14)	

D. Uncertainties relating to human observations (ranked per order of priority)		
Uncertainty	**Scenario of maximum probability that the RP for acute toxicity when combined with an MOE of at least 100 will be higher (lower risk)**	**Scenario of maximum probability that the RP for acute toxicity when combined with an MOE of at least 100 will be lower (higher risk)**
Substances used and read across: Calculation of a group effect of no concern for acute toxicity/use of potency factors – Table 1, Section 1.2.1, – Section 3.1.5, Table 11 and Appendix A – Section 3.2.1, Table 15	With the exception of rhodojaponin III, all grayananes known to be present in 'certain honey' (Table 1) appear to have similar or lower potency than GTX I and III (see Section 3.1.5 and Appendix A). *Rhododendron* honeys from the Turkish Black Sea region are rich in GTX I and III and contain low or no levels of rhodojaponin III.	Some honeys have high levels of rhodojaponin III (see Section 3.2.1, Table 15). *Rhododendron* honeys from Nepal may contain high levels of rhodojaponin III.
Severity of the effect: Uncertainty relating to low dose-effects after consumption of 'certain honey'		The available data refer to intoxication reports, i.e. after severe life-threatening effects following consumption of mad honey; relevant reports on acute adverse effects in humans from consumption of 'mad honey' which do not end to the hospital are not found. If adverse (but not life-threatening) effects are manifested in lower doses, then a lower RP for acute toxicity would apply for grayananes. Adverse low dose effects may be unspecific and not seen in causal relationship with honey consumption by affected persons.

Table E.2: List of minor uncertainties

Main group	Sub-group	Overarching questions	Sources of uncertainty in the opinion on grayananes in 'certain honey'
Chemical composition and analytical methods	**Dosing and chemical composition**	Uncertainty in the chemical composition of the material administered to the experimental animals.	a) Purity of the single substance tested not always defined in the studies. b) The impurities and unidentified compounds of the tested substance, and/or botanical material and/or the honey tested
			Physicochemical properties of GTX III versus the hemi (ethyl acetate), e.g. the free GTX III (may be less soluble and bioavailable) (read across).
	Analytical methods		Certified reference standards not commercially available for most grayananes.

Main group	Sub-group	Overarching questions	Sources of uncertainty in the opinion on grayananes in 'certain honey'
Hazard identification and characterisation	**ADME**	Is there uncertainty in any aspect of ADME?	Uncertainty related to the elimination of grayananes.
	Mixture group membership and interactions	Is there uncertainty on the extent and profile of effects due to co-exposure (e.g. metabolites, interaction of chemicals, combined effects)?	Interaction/combined effects between grayananes and with other substances.
	Selection of reference point from Animal studies	Are there uncertainties in the selection of the RP that are not covered by the BMD confidence interval e.g. is model uncertainty covered?	Uncertainty in the confidence intervals of the selected BMR.
Consumption data	**Data reporting**	Is there uncertainty in the consumption data due to e.g. classification, bw, memory errors etc.?	Memory errors and capacity to report details in dietary surveys, possible under and over-reporting.
		Is there uncertainty in consumption data, e.g. due to methodology of the dietary survey, weekdays, national recipes?	• Dietary survey methodology (dietary record vs 24-hour recall), dietary software, interview options (place, face to face vs telephone and background of the interviewers) and use of portion-size measurement aids for the estimation of portion sizes. • Sampling frame, method and design of the dietary surveys. • Representativeness over different weekdays and seasons within dietary survey. • Sample size and response rate of the dietary surveys.
	Representativeness of the data	Is there uncertainty in consumption data, e.g. representativeness of the countries, special population groups, sample size and response rates.	Sampling frame, method and design of the dietary surveys.
			Representativeness over different weekdays and seasons within dietary surveys.
			Availability of adequate number of consumption surveys and adequate number of subject days per age group.
			Availability of food consumption data for the general population, including consumers only of specific foods of special interest.
			Sample size and response rate of the dietary surveys.
Exposure estimates	**Statistical models**	Is there uncertainty in consumption data, e.g. due to methodology of the dietary survey, weekdays, national recipes?	Consumers loyalty to specific brands or products not considered, local consumption.
Risk characterisation	**Uncertainty factors**	Is there uncertainty on the selected (default or specific) UFs?	Inter-species differences not correctly covered by standard UFs (for animal data only).
			Use of uncertainty factors to cover data gaps.

Annex A – Protocol

The file containing the protocol on grayananes in 'certain honey' is available under the Supporting Information section on the online version of the scientific output.

Annex B – Plant list of Ericaceae species

The excel file containing the plant list of Ericaceae species that have been investigated for grayanane content or that have been listed for ethnopharmacological use is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.7673435

Annex C – Consumption data statistics

The excel file containing the information on the dietary surveys included in the assessment, the detailed acute consumption statistics per country, survey and age group calculated in mg/kg bw per day and in mg/kg bw per eating event is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.7673456

Annex D – Uncertainty analysis: EKE Protocol

The file containing the EKE protocol on grayananes in 'certain honey' is available under the Supporting Information section on the online version of the scientific output.