The Director General

Maisons-Alfort, 1 June 2023

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the development of a toxicity reference value (TRV) for palytoxin (CAS No 77734-91-9)

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 1 June 2023 shall prevail.

On 3 December 2021, ANSES received a formal request from the Directorate General for Health (DGS) and the Directorate General for Food (DGAL) to conduct the following expert appraisal: request for an opinion on the risks to human health associated with the proliferation of *Ostreopsis* spp. on the Basque coast.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In recent decades, dinoflagellates (unicellular eukaryotes) of the genus *Ostreopsis* have been identified in the coastal areas of several countries (Accoroni and Totti 2016; Aligizaki *et al.,* 2008; Amzil *et al.,* 2012; Ciminiello *et al.,* 2006; Del Favero *et al.,* 2012; Fraga *et al.,* 2017; Funari, Manganelli and Testai 2015; Santos *et al.,* 2019). When they are found in sea spray or seawater, these benthic microalgae can cause cases of human poisoning (Patocka *et al.* 2018; Walsh 2017). Exposure can occur via the respiratory route (contact with sea spray when walking, monitoring beaches, swimming or engaging in boating activities, etc.), through dermal contact with seawater or macroalgae (when swimming and/or engaging in boating activities) or through ingestion (of seawater when swimming or boating, or of seafood contaminated by toxins produced by *Ostreopsis* spp.). Clinical manifestations such as coughing, rhinorrhoea,

irritation of the ENT region (ear, nose, and throat) and eyes, headaches, fever, breathing difficulties, nausea, vomiting, diarrhoea, abdominal pain, myalgia, redness or itching have all been observed in people exposed to *Ostreopsis* (Berdalet *et al.,* 2022). Some case reports also mention cardiovascular problems (Hoffmann *et al*., 2008; Deeds *et al*., 2010; Wu *et al*., 2014).

Ostreopsis blooms are also linked to the mass mortality of certain invertebrates (Sansoni *et al*., 2003; Totti *et al*., 2010; Ciminiello *et al*., 2006, 2012, 2014). The toxins produced by *Ostreopsis* have been detected in a wide range of seafood products (Aligizaki *et al*., 2008; Bire *et al.*, 2013, 2015; Brissard *et al*., 2014; EFSA 2009) from the Mediterranean Sea and may therefore pose a potential risk of food poisoning to humans.

In France, *Ostreopsis* has been observed on the Mediterranean coast repeatedly since 2005 (observations by Ifremer[1] as part of REPHY, the observation and monitoring network for phytoplankton and hydrology in coastal waters). During the summer of 2021, a major *Ostreopsis* bloom episode was also reported on the Basque coast from late July to late September. Nearly 674 people[2] suffered poisoning symptoms after visiting beaches along these coastlines (Paradis and Labadie, 2022). A few similar cases of poisoning had already been reported in the summer of 2020. It is therefore possible that this emerging phenomenon could become recurrent and increase along the Atlantic coast.

Initial investigations by Ifremer revealed the presence of two species of microalgae of the genus *Ostreopsis*: *O*. cf. *siamensis* and *O*. cf. *ovata* (Amzil *et al.,* 2021). While *O*. cf. *siamensis* has been observed in the area since 2018, the detection of *O*. cf. *ovata* in this part of the Bay of Biscay is a new development, considering that blooms of this species are known to cause disorders similar to those observed in the Mediterranean since the 2000s.

O. cf. *ovata* is known to produce toxins from the palytoxin group (PLTX), mainly ovatoxins (Amzil *et al.,* 2012; Brissard *et al.,* 2015; Ciminiello *et al.,* 2008; Ciminiello *et al.,* 2012; Ciminiello *et al.,* 2010; García-Altares *et al.,* 2015; Gemin 2020; Tartaglione *et al.,* 2017). These compounds could be responsible for the symptoms observed in humans (Illoul *et al.,* 2012; Tichadou *et al.,* 2010), especially as some of them have been detected in aerosols (Ciminiello *et al.,* 2014; Medina-Pérez *et al.,* 2021).

On 3 December 2021, the DGS and DGAL issued a formal request to ANSES for an opinion on the risks to human health associated with the proliferation of *Ostreopsis* spp. along the entire French coastline. The Agency redefined the scope and title of the formal request in order to focus on the risks associated with the proliferation of *Ostreopsis* on the Basque coast. The purpose of this formal request was firstly to update knowledge on microalgae of the genus *Ostreopsis*, for which the Agency had already issued two opinions in 2007 and 2008 (Afssa, 2007; 2008), and secondly to establish specific recommendations for the Atlantic coast and, if appropriate, to update the specific recommendations for the Mediterranean coast established in 2007 and 2008.

As part of the expert appraisal contract drawn up on 20 December 2021, the questions in the formal request were reformulated by ANSES according to two themes: the first concerned the review of knowledge and the second the drafting of recommendations, including the proposal of short- and medium-term toxicity reference values (TRVs) for the oral, respiratory and dermal routes for the reference toxin. This opinion deals solely with the toxicity of PLTX and the

[1] The French Research Institute for Exploitation of the Sea
[2] Number of cases recorded by the Bordeaux poison control centre in late September 2021

selection or establishment of short- and medium-term TRVs for the oral, respiratory and dermal routes.

2. ORGANISATION OF THE EXPERT APPRAISAL

ANSES entrusted examination of this request to the "*Ostreopsis*" Working Group (WG), reporting to the Expert Committee (CES) on "Water".

The Expert Committee on "Health Reference Values" (CES VSR) was asked to propose oral, respiratory and dermal TRVs for PLTX. Rapporteurs were appointed to analyse data on the substance's toxicity. The methodological and scientific aspects of the work were presented to the CES VSR and validated at its meeting of 9 March 2023. The report takes into account the comments and additional information provided by CES members.

This work was therefore conducted by a group of experts with complementary skills.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

3. ANALYSIS AND CONCLUSIONS OF THE CES VSR

3.1. General information on palytoxin

PLTX was first isolated in 1971 from the cnidarian hexacorallia *Palythoa toxica* (tropical coral) (Moore and Scheuer, 1971). PLTX and its analogues are thought to be synthesised by bacteria (Moore, Helfrich and Patterson, 1982; Seemann *et al.,* 2009) or microalgal symbionts of the coral *Palythoa* (Maeda *et al*., 1985). PLTX and 42-hydroxy-PLTX have also been detected in *Trichodesmium* sp., a tropical marine cyanobacteria (Kerbrat *et al.*, 2011).

The physico-chemical properties are provided in the report.

3.2. Summary of the toxicological data

The toxicity of PLTX has been studied in numerous animal species (mainly rodents, but also lagomorphs, dogs and monkeys), via several routes of administration. The data available in the literature are disparate due to the different methodologies used, the origin of the PLTX and the differences in genetic strain, sex and age of the animals used. The onset kinetics, type and intensity of the symptoms differ according to the route of administration considered.

Given that the vast majority of human exposure to PLTX is through the inhalation of toxic aerosols and the ingestion of contaminated seafood, these two routes of exposure should be used to develop TRVs. Experimental animal data on the toxicity of PLTX administered by inhalation and orally were published recently. Exposure by the dermal and ocular routes should not be underestimated, however, especially among amateur and/or professional aquarists, who may be more intensely exposed by both the inhalation and dermal routes.

3.2.1. Toxicokinetics

Few data have been published on the kinetic parameters associated with exposure to PLTX. Given the systemic damage observed in toxicity studies by inhalation and gavage, it appears

that the toxin can cross the pulmonary and intestinal barriers and be distributed to various organs. However, it is not possible to determine whether this crossing of the pulmonary and intestinal barriers occurs with or without alteration to these epithelia. No data are available on the metabolism and elimination of PLTX, regardless of the route of administration.

3.2.2. Acute toxicity

- Human data

There are few available clinical observations of PLTX poisoning through the consumption of fish or shellfish; these mainly relate to the consumption of tropical species in Asia.

For the respiratory and dermal routes, the available data mainly come from clinical observations following exposure due to cleaning aquariums containing soft corals of the genera *Zoanthus* and *Palythoa*, but also during beach activities (CAPTV, 2022; Deeds *et al*., 2018; Hoffman *et al*., 2008; CCTV, 2018). Symptoms are mainly eye, skin and respiratory irritation, and/or flu-like symptoms (headache, myalgia, hyperthermia, tremors, and even nausea and vomiting).

- Animal data

Studies are available on the intravenous (IV) (Ito *et al*., 1982; Deeds and Schwartz, 2010; Kockskämper *et al*., 2004), intraperitoneal (IP) (Ito *et al*., 1996; Rhodes *et al*., 2002; Riobó *et al*., 2008; Wiles *et al*.,1974; Poli *et al*., 2018), oral (Munday *et al*., 2008; Sosa *et al*., 2009; Boente-Juncal *et al*., 2020a), respiratory (Wiles *et al*., 1974; Poli *et al*., 2018; Ito & Yasumoto, 2009) and dermal routes (Wiles *et al*., 1974; Fujiki *et al.*, 1986).

The IV and IP studies are described in the report.

- **Oral route**

Three recent studies showed that the 24-hour LD_{50}[3] of PLTX in mice by gavage was estimated to be respectively 510 µg/kg bw ($CI_{95\%}$ = 311-809 µg/kg bw), 599 µg/kg bw ($CI_{95\%}$ = 508-707 µg/kg bw) and 767 µg/kg bw ($CI_{95\%}$ = 549-1039 µg/kg bw) (Munday *et al.*, 2008; Boente-Juncal *et al.*, 2020a; Sosa *et al*., 2009). These values are far higher than the LD_{50} values published for the IV and IP routes. This could be explained by the fact that PLTX is a large hydrophilic compound whose absorption would be limited in the gastrointestinal tract and which would be partly degraded by stomach acid. The toxicity (LD_{50}) of PLTX for mice seems to be even lower with exposure by voluntary ingestion (mixed with feed) compared with exposure by the intraperitoneal route or by gavage, since no effect was observed at a dose of 2500 µg/kg bw (Munday, 2006). There is no relationship between the administered dose and the survival time of exposed animals (Sosa *et al*., 2009).

Following a 16-hour fast, Sosa *et al*. exposed female CD-1 mice (n = 5/dose, 18-20 g, 4 weeks old) by gavage (10 mL/kg) to single PLTX[4] doses of 0, 300, 424, 600, 848, 1200 and 1697 µg/kg bw (dissolved in phosphate buffer (PBS)) with observation over 24 hours (Sosa *et al.*, 2009). The most frequent symptoms observed in animals that did not survive the observation period were spasms (location not specified) (86%), paralysis mainly involving the hind limbs (80%), respiratory distress (70%), scratching (57%) and jumping (50%). Scratching was

[3] The LD_{50} (median lethal dose) is the statistically deduced single dose expected to cause the death of 50% of the animals to which the substance has been administered.
[4] Isolated from *P. tuberculosa*, Wako, purity >90%

observed recurrently and at every dose (except in unexposed mice). These symptoms are comparable to those observed after IP exposure. The neurotoxic nature of PLTX was demonstrated by its indirect effects (although the mechanisms are not clearly understood) on the contractile function of skeletal muscles, as well as on respiratory muscles. Histological alterations in various organs (stomach, liver, pancreas, kidney) were also observed, both in mice that died during the 24-hour observation period and in mice that survived. In particular, histological analysis of the dead mice almost systematically showed effects on the liver and pancreas, such as a sharp reduction in, or even absence of, liver glycogen. In the pancreas, a reduction in enzyme secretion from the glandular acini was also observed. According to the symptoms, the toxin targeted the skeletal and heart muscles. The authors proposed a NOAEL[5] of 300 µg/kg bw for PLTX after acute oral administration in mice (LD_{50} = 767 µg/kg bw; $CI_{95\%}$ = 549-1039 µg/kg bw).

Ito and Yasumoto exposed male ICR mice (3-4 weeks old, n = 4), without fasting, to commercially sourced PLTX[6] administered by gavage in 200 µg/kg bw saline, and monitored mortality over 24 hours (Ito & Yasumoto, 2009). A control group was compared with the treated groups (n = 6). PLTX was also tested at 200 and 500 µg/kg bw (n = 4) with monitoring over 2 hours. The dose of 200 µg/kg bw was not lethal. *Post-mortem* analysis of the mice at this dose revealed slight damage to the stomach, small intestine, lungs and kidneys. The authors also noted a slight accumulation of gastric acid (or secretions) in the stomach. These effects, described by the authors as inflammatory, appeared fairly quickly (within 2 hours). On the basis of these observations, the authors proposed a LOAEL[7] of 200 µg/kg bw for PLTX, in contrast to the previous study.

Lastly, in the same study, PLTX was administered to mice sublingually at a dose of around 200 µg/kg bw (176-235 µg/kg bw). This exposure was designed to mimic food poisoning, in which the toxin comes into contact with the mucous membranes of the mouth and oesophagus before reaching the rest of the digestive tract. The toxin was applied in a very small volume (2 µL) to prevent it from being swallowed. The mice were "inactive" and hyperventilated. *Post-mortem* examination of the organs revealed slight bleeding in the lungs, which appeared 30 minutes after exposure, followed by interstitial inflammation after 1.5-8 hours, and then destruction of the pulmonary alveoli within 8-24 hours. Gastrointestinal tissue appeared yellowish, fragile and swollen at 30 minutes. The villi of the small intestine were damaged at the apical part and secreted mucus. In the kidneys, the authors observed glomerular atrophy after 8-24 hours. PLTX administered via the oral cavity reached the systemic circulation and the aforementioned organs, where it induced structural and functional disruption that was far more severe than with *per os* administration. This suggests that PLTX is partially degraded as it passes through the digestive tract.

In the study by Boente-Juncal *et al.*, female Swiss mice (n = 3 to 9, 18-21 g, 4 weeks old at the start of the experiment) were exposed by gavage, without fasting, to PLTX[8] doses of 0, 15, 36, 100, 350, 500, 750, 1200 µg/kg bw (in saline solution) (Boente-Juncal *et al.*, 2020a). The animals were monitored every 2 hours until the end of the observation period (96 hours). The lethality study determined an LD_{50} of 599.3 µg/kg bw ($CI_{95\%}$ = 508-707 µg/kg bw), close to the values of the two previous studies. At the lethal dose (1200 µg/kg bw), the mice died within two hours of gavage administration of the toxin and showed signs of distress (ataxia, lethargy,

[5] No observed adverse effect level
[6] Wako, with no mention of purity
[7] Lowest observed adverse effect level
[8] Extracted from *P. tuberculosa*, Wako, 88.9% pure

kyphosis, cyanosis, dyspnoea, piloerection) and abdominal pain. These signs diminished as the doses were reduced. At the dose of 750 µg/kg bw, a mouse that survived for 9 hours had intense haemorrhaging in the abdominal cavity. Based on these observations, the authors proposed a NOAEL of 15 µg/kg bw, far from the value proposed by Sosa *et al*. in 2009 (300 µg/kg bw). In the aforementioned study, mice exposed to 15 µg/kg bw of PLTX showed no signs of pain or any other symptoms and remained healthy for the entire 96-hour observation period. In contrast to the study by Sosa *et al*. (2009), the mice did not scratch or jump. One of the differences between the two studies was the free access to food and water during treatment (Boente-Juncal *et al*., 2020a), whereas in the earlier study, the animals were deprived of food for 16 hours before and 2 hours after treatment (Sosa *et al*., 2009). Biochemical blood tests were also conducted on mice exposed to the toxin and compared with the control mice. PLTX did not alter blood glucose or cholesterol levels. However, plasma concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) increased significantly after administration of PLTX at doses of 36 µg/kg bw and above. Low doses of PLTX did not cause any significant changes in blood sodium and chloride levels; only a significant increase was observed at 300 µg/kg bw (for Na^+ and Cl^-) while at 1200 µg/kg bw only a decrease in blood sodium levels was observed. Lastly, an increase in blood potassium levels was observed at high doses (750 and 1200 µg/kg bw). Ultrastructural analysis of the liver and kidneys was performed on control mice and mice treated with 500 and 750 µg/kg bw PLTX. Liver damage was visible after 96 hours. The effects of PLTX were also seen in the kidneys. At 750 µg/kg bw (but not at 500 µg/kg bw), PLTX caused alterations in nephron structure: podocyte nuclei were irregular and individual podocytes fused. Boente-Juncal *et al*. pointed out that these kidney effects may be related to the effects observed in humans following PLTX poisoning (Wu *et al*., 2014).

More recently, Sosa *et al*. exposed female CD-1 mice (n = 8) by gavage, without fasting, to single PLTX[9] doses of 0, 30, 90 and 270 µg/kg bw and monitored them for 24 hours (Sosa *et al*., 2022). Observed mortality was 0/16 for control animals, 0/8 at 30 µg/kg bw, 2/8 at 90 µg/kg bw and 2/8 at 270 µg/kg bw. After 24 hours of monitoring, no signs of toxicity were observed at the dose of 30 µg/kg bw. At the dose of 90 µg/kg bw, clinical observations included scratching (1/8), piloerection (2/8), muscle spasms (2/8) and abdominal swelling (1/8). At the dose of 270 µg/kg bw, the authors observed scratching (1/8), piloerection (2/8), sedation (2/8), tremors (2/8), agitation (2/8), paralysis (3/8), dyspnoea (2/8), muscle spasms (2/8) and abdominal swelling (1/8).

- **Respiratory route (inhaled, intratracheal, intranasal instillation)**

In male Wistar rats (n = 21) exposed to PLTX (aqueous solution with 50% ethanol) by the intratracheal route, the LD_{50} values at 24 hours, 5 and 10 days were estimated to be respectively 0.36 µg/kg bw ($CI_{95\%}$ = 0.23-0.55 µg/kg bw), 0.31 µg/kg bw ($CI_{95\%}$ = 0.17-0.54 µg/kg bw) and 0.18 µg/kg bw ($CI_{95\%}$ = 0.09-0.38 µg/kg bw). The signs of toxicity reported were similar to those observed with an IV injection, but with greater respiratory difficulties and wheezing (Wiles *et al.*, 1974).

In the study by Poli *et al.*, female Fischer rats (n = 15) were exposed to PLTX by inhalation (Poli *et al*., 2018). The LD_{50} of PLTX was 0.041 µg/kg bw. Histological lesions were observed in many tissues 12 hours after exposure, including:

- lung tissue in all rats (moderate to severe lesions),

[9] Wako Chemicals GmbH, purity >90%

- nasal tissue (degeneration of the epithelium, mild to moderate necrosis with neutrophilic inflammation),
- tracheal tissue (necrosis, degeneration and/or loss of epithelium),
- liver tissue (marked congestion of the centrilobular zone without any significant change in the hepatocytes),
- lymphoid tissue (thymus, spleen, mesenteric and/or mandibular lymph nodes) (necrosis and/or mild to moderate apoptosis of lymphocytes and/or depletion),
- cardiac tissue (mild degeneration of cardiomyocytes in the right ventricle and/or right papillary muscle, acute necrosis with increased cytoplasmic eosinophilia and nuclear pyknosis with or without mineral precipitation),
- renal tissue (degeneration/necrosis of the renal tubular epithelium in some rats),
- salivary gland tissue (degeneration/necrosis of the epithelium of the mandibular salivary gland duct in some rats).

Twenty-four and 36 hours after exposure, all the rats had lesions similar to those observed at 12 hours in all these tissues and organs, with a degree of severity that increased with time.

Intratracheal instillation of PLTX in mice caused alveolar haemorrhage, pulmonary oedema, gastrointestinal erosion and glomerular atrophy (Ito & Yasumoto, 2009). Death occurred following paralytic symptoms at a dose of 2 µg/kg bw in mice and 5 to 7.5 µg/kg bw in rats (observation time not specified). At a sublethal dose (1 µg/kg bw), the rodents were paralysed and could not move for 1 to 2 hours, before recovering. After 24 hours, the animals' behaviour was normal, but histological analysis revealed multiple tissue lesions (lungs, gastrointestinal tract and kidneys).

After euthanasia, lung and liver lesions were observed in rats (females) exposed by intranasal instillation to an aerosol dose of 0.05 µg/kg bw PLTX (Poli *et al.*, 2018). Pulmonary (lung congestion, consolidation of lobes and/or areas of haemorrhage) and hepatic effects (hepatocyte lesions, areas of haemorrhage, congestion) were observed. Some rats had epistaxis (nasal haemorrhage) and/or red scabs around the eyelids and nostrils (Poli *et al.*, 2018).

- **Dermal route**

Following intradermal injection of 1 and 10 µg/mL PLTX (i.e. 0.11 and 0.55 µg of toxin) in several species (rats, guinea pigs and rabbits), skin whitening occurred at the injection site within 15 to 30 minutes, indicating acute vasoconstriction of the blood capillaries. After 1 to 2 hours, oedema and erythema were observed around the initial whitened area. Wiles *et al.*, (1974), found no signs of toxicity or lethality due to systemic effects of PLTX. Macroscopic and microscopic *post-mortem* examinations of the animals revealed focal necrosis at the injection site with local inflammation. The authors also observed kidney necrosis and alterations to the pulmonary vessels. These data suggest that some PLTX was distributed systemically and affected internal organs (lungs, kidneys), which corroborates studies of oral exposure to PLTX.

For the percutaneous route, application of 0.05 or 0.1 mL of PLTX (5 µg/mL) to the skin of a rabbit induced the appearance of a whitened and slightly swollen area at the application site after 1 to 2 hours (Wiles *et al.*, 1974). After 4 to 5 hours, the authors observed oedema and necrosis of the bleached area and the peripheral inflamed area. Although the study was carried out on a single animal, this result suggests that PLTX acts by inducing necrotic and inflammatory effects of the skin. A more recent study tested different doses of PLTX extracted from *P. tuberculosa* with no mention of purity. The quantities of PLTX used were 0.3, 3.3, 8.25, 33 and 165 µg, applied topically to the ears of mice (Fujiki *et al.*, 1986). The irritant effect of PLTX, measured as the amount of toxin inducing erythema in the ears of 50% of the mice

tested 24 hours after application, was 0.02 µg/ear. The mice died within 4 hours of application of 33 or 165 µg of PLTX to the ear.

3.2.3. Skin and eye irritation

In humans, dermatological symptoms, mainly dermatitis, have been associated with exposure of the skin to PLTX during *Ostreopsis* blooms (Pelin *et al*., 2011). *In vivo* experimental data would be needed to characterise the effects of PLTX on the skin.

In animals, ocular effects were detected in rabbits after conjunctival instillation of PLTX at 0.1, 0.2 and 0.4 µg/kg bw without rinsing (Wiles *et al.,* 1974), and included moderate eye watering, irritation, oedema and conjunctivitis after 4 hours of exposure. At 24 hours, the affected eyes were completely closed, with an exudate of pus and blood, and severe conjunctivitis with corneal oedema, ulceration and opacity. The highest concentrations of PLTX caused irreversible damage. With the lower doses, recovery was rapid and complete.

3.2.4. Sensitisation

No human or animal studies were identified in the literature.

3.2.5. Repeated and chronic toxicity

- **Human data**

Repeated human exposure to PLTX can potentially occur through the consumption of contaminated seafood, mainly by populations living in coastal areas. Repeated exposure over a limited period is therefore likely. A few experimental studies have investigated the oral toxicity of PLTX after repeated administration. However, no repeated or chronic toxicity studies of PLTX by the oral, inhalation (inhalation or intratracheal instillation) or dermal routes are available in humans.

- **Animal data**

In animals, only studies by the oral (Del Favero *et al*., 2013; Boente-Juncal *et al*., 2020b), dermal (Fujiki *et al.,* 1986) and IP routes (Ito *et al.,* 1997 – described in the report) are available.

  - **Oral route**

In the study by Del Favero *et al*., PLTX extracted from *P. tuberculosa* (Wako, over 90% pure dissolved in PBS) was administered by gavage at doses of 0, 30, 90 and 180 µg/kg bw/d, for 7 consecutive days, to groups of six female CD1 mice followed by a 2-week recovery phase (without intervention) (Del Favero *et al*., 2013). During treatment, mortality rates of 33, 33 and 83% respectively were reported at the doses of 30, 90 and 180 µg/kg/d. During the recovery phase, one mouse in the lowest dose group and two in the intermediate dose group died. In addition, the weight of the mice fell by up to 40% in all the treated groups. This weight loss was associated with a reduction in food consumption from the second day. Just before death, the mice showed the following symptoms: loss of motor coordination, hyporeactivity, general sedation and loss of reflexes. In some animals, paralysis of the lower limbs was observed. Death was preceded by breathing difficulties and panting. Independently of the lethal effects, the abdomen was dilated from the third day of treatment, which affected more mice at the lowest dose (4/6) than at the intermediate (3/6) and high doses (1/6). Unlike the liver, lungs and brain, an increase in the relative weight of the kidneys, spleen and heart was observed

only at the highest dose (180 µg/kg bw/d). In the lungs, histological alterations (mild to severe alveolar oedema sometimes associated with acute inflammation and necrosis) were observed in two of the six mice that died prematurely, irrespective of the dose. Alterations to heart muscle (separation of muscle fibres) at the doses of 90 and 180 µg/kg bw/d, and to a lesser extent at 30 µg/kg bw/d, were also reported.

In the second experiment, carried out to determine a NOAEL, groups of eight mice were exposed to 0, 3 or 30 µg/kg bw/d for 7 consecutive days (Del Favero *et al.*, 2013). As before, four mice per group were euthanised 24 hours after the last gavage. The remaining animals were monitored for a further 2 weeks to assess the reversibility of symptoms. No mortality was observed during this second experiment. No abnormalities or clinical signs were observed in the group treated with 3 µg/kg bw/d, whereas mice treated with 30 µg/kg bw/d showed transient mild signs of hypoactivity and sporadic scratching episodes. In addition, two mice had swollen abdomens. No change in weight was observed at 3 µg/kg bw/d, whereas a 25% decrease associated with a reduction in food consumption was reported at 30 µg/kg bw/d. During the recovery period, weight and food intake returned to normal. No effect was observed on the relative weights of the various organs (liver, kidneys, lungs, spleen, brain and heart).

This study shows that daily oral administration of PLTX for 7 days can be lethal from 30 µg/kg bw/d, a dose 17 times lower than the LD_{50} after single administration. The fact that mortality can be observed several days after exposure indicates that the toxic effects induced by repeated exposure are not entirely reversible and can prove fatal. The authors proposed a NOAEL of 3 µg/kg bw/d and a LOAEL of 30 µg/kg bw/d. At this LOAEL, the lungs, heart, liver and gastrointestinal tract are major targets for PLTX.

In the study by Boente-Juncal *et al.*, the repeated toxicity of PLTX extracted from *P. tuberculosa*[10] was assessed in female Swiss mice (n = 3-10/group) exposed by gavage for a period of 28 days to doses of 0.03, 0.1, 0.3, 1, 3.5 and 10 µg/kg bw/d (Boente-Juncal *et al.*, 2020b). An LD_{50} of 0.44 µg/kg bw was calculated. At 10 µg/kg bw/d, no mice survived after 18 days of treatment, whereas at 3.5 and 0.3 µg/kg bw/d, mortality rates of respectively 75% and 43% were observed. The first deaths only occurred later on (after 17 days of treatment) for the 0.3 µg/kg bw/d dose. At 0.03 µg/kg bw/d, no mortality was observed. Survival times differed considerably from one animal to another and for each dose of toxin.

At the dose of 0.03 µg/kg bw/d, no symptoms suggestive of PLTX poisoning were observed. At the dose of 0.1 µg/kg bw/d, typical symptoms (lethargy, piloerection, abdominal pain, facial oedema, kyphosis) were observed. Mice exposed to 1 and 3.5 µg/kg bw/d suffered weight loss. The onset of symptoms was dose-dependent. Certain symptoms only appeared at higher doses, such as ataxia, dyspnoea and circular movements (from 1 µg/kg bw/d) or vocalisations (from 3.5 µg/kg bw/d).

No macroscopic changes were observed at the dose of 0.03 µg/kg bw/d. On the other hand, abdominal swelling and the presence of gas and mucus in the stomach and intestines were frequently observed in some of the animals treated with higher doses. On the last day of treatment, the cumulative quantity of urine was lower for mice treated with doses of 1 and 3.5 µg/kg bw/d. No effect on the cumulative mass of faeces was observed.

Repeated exposure produced significant effects on blood and urine biochemical parameters after 28 days or just after the animal's death. The four enzymes (ALT, AST, CK, LDH) had increased levels at 0.1 and 0.3 µg/kg bw/d. An increase in AST and ALT was also observed at 1 and 3.5 µg/kg bw/d. Significant variations in blood sodium levels (decrease at the dose of

[10] Wako, 88.9% pure

0.3 µg/kg bw/d) and blood potassium levels (increase at the doses of 0.03, 0.1 and 0.3 µg/kg bw/d, which the CES experts considered to be within the normal physiological ranges for this species) and consequently in the Na+/K+ ratio, were observed. Blood chloride levels were also elevated for the doses of 0.03 to 1 µg/kg bw/d.

Lastly, macroscopic alterations in the digestive tract and ultrastructural alterations in the stomach were observed from the dose of 0.1 µg/kg/d. During the 28-day exposure period, mice receiving a daily dose of 0.03 µg/kg bw/d of PLTX showed no significant changes. The authors proposed a NOAEL of 0.03 µg/kg bw/d.

- **Dermal route**

No *in vivo* studies on mammals or humans are available on the possible toxic effects of PLTX by the dermal route.

3.2.6. Effects on reproduction and development

No *in vivo* studies on mammals or humans are available on the effects of PLTX on reproduction or development.

3.2.7. Genotoxicity

PLTX was negative in the Ames mutagenesis test on *Salmonella* Typhimurium strains with or without metabolic activation. Moreover, it did not act as an initiator in the *in vitro* BALB/c 3T3 cell transformation assay, or on mouse skin *in vivo* (Fujiki et al., 1986; Munday, 2011).

3.2.8. Carcinogenicity

No human studies are available on the carcinogenicity of PLTX.

In animals, following repeated administration to the skin of mice (0.5 µg, twice a week for 30 weeks), PLTX did not induce any tumours but produced systemic toxic effects: only 8 out of 15 mice survived to week 30. On the other hand, if the same application procedure was followed after an initiation stage with 100 µg of dimethylbenz(a)anthracene (DMBA), 62.5% of the mice developed tumours (with an average of 1 tumour per mouse): seven papillomas and one early invasive epithelioid carcinoma. The total dose of PLTX required for a 50% incidence of tumours was estimated to be 15 µg (Fujiki *et al.,* 1986). This article highlights the tumour-promoting effect of PLTX on the skin.

3.2.9. Mechanism of action

PLTX inhibits the normal function of the Na/K-ATPase pump by temporarily transforming it into an ion channel, allowing transmembrane transport of ions according to their concentration gradient (Takeuchi *et al*., 2009). Monovalent cations (Na^+, K^+) can therefore pass through the ion channel generated by PLTX (Habermann, 1989; Scheiner-Bobis *et al*., 2002).

The Na/K-ATPase pump is a protein heterocomplex consisting of two α and β subunits, and a regulatory γ subunit. Each α and β subunit has different isoforms whose expression profile differs according to tissue, and even according to sex and age. The interindividual variability in response to inhalation poisoning with PLTX could be explained by the existence of genetic variants of Na/K-ATPase (Pelin *et al*., 2020).

Pelin *et al*. showed in HaCaT cells (keratinocytes) that PLTX's cytotoxicity is not counteracted in the presence of oxidative stress inhibitors[11], indicating that oxidative stress is not the main mechanism of this toxicity (Pelin *et al*., 2013). PLTX toxicity is due to the alteration of normal ion homeostasis in excitable and non-excitable cells (Wu, 2009; Rossini & Bigiani, 2011).

The interaction of PLTX with Na/K-ATPase initially induces a change in membrane ion permeability, which then leads to depolarisation and Ca^{2+} influx (Amano *et al*., 1997; Schilling *et al*., 2006), followed by cell lysis in different cell types (Satoh *et al*., 2003; Schilling *et al.,* 2006; Munday, 2011). Many of the cellular effects of PLTX are linked to this increase in intracellular Ca^{2+}.

Although no effect of PLTX on non-gastric human H/K-ATPase has been reported (Guennoun *et al.,* 2007), other authors have shown that PLTX can target H/K-ATPase in the colon, apparently converting it into a channel (Scheiner-Bobis *et al.,* 2002).

3.2.10. Sensitive populations

The available information on human exposure to PLTX (CAPTV Bordeaux, 2022) shows that there are no significant variations in symptoms depending on the age group or sex of the exposed persons. The observed symptoms are mainly coughing, oropharyngeal pain and acute rhinitis, regardless of age. However, there is a strong presumption of statistical association between the level of symptom severity and at least one pre-existing medical condition (allergic rhinitis, asthma, allergies, smoking) compared with people with no medical history.

Taken together, this evidence suggests that people with respiratory disorders (asthma, allergic rhinitis), allergies or a history of smoking may be more sensitive to exposure to inhaled PLTX.

3.3. Overview of TRVs

Only EFSA proposed in 2009 an acute reference dose (ARfD) by ingestion of 0.2 µg/kg bw for PLTX and another related toxin (ostreocin-D) based on the 2009 study by Ito and Yasumoto, which identified a LOAEL of 200 µg/kg bw in mice for PLTX, supported by the study of Sosa *et al*. (2009), which showed a NOAEL of 300 µg/kg bw in mice for PLTX.

Table 1: EFSA's ARfD (EFSA, 2009)

Organisation (year)	Critical effect Key study	PoD (Point of departure)	Uncertainty factors	TRV
EFSA (2009)	Accumulation of gastric fluids and multivisceral damage (stomach, intestines, lungs and kidneys) Ito and Yasumoto (2009)	LOAEL = 200 µg/kg bw	1000 UF_L = 10 UF_A = 10 UF_H = 10	ARfD = 0.2 µg/kg bw

No medium- or long-term TRVs for the oral route and no short-, medium- or long-term TRVs for the inhalation and dermal routes have been identified for PLTX.

[11] Non-selective inhibitor of NADPH oxidase (NOX), diphenyleneiodonium chloride and methyl-L-arginine acetate (inhibitor of nitric oxide synthase (NOS))

3.4. Proposed short-term oral TRV

3.4.1. Choice of the critical effect

Symptoms observed following oral exposure to PLTX in mice include lethargy, ataxia, abdominal pain, dyspnoea and piloerection. These symptoms are consistent with clinical data from poisonings due to *Ostreopsis* blooms. **The CES selected as the critical effect all these symptoms appearing after acute exposure to PLTX.**

3.4.2. Analysis of the existing short-term TRVs

In 2009, EFSA proposed an ARfD of 0.2 µg/kg bw for PLTX and another toxin in the same group (**Table 1**). **The CES decided not to select EFSA's TRV** given that the 2009 study by Ito and Yasumoto had numerous methodological biases (small number of animals, single dose tested, ToxRtool score of 3), and that other studies showed effects at lower doses. **The CES therefore proposed establishing a short-term TRV.**

3.4.3. Choice of the key study

The critical analysis and ToxRtool[12] scores of the five available short-term studies are shown in Table 2.

Table 2: Critical analysis of short-term oral studies

References	ToxRtool score	Critical analysis
Wiles *et al.,* 1974	3	Numerous methodological limitations, including the absence of a control group of animals and a PLTX solution diluted with 50% ethanol.
Sosa *et al.,* 2009	1	Study deemed to be of good quality. It identified a NOAEL of 300 µg/kg/d and a LOAEL of 424 µg/kg/d. Limitations: - symptoms were only monitored for 24 hours, whereas the effects of PLTX can be delayed over time - the doses tested were relatively high (the lowest dose tested was 300 µg/kg)
Ito and Yasumoto, 2009	3	Methodological limitations: small number of animals, unspecified purity of PLTX.
Boente-Juncal *et al.*, 2020a	1	Study deemed to be of good quality. It identified a NOAEL of 15 µg/kg bw on the basis of clinical observations made over a long monitoring period (96 hours) that are well documented in mice (dyspnoea and abdominal pain) at a dose of 36 µg/kg bw.
Sosa *et al.,* 2022	1	Study deemed to be of good quality. It identified a NOEL of 30 µg/kg bw on the basis of the effects observed at a dose of 90 µg/kg bw (scratching, sedation, muscle spasms). Limitation: symptoms were only monitored for 24 hours, whereas the effects of PLTX may be delayed over time.

12 ToxRTool is a tool for assessing the reliability of toxicological data, making the decision-making process for assigning reliability categories more transparent and harmonised. Scores: 1 = reliable without restrictions, 2 = reliable with restrictions, 3 = not reliable, 4 = not assignable.

Among these studies, three are of equivalent methodological quality, with a ToxRtool score of 1: the 2009 and 2022 studies by Sosa et *al*., and the 2020a study by Boente-Juncal *et al.* The Sosa *et al*. team administered a single dose of PLTX to animals orally with 24-hour post-exposure monitoring (Sosa *et al*., 2009 and 2022), while the Boente-Juncal *et al*. team administered a single dose of PLTX to animals orally but with longer monitoring of 96 hours. Human clinical data have shown that effects can be delayed following exposure to PLTX (CAPTV Bordeaux, 2022). In the 2009 study by Sosa *et al*., the ratio between the LD_{50} and the NOEL was 2.56, whereas this ratio was 40 in the study by Boente-Juncal *et al*., highlighting the more precise estimate of the NOAEL in the latter study. On the basis of this evidence, **the CES selected the study by Boente-Juncal *et al*. (2020a) as the key study**.

The methodological approaches of the studies available in the literature focus more on determining the LD_{50} than the NOAEL or LOAEL. The experts expressed regret that the effects investigated in these studies were limited to lethargy, abdominal pain, dyspnoea and ataxia, and that the studies did not cover effects that may appear at lower doses, or potentially more serious effects such as cardiotoxicity.

3.4.1. Choice of the point of departure

According to the ANSES method for developing TRVs (ANSES, 2017), establishment of a benchmark dose (BMD) is to be preferred. If the available data mean that this is not possible, a NOAEL is selected as the second choice PoD and a LOAEL as the last option. The BMD approach was followed, but was not adopted given the lack of robustness of the data (small number of animals used in the study) and the large confidence interval around the BMD.

The CES therefore adopted the NOAEL described in the 2020a study by Boente-Juncal *et al*. of 15 µg/kg bw in mice as the PoD.

NOAEL = 15 µg/kg bw

3.5. Allometric adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability. A human equivalent dose (HED) was calculated, using the following equation:

$$\text{Human equivalent dose} = \text{Animal dose} \times (\text{Animal weight} / \text{Human weight})^{1/4}$$

The average weight of the mice was 22 g, based on the range of mouse weights reported in the study (18-22 g). The average human weight used for the calculation was 70 kg.

The adjusted PoD is: **$NOAEL_{HED}$ = 2 µg/kg bw**

3.5.1. Choice of uncertainty factors

The TRV was calculated from the 2020a study by Boente-Juncal *et al.* using the following uncertainty factors (ANSES, 2017):

- Inter-species variability (UF_A): $UF_{A\text{-}TD}$ = 2.5. The allometric adjustment performed enabled a human equivalent dose to be calculated. To account for toxicodynamic

variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to WHO-IPCS recommendations (WHO-IPCS, 2005) and based on ANSES's practices (ANSES, 2017).

- Inter-individual variability (UF_H): 10. Because there were no scientific data available to reduce the default value, the value of 10 was used.
- Use of a point of departure ($UF_{B/L}$): 1
- Inadequacy of the data (UF_D): 1

An overall uncertainty factor of 25 was therefore used for establishing the TRV.

3.5.2. Proposed short-term oral TRV and confidence level

TRV = 0.08 µg/kg bw/d

As this is a TRV for single exposure by the oral route, the value should be considered as an acute TRV.

The **overall confidence level moderate/low** was assigned to this TRV, based on the following criteria: type and quality of the body of data (moderate confidence level), choice of the critical effect and mode of action (moderate confidence level), choice of the key study (low confidence level) and choice of the PoD (low confidence level).

The methodological approaches of studies available in the literature focus more on determining the LD_{50} than the NOAEL or LOAEL. The experts expressed regret that the effects investigated in these studies were limited to lethargy, abdominal pain, dyspnoea and ataxia, and that the studies did not cover effects at lower doses, or potentially more serious effects such as cardiotoxicity.

3.6. Proposed short-term TRV by inhalation

Two acute respiratory toxicity studies are available: Wiles *et al.* 1974 by the intratracheal route and Poli *et al*. 2018 by the inhalation route. As the 1974 study by Wiles *et al*. was of limited methodological quality (inadequate route of exposure: intratracheal and a PLTX solution diluted with 50% ethanol), the CES did not select it as the key study. Although the 2018 study by Poli *et al*. is the only one available for this route, the CES considers that it cannot be selected as the key study due to numerous methodological limitations (absence of controls, unclear exposure design that causes confusion in interpretation, study of the effect of time on a single dose) and the inconsistency of the relationship between the toxicity of the inhalation route and the intraperitoneal route, which does not correspond to the other studies.

Given the lack of human data and the inadequate quality of the animal data, it was not possible to propose a TRV for short-term exposure for the inhalation route. In addition, given the lack of toxicokinetic data and the wide disparity in LD_{50} results observed in animals for the different exposure routes, the CES did not deem it relevant to extrapolate from one route to another.

The CES drew attention to the lack of data for this type of exposure, which is a major route of exposure for humans.

3.7. Proposed short-term TRV by the dermal route

Due to the physico-chemical characteristics of PLTX (high molecular weight and water solubility), the dermal route via local exposure is not expected to be a systemic route for PLTX. Considering this evidence and the limitations (topical and intradermal administration, single dose) of the studies described for this route (Wiles *et al*., 1974; Fujiki *et al*., 1986), the CES did not select these studies as key studies. **No TRV for short-term exposure could therefore be proposed for the dermal route. In addition, given the lack of toxicokinetic data and the wide disparity in LD_{50} results observed in animals for the different exposure routes, the CES did not deem it relevant to extrapolate from one route to another.**

3.8. Proposed medium-term oral TRV

3.8.1. Choice of the critical effect

The symptoms observed following repeated oral exposure to PLTX in mice for 28 days were lethargy, ataxia, abdominal pain, dyspnoea and piloerection. These effects are consistent with all the observations made in animals, regardless of the duration of exposure.

The CES selected as the critical effect all the symptoms observed following repeated exposure to PLTX.

3.8.2. Analysis of the existing medium-term TRVs

In the absence of a medium-term TRV, **the CES proposed establishing such a TRV**.

3.8.3. Choice of the key study

The only repeated toxicity study with a duration of exposure compatible with the derivation of a medium-term value is the 2020b study by Boente-Juncal *et al*. This was deemed to be of good quality (ToxRtool score 1), although it was carried out on a small number of animals (the number varied according to the dose). The data show that during the 28-day exposure period, mice receiving a daily dose of 0.03 µg/kg bw/d of PLTX showed no major adverse effects. The only statistically significant effect was hyperkalaemia (2 out of 5 mice), which the experts considered to be within the usual physiological values for this species. This study therefore identified a NOAEL of 0.03 µg/kg and a LOAEL of 0.1 µg/kg on the basis of all the effects observed at this dose. These results enabled the authors to propose a NOAEL of 0.03 µg/kg bw. **The CES therefore selected the study by Boente-Juncal *et al*. as the key study (Boente-Juncal *et al*., 2020b)**. Nevertheless, the experts expressed regret that the effects investigated in this study were limited to lethargy, abdominal pain, dyspnoea and ataxia, and that the study did not cover effects at lower doses, or potentially more serious effects such as cardiotoxicity.

3.8.4. Choice of the point of departure

The recommendations in the method suggest giving preference to establishing a benchmark dose (BMD) whenever available data allow, selecting a NOAEL as the second choice and a LOAEL as the last option (ANSES, 2017).

The data from this study by Boente-Juncal *et al*. and the dose-response relationship of the effects observed did not make it possible to establish a BMD.

The CES therefore selected the NOAEL as the point of departure, i.e. a **NOAEL of 0.03 µg/kg bw/d**.

3.8.5. Allometric adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability. A human equivalent dose (HED) was calculated, using the following equation:

$$\text{Human equivalent dose} = \text{Animal dose} \times (\text{Animal weight} / \text{Human weight})^{1/4}$$

The average weight of the mice in the study was 27 g. The weight used in the calculation for humans was 70 kg.

$$\mathbf{NOAEL_{HED} = 0.004\ \mu g/kg\ bw/d}$$

3.8.6. Choice of uncertainty factors

The TRV was calculated from the 2020b study by Boente-Juncal *et al.* using the following uncertainty factors (ANSES, 2017):

- Inter-species variability (UF_A): 2.5

The dose adjustment performed enabled a human equivalent dose to be calculated. To account for toxicodynamic variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to WHO-IPCS recommendations (WHO-IPCS, 2005) and based on ANSES's practices (ANSES, 2017).

- Inter-individual variability (UF_H): 10

Because there were no scientific data available to reduce the default value, the value of 10 was used.

- Use of a point of departure ($UF_{B/L}$): 1
- Inadequacy of the data (UF_D): 1

An overall uncertainty factor of 25 was therefore used for establishing the TRV.

3.8.7. Proposed iTV for repeated oral exposure and confidence level

As the data available for repeated exposure were limited (only one study available), the CES decided to establish an indicative toxicity value (iTV) rather than a TRV for which an additional UF_D would have to be applied. The iTV is an indicative value that is less robust than the TRV and therefore has a low confidence level.

iTV = 0.0002 µg/kg bw/d, i.e. **0.2 ng/kg bw/d**

3.9. Proposed medium-term TRV by inhalation

Given the lack of human and animal data, it was not possible to propose a TRV for medium-term exposure for the inhalation route. In addition, given the lack of toxicokinetic data and the

wide disparity in LD_{50} results observed in animals for the different exposure routes, the CES did not deem it relevant to extrapolate from one route to another.

3.10. Proposed medium-term TRV by the dermal route

Given the lack of human and animal data, it was not possible to propose a TRV for medium-term exposure for the dermal route. In addition, given the lack of toxicokinetic data and the wide disparity in LD_{50} results observed in animals for the different exposure routes, the CES did not deem it relevant to extrapolate from one route to another.

3.11. CES conclusion and recommendations

An acute oral TRV (single exposure) was developed for PLTX on the basis of a NOAEL derived from the 2020a study by Boente-Juncal *et al*. with a moderate-low confidence level.

A medium-term oral iTV was developed after repeated oral exposure on the basis of a NOAEL derived from the 2020b study by Boente-Juncal *et al.* with, by definition, a low level of confidence. In view of the very limited database (only one study available), an iTV is established by ANSES when all the conditions required for establishing a TRV are not met (insufficient data, doubts about the harmful nature of the effect and/or time and/or resource constraints). This pragmatic approach aims to provide a temporary response to the health risk assessment objective pending sufficient qualitative and/or quantitative data, and can therefore help meet the expectations of risk managers in the presence of documented exposure situations. This iTV can only be used to respond to the specific situation and context that justified its establishment.

The duration of application of a medium-term TRV (or iTV) is usually defined as 15 to 364 days. However, in this specific case, the iTV will be applied for a period of several weeks, in line with the duration of *Ostreopsis* bloom episodes generally observed in France.

The CES was unable to develop short- and medium-term TRVs for the respiratory and dermal routes for palytoxin due to a lack of data for these routes.

Table 3: TRV for palytoxin

Type of TRV (duration of application)	Body establishing the value (date)	Critical effect (key study)	Establishment		TRV
			PoD	**UF**	
Acute TRV by the oral route (single exposure)	ANSES (2023)	All symptoms observed (lethargy, ataxia, abdominal pain, dyspnoea, piloerection) Boente-Juncal *et al*. (2020a): study in mice exposed to a single dose, 96-hour observation period	NOAEL = 15 µg/kg <u>Allometric adjustment</u> $NOAEL_{HED}$ = $15*(0.022/70)^{0.25}$ = 2 µg/kg/d	25 UF_{A-TK} = 1 UF_{A-TD} = 2.5 UF_H = 10 $UF_{B/L}$ = 1 UF_D = 1	**0.08 µg/kg/d** **Confidence level: moderate-low**
Medium-term oral iTV (a few weeks)	ANSES (2023)	All symptoms observed (lethargy, ataxia, abdominal pain, dyspnoea, piloerection) Boente-Juncal *et al*. (2020b): study in mice exposed to doses of PLTX for 28 days	NOAEL = 0.03 µg/kg bw LOAEL = 0.1 µg/kg bw <u>Allometric adjustment</u> $NOAEL_{HED}$ = $0.03*(0.027/70)^{0.25}$ = 0.004 µg/kg/d	25 UF_{A-TK} = 1 UF_{A-TD} = 2.5 UF_H = 10 UF_S = 1 $UF_{B/L}$ = 1 UF_D = 1	0.0002 µg/kg bw/d or **0.2 ng/kg/d** **Confidence level: low**
Short- and medium-term TRV by the respiratory route	ANSES (2023)	**No TRV due to a lack of data**			
Short- and medium-term TRV by the dermal route	ANSES (2023)	**No TRV due to a lack of data**			

In light of the information set out in this opinion, the CES recommends that further inhalation toxicity studies be carried out with PLTX and that measurements be taken of PLTX concentrations in aerosols.

Given the heterogeneity of the values, which prevents any extrapolation from one route to another, and the lack of toxicokinetic data, the CES also recommends that toxicokinetic and repeated administration toxicity studies be carried out.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the proposed oral TRVs established for palytoxin.

The Agency reiterates that a toxicity reference value (TRV) is a toxicological indicator for qualifying or quantifying a risk to human health. TRVs enable the potential health effects of exposure to substances to be assessed. They can be used as part of quantitative health risk assessments (QHRAs) carried out at population level, in a given exposure context, and thus help in the choice of risk management measures.

Pr Benoit Vallet

KEY WORDS

Toxicity reference value, TRV, marine toxins, *Ostreopsis*, palytoxin, acute, repeated toxicity

SUGGESTED CITATION

ANSES. (2023). Opinion on the development of a TRV for palytoxin. (Request No 2021-SA-212). Maisons-Alfort: ANSES, 19 p.