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# Manual for using REPHYTOX Data. Information to improve the understanding of REPHYTOX data files available to scientists and the public

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# Fact sheet

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Manual for using REPHYTOX Data. Information to improve the understanding of REPHY data files available to scientists and the public

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**Abstract**
REPHYTOX (Monitoring Network for Phycotoxins in marine organisms) is a network implemented by Ifremer. The data acquired by REPHYTOX have been banked since 1987 in the **Quadrige** database. This manual is intended for users of REPHYTOX data, which are made available on the internet from **SEANOE**. The **Quadrige** database is a component of the French Water Information System and its mission is to manage and exploit data from numerous coastal monitoring networks. **SEANOE** (Sea scientific open data publication) is a publisher of scientific data in the field of marine sciences. The REPHYTOX dataset available in SEANOE makes available all REPHYTOX data for the French metropolis, for years prior to the current year, in the form of fixed files, with an annual update. The dataset is associated with a DOI: http://doi.org/10.17882/47251.
This manual is intended to improve the understanding of REPHYTOX data for best use. It explains for example the fields present in the files, gives indications on the way the data were entered, and provides the elements to be taken into account for the treatment of these data.

**Key words**
REPHYTOX, data, Quadrige, SEANOE, DOI, phycotoxins, DSP, PSP, ASP, palytoxins, shellfish, coastal waters

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# Summary

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# Preamble

REPHY data are published without any warranty, express or implied. The user assumes all risk arising from his/her use of data. REPHYTOX data are intended to be research-quality and include estimates of data quality and accuracy, but it is possible that these estimates or the data themselves contain errors. It is the sole responsibility of the user to assess if the data are appropriate for his/her use, and to interpret the data, data quality, and data accuracy accordingly. Authors welcome users to ask questions and report problems:

- REPHY-REPHYTOX national coordination: coord.rephy@ifremer.fr
- or Quadrige administration unit: q2suppor@ifremer.fr

# Introduction

REPHYTOX (Monitoring Network for Phycotoxins in marine organisms) is a network implemented by Ifremer: http://envlit.ifremer.fr/surveillance/phytoplancton_phycotoxines.

The data acquired by REPHYTOX have been banked since 1987 in the **Quadrige** database. This manual is intended for users of REPHYTOX data, which are made available on the internet from **SEANOE**.

The **Quadrige** database is a component of the French Water Information System and its mission is to manage and exploit data from numerous coastal monitoring networks. **SEANOE** (Sea scientific open data publication) is a publisher of scientific data in the field of marine sciences. The REPHYTOX dataset available in SEANOE makes available all REPHYTOX data for the French metropolis, for years prior to the current year, in the form of a fixed file, with an annual update. The dataset is associated with a DOI: http://doi.org/10.17882/47251. The file is in csv format

This manual is intended to improve the understanding of REPHYTOX data for best use. It explains for example the fields present in the files, gives indications on the way the data were entered, and provides the elements to be taken into account for the treatment of these data.

REPHYTOX data only exists in metropolitan France.

# Data structure

The column headers include in their label the structure specific to the Quadrige database with the hierarchy:
**monitoring location / survey / sampling operation / sample / result**

A monitoring location is defined by geographical coordinates. A survey may be described as a monitoring location / date / time, on which one or more sampling operations are performed. A sampling operation may be described as an action to collect shellfish sample (s). A sample is defined by its matrix.

The fields present in the SEANOE files are the same for all parameters used for toxin data.

The fields present in SEANOE are described in **Table 1**. Since most of the fields are mandatory for data entry, they are always present in the files. Those that are optional are indicated in **Table 1**.

**Table 1. Detailed explanations of the fields in the SEANOE files for REPHYTOX data**

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Lieu de surveillance : Entité de classement : Libellé<br>Monitoring location: Classification entity: Name | Code and name of the "marine area" to which the monitoring location belongs. The marine areas are the result of Quadrige's own zoning of the French coastal waters. They are numbered from north to south in the Channel and Atlantic, and from West to East in the Mediterranean. |
| Lieu de surveillance : Identifiant<br>Monitoring location: Identifier | Non-significant identifier assigned by the Quadrige system to uniquely identify a monitoring location |
| Lieu de surveillance : Mnémonique<br>Monitoring location: Mnemonic | The mnemonic is an informative and unique identifier. The mnemonic is constructed as follows:<br>. code of the marine area<br>. P, L or S depending on whether it is a point, linear or surface monitoring location<br>. order number of the monitoring location in the marine area<br>For example: 002-P-024, 079-S-075<br>N.B. there are point and surface monitoring locations in the REPHYTOX 1987-2016 dataset. The polygons of the surface locations can be retrieved by downloading the geographical layers of the REPHYTOX locations on: http://www.ifremer.fr/surval2/ |
| Lieu de surveillance : Libellé<br>Monitoring location: Name | Name in text |
| Passage : Identifiant interne<br>Survey: Internal identifier | Non-significant identifier assigned by the Quadrige system to uniquely identify a survey**. A survey may be described as a monitoring location / date / time, on which one or more sampling operations are performed.** |
| Passage : Date<br>Survey: Date | In the format dd / mm / yyyy |
| Passage : Année<br>Survey: Year | In the format yyyy |
| Passage : Mois<br>Survey: Month | In the format mm |
| Passage : Heure<br>Survey: Time | French "real" time ("watch" time). For example, depending on the season in France: UTC (CUT) + 1 in winter, UTC (CUT) + 2 in summer. Optional field. |
| …… | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Coordonnées passage : Coordonnées minx<br>Survey: coordinates: minx coordinates | These coordinates are used to specify the exact location of the survey, if it is slightly different from the monitoring location.<br>For surveys on point monitoring locations, in most cases these coordinates are the same as the monitoring location, and are automatically generated by the system. For point monitoring locations: minx = maxx and miny = maxy.<br>For surveys on surface monitoring locations : (i) either the coordinates of the survey have been specified to define a punctual survey, and then minx = maxx, miny = maxy, (ii) they have not been specified and then all these four coordinates of survey determines the bounding box of the surface location; to retrieve the exact polygon of the monitoring location, it is necessary to download the geographical layers of the REPHYTOX locations on: http://www.ifremer.fr/surval2/ |
| Coordonnées passage : Coordonnées maxx<br>Survey: coordinates: maxx coordinates | |
| Coordonnées passage : Coordonnées miny<br>Survey: coordinates: miny coordinates | |
| Coordonnées passage : Coordonnées maxy<br>Survey: coordinates: maxy coordinates | |
| Passage : Date de validation<br>Survey: Validation date | The validation of the survey indicates that the survey information has been correctly entered and checked.<br>In the SEANOE files, only the validated surveys are present, this field is thus systematically filled. |
| Passage : Niveau de qualité<br>Survey: Quality flag | A quality flag is awarded if the survey has gone through a qualification process: good, doubtful or bad.<br>In the SEANOE files, only those surveys are available: not yet qualified, qualified with a "good" flag, or qualified with a "doubtful" flag if the samples and results attached to it are not doubtful. |
| Passage : Date de qualification<br>Survey: Qualification date | Fields completed if the survey has gone through a qualification process.<br>In the case of surveys which were qualified as doubtful or bad, the qualification comment has been imperatively completed, and explains the doubtful or bad flag. |
| Passage : Commentaire de qualification<br>Survey: Qualification comment | |
| Prélèvement : Identifiant interne<br>Sampling operation : Internal identifier | Non-significant identifier assigned by the Quadrige system to uniquely identify a sampling operation. **A sampling operation may be described as an action to collect shellfish sample (s).** |
| …… | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Prélèvement : Service préleveur : Code<br>Sampling operation: Sampler laboratory: Code | Code and name of the organization, structure or laboratory that collected the sample. Not nominative |
| Prélèvement : Service préleveur : Libellé<br>Sampling operation: Sampler laboratory: Name | |
| Libellé de l'engin de prélèvement<br>Sampling operation: Sampling equipment | Name in text |
| Prélèvement : Niveau<br>Sampling operation: Depth level | Indication on the level of sampling in the water column, for example: "emerged" or "bottom" or "surface (0-1m)". |
| Prélèvement : Immersion<br>Sampling operation: Immersion | Accurate information on the depth of sampling, in meters. Optional field. |
| Prélèvement : Immersion Min<br>Sampling operation: Immersion Min | If the sampling is done at several levels (for example over the entire height of a “bouchot”), the immersion is then replaced by a minimum immersion and a maximum immersion. Optional fields. |
| Prélèvement : Immersion Max<br>Sampling operation: Immersion Max | |
| Prélèvement : Symbole de l'unité d'immersion<br>Sampling operation: Immersion unit symbol | The immersion unit symbol is always in "meter" |
| Prélèvement : Date de validation<br>Sampling operation: Validation date | The validation of the sampling operation indicates that the sampling operation information has been correctly entered and checked.<br>In the SEANOE files, only the validated sampling operations are present, this field is thus systematically filled. |
| …… | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Prélèvement : Niveau de qualité<br>Sampling operation: Quality flag | A quality flag is assigned if the sampling operation has gone through a qualification process: good, doubtful or bad.<br>In the SEANOE files, only those surveys are available: not yet qualified, qualified with a "good" flag, or qualified with a "doubtful" flag if the samples and results attached to it are not doubtful. |
| Prélèvement : Date de qualification<br>Sampling operation: Qualification Date | Fields filled in if the sampling operation has gone through a qualification process.<br>In the case of sampling operations which were qualified as doubtful or bad, the qualification comment has been imperatively completed, and explains the doubtful or bad flag. |
| Prélèvement : Commentaire de qualification<br>Sampling operation: Qualification comment | |
| Echantillon : Identifiant interne<br>Sample: Internal identifier | Non-significant identifier assigned by the Quadrige system to uniquely identify a sample. **A sample is defined by its matrix**. |
| Echantillon : Libellé du support<br>Sample: Matrix name | For the REPHYTOX data, the matrix generally used is "Bivalve". Some samples however exist with the matrices: "Echinoderm" or "Gastropod". |
| Echantillon : Libellé du taxon support<br>Sample: Name of the taxon being analyzed | Latin name of the organism (shellfish most often) on which the analysis is carried out. Based on the WoRMS nomenclature. |
| Echantillon : Commentaire<br>Sample: Comment | Comment free, optional |
| Echantillon : Date de validation<br>Sample: Validation date | The validation of the sample indicates that the sample information has been correctly entered and checked.<br>In the SEANOE files, only the validated samples are present, this field is thus systematically filled. |
| ….. | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Echantillon : Niveau de qualité<br>Sample: Quality flag | A quality flag is assigned if the sample has gone through a qualification process: good, doubtful or bad.<br>In the SEANOE files, only those samples are available: not yet qualified, or qualified with a "good" flag |
| Echantillon : Date de qualification<br>Sample: Qualification date | Fields filled in if the sample has gone through a qualification process.<br>In the case of samples which were qualified as doubtful or bad, the qualification comment has been imperatively completed, and explains the doubtful or bad flag. |
| Echantillon : Commentaire de qualification<br>Sample: Qualification Comment | |
| Résultat : Identifiant<br>Result: Identifier | Non-significant identifier assigned by the Quadrige system to uniquely identify a result |
| Résultat : Service analyste : Code<br>Result: Analyst laboratory: Code | Code and name of the organization, structure or laboratory that did the analysis. Not nominative |
| Résultat : Service analyste : Libellé<br>Result: Analyst laboratory: Name | |
| Résultat : Code paramètre<br>Result: Parameter code | Code and name of the measured parameter. Parameters are detailed in the chapters below.<br>For REPHYTOX data, two types of parameters can be used: quantitative parameters (the majority) leading to numerical results, and qualitative parameters leading to results in textual form |
| Résultat : Libellé paramètre<br>Result: Parameter name | |
| Résultat : Libellé support<br>Result: Matrix name | For the REPHYTOX data, the matrix generally used is "Bivalve". Some samples however exist with the matrices: "Echinoderm" or "Gastropod". |
| Résultat : Libellé fraction<br>Result: Fraction name | For REPHYTOX data, analyses can be done on different fractions of shellfish, for example: "total flesh" or "digestive gland (hepatopancreas)" or "muscle + gonad".<br>"Sans objet" (="Not applicable") fractions should be considered as an absence of fraction information. |
| …… | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Résultat : Libellé méthode<br>Result: Method name | Name in text |
| Résultat : Symbole unité de mesure associé au quadruplet<br>Result: Symbol of the unit of measure associated with the quadruplet | A "quadruplet" is the combination of a parameter, a matrix, a fraction and a method. This mandatory association for data entry prohibits inconsistent entries. A quadruplet is associated with a single unit of measure |
| Résultat : Libellé unité de mesure associé au quadruplet<br>Result: Name of the unit of measure associated with the quadruplet | |
| Résultat : Valeur de la mesure<br>Result: Value of the measurement | Numeric value of the measurement.<br>This field is only filled for quantitative parameters. |
| Résultat : Libellé précision<br>Result: Additional information on measurement value | Depending on the case, this additional information can be: "> value", "<value", "Inf. LD "," Inf LQ ". The last two labels correspond to "below the Limit of Detection" and "below the Limit of Quantification", respectively. These LDs and LQs depend on: the method, the analysis instrument, and the analysis laboratory.<br>Optional field. This field is only filled for quantitative parameters. |
| Résultat : Valeur qualitative<br>Result: Qualitative value | Result in textual form.<br>This field is only filled for qualitative parameters. |
| Résultat : Commentaires<br>Result: Comment | Comment free, optional |
| Résultat : Date de validation<br>Result: Validation date | The validation of the result indicates that the result information has been correctly entered and checked.<br>In the SEANOE files, only the validated results are present, this field is thus systematically filled. |
| …… | |

| Column header in SEANOE files (French and English translation | Additional explanation for REPHYTOX data |
|---|---|
| Résultat : Niveau de qualité<br>Result: Quality flag | A quality flag is assigned if the result has gone through a qualification process: good, doubtful or bad.<br>In the SEANOE files, only those results are available: not yet qualified, or qualified with a "good" flag |
| Résultat : Date de qualification<br>Result: Qualification date | Fields filled in if the result has gone through a qualification process.<br>In the case of results which were qualified as doubtful or bad, the qualification comment has been imperatively completed, and explains the doubtful or bad flag. |
| Résultat : Commentaire de qualification<br>Result: Qualification comment | |

# Data information

The data described here were acquired according to the REPHYTOX protocols, which may have evolved over time.

The following data are examined successively: (i) lipophilic toxins including diarrheic toxins, (ii) paralytic toxins (PSP), (iii) amnesic toxins (ASP), (iv) palytoxins-ovatoxins.

These data are usually compared to thresholds or limits, which are:

- the sanitary threshold: this is the threshold defined in the European regulatory texts, which corresponds to the value not to be exceeded for the shellfish analyzed to be consumable
- the limit of detection: it is the lowest value that can be **obtained** in the context of the analysis, it depends on the method, the analytical machine and the analysis laboratory
- the limit of quantification: it is the lowest value that can be **quantified** in the context of the analysis, it depends on the method, the analytical machine and the analysis laboratory

An analysis result must never be equal to "0", since it is never certain that the toxins are totally absent from the sample when the measurement gives a result below the detection limit, since it depends on the performance of the analytical tool used. The limit of quantification is always greater than the limit of detection: a result between these two limits indicates that the toxins are present in a very small quantity, but that they cannot be precisely quantified.

In general, since the toxin data have not yet gone through a qualification process:

- the results equal to "0" must be interpreted as results below the detection limit
- in case of inconsistency between the numerical value and the “Additional information on measurement value” (=”Libellé precision”), the numerical value must be preferred

## Lipophilic toxin data including diarrheic toxins

Until 2009 inclusive, lipophilic toxins were analyzed by mouse bioassay. Since 2010, chemical analyzes by CL/-SM/-SM (LC/-MS/-MS Liquid Chromatography coupled with Tandem Mass Spectrometry) have replaced mouse bioassays for regulatory monitoring. However, bioassays are still used in the context of a vigilance system, for the detection of any known or unknown or emerging toxin analogues.

The parameters corresponding to the two types of analysis are detailed successively below.

### Mouse bioassay

The mouse bioassay was the reference method until 2009 inclusive:

- all results present in data for this method over the period 1987-2009 are therefore regulatory monitoring results
- the results present from 2010 (included) concern non-regulatory data, acquired as part of a vigilance system, concerning no more than 15 sampling locations throughout the French coastline

Mouse bioassays are qualitative analyses, leading to a binary result (favorable or unfavorable). They consist in interpreting the 24-hour survival of three mice injected with shellfish extract: the death of two or three mice in less than 24 hours leads to an unfavorable result, whereas the favorable result is obtained with the death of a single mouse or the survival of the three mice.

As a qualitative analysis, there is no limit of detection or quantification.

The survival data for each of the three mice, however, are available, as well as the symptoms, as these data may have been useful for some studies. However, it is not recommended to use these detailed survival times to classify the results between them.

The parameters used for mouse bioassays are described below.

## Parameters for the final result of the bioassay

The final result is calculated as the average or the median survival time of each mouse: the average was used until 2002, and then was replaced at that date by the more relevant median. The two corresponding parameters are as follows:

**SVSOUMOY –** « Temps de survie moyen souris » **= Average survival time**

**SVSOUMED –** « Temps de survie médian souris » **= Median survival time**

For these two parameters, the unit is in minutes and:

- a result> = 1440 min (= 24 H) corresponds to a favorable result, *i.e.* an absence of toxins or a presence of toxins lower than the regulatory sanitary threshold
- a result <1440 min corresponds to an unfavorable result, *i.e.*with presence of toxins above the sanitary threshold

*Note that the rule of calculation of the SVSOUMOY parameter was adapted to respect the interpretation of the bioassay (described above): the average was calculated only if at least two mice had died, otherwise the result was equal to 1440. For SVSOUMED, the simple calculation of the median consistently gives a result consistent with this interpretation.*

## Parameters for the detailed results of the bioassay

These detailed results are in the same sample as the final result: the sample is identified by the field « Echantillon : Identifiant interne » (=Sample: internal identifier), and corresponds to a single species of shellfish.

For each mouse, the survival times and symptoms are detailed with the following parameters:

**SVSOURI1 –** « Temps survie 1ère souris » **= Survival time 1st mouse**

**SVSOURI2 –** « Temps survie 2ème souris » **= Survival time 2nd mouse**

**SVSOURI3 –** « Temps survie 3ème souris » **= Survival time 3rd mouse**

**SVSOURIS –** « Temps survie souris » **= Survival time**

The mice are generally differentiated by 1, 2 and 3, which normally makes it possible to associate the survival time with the symptoms described below. For older data, the SVSOURIS parameter is repeated three times in the sample.

The unit of these parameters is the minute.

**SYMPTSOU1 –** « Symptômes 1ère souris lors d'un test toxines lipophiles » **= Symptoms 1st mouse during a mouse bioessay**

**SYMPTSOU2 –** « Symptômes 2ème souris lors d'un test toxines lipophiles » **= Symptoms 2nd mouse during a mouse bioessay**

**SYMPTSOU3 –** « Symptômes 3ème souris lors d'un test toxines lipophiles » **= Symptoms 3rd mouse during a mouse bioessay**

**SYMPTSOU –** « Symptômes souris lors d'un test toxines lipophiles » **= Mouse symptoms during a mouse bioessay**

Mice are generally differentiated by 1, 2 and 3, which normally makes it possible to associate the symptom with the survival time described above. For older data, the SYMPTSOU parameter only appears once in the sample and corresponds to the symptoms of the three mice taken together.

These parameters are qualitative, the possible values being:

- For the SYMPTSOU parameter
  - « typiques sans diarrhée » = **typical without diarrhea**
  - « typiques avec diarrhée » = **typical with diarrhea**
  - « atypiques : neurol. convulsion » = **atypical: neurological, convulsions**
- For parameters SYMPTSOU1, SYMPTSOU2, SYMPTSOU3
  - « typique sans diarrhée » = **typical without diarrhea**
  - « typique avec diarrhée » = **typical with diarrhea**
  - « atypique : neurologique, convulsion » = **atypical: neurological, convulsion**
  - « survie affaiblie » = **weakened survival**
  - « non observée : mort nocturne » = **not observed: nocturnal death**

## Fractions

Several fractions are present in the 1987-2016 dataset for these parameters, the recommendations for use are as follows:

- « Glande digestive (hépatopancréas) » = **digestive gland (hepatopancreas)**
  - no problem, it was the official fraction for the regulatory bioassay, it is also the fraction used for vigilance since 2010
- « Chair totale égouttée » = **total flesh drained**
  - no problem, it is a fraction which was used and validated between 1999 and 2009 for some shellfish (in particular the pectinids and small shellfish like clams, donax, etc)

- « Sans objet » = **not applicable**
    - since the toxin data have not yet gone through a qualification process, the fraction, although not specified, can be considered as correct
- « Chair + liquide intervalvaire » = **flesh + inter-valve fluid**, « Chair sans glande digestive » = **flesh without digestive gland**, « Muscle + gonade » = **Muscle + gonad**
    - these three fractions are not correct or correspond to very particular studies, in the doubt better not to use these data

## Methods

Different methods are used in the 1987-2016 dataset for these parameters, reflecting the evolution of toxin monitoring legislation. Precautions for the use of data with these different methods are provided in **Table 2**.

**Table 2. The different methods used for the detection of lipophilic toxins by mouse bioassay, and the precautions for use**

| Key words in the method name | | Concerned results | Additional information | Recommendations for using the results |
|---|---|---|---|---|
| In French | In English | | | |
| toxicité globale acétone | overall toxicity acetone | 1987 -2000 | Method for detecting all lipophilic toxins, therefore nonspecific diarrheic toxins | Use with caution |
| toxicité DSP dichlorométhane | DSP toxicity dichloromethane | 1987 - 2008 | Method for more specifically detecting diarrheic toxins | No problem |
| toxicité atypique méthanol | atypical toxicity methanol | 1994 - 2001 | Method for detecting atypical, non-diarrheic toxins | Too specific, do not use, at least with other results |
| toxines lipophiles Yasumoto 84 modifié | modified Yasumoto 84 lipophilic toxins | 2002 - 2015 | Method for more specifically detecting diarrheic toxins | No problem |
| toxines lipophiles Hannah et al. 1995 | lipophilic toxins Hannah et al. 1995 | 2003 - 2009 | Method reserved for the bioessays carried out on the whole flesh, for certain shellfish, like the scallops | No problem |
| ANSES LNRBM-LIP 01 | ANSES LNRBM-LIP 01 | From 2015 | Official method of ANSES (French national reference laboratory for toxins) for vigilance | Can be used, knowing that they are not regulatory monitoring results |

## Chemical Analysis by CL/-SM/-SM (LC/-MS/-MS Liquid Chromatography Coupled to Tandem Mass Spectrometry)

The chemical analysis by CL/-SM/-SM (LC/-MS/-MS ) is the reference method since 2010 included:

- all results present in the dataset for this method from 2010 are therefore regulatory monitoring results
- the results present in 2008 and 2009 data concern non-regulatory data, acquired for particular studies with a view to preparing the transition between bioassay and chemical analysis

These chemical analyses give quantitative results, with limits of detection and quantification.

### Parameters

28 toxins have been identified and quantified since 2010, but only some of them are regulated: they are grouped into three families made up of the sums of some of these toxins, corrected by their TEFs (Toxic Equivalent Factor). The 31 parameters (28 identified toxins + 3 families) that can be used for lipophilic toxins are listed in **Table 3**.

**Table 3. The parameters present in the data corresponding to the lipophilic toxins analyzed by CL/-SM/-SM (LC/-MS/-MS) between 2008 and 2016**

| Parameters present in the dataset from 2010 | | Parameters present in the dataset 2008-2009 |
|---|---|---|
| **Individual toxins** | | |
| 45-OH-homo-YTX | 45-hydroxy-homo-Yessotoxine | |
| 45-OH-YTX | 45-hydroxy-Yessotoxine | YES |
| PTX-2sa-épi | Acide 7-épi seco-Pectenotoxine-2 | YES |
| AO-libre | Acide Okadaïque libre | YES |
| AO-total | Acide Okadaïque total | YES |
| PTX-2sa | Acide seco-Pectenotoxine-2 | YES |
| AZA-1 | Azaspiracide-1 | YES |
| AZA-2 | Azaspiracide-2 | YES |
| AZA-3 | Azaspiracide-3 | YES |
| COOH-homo-YTX | Carboxy-homo-Yessotoxine | |
| COOH-YTX | Carboxy-Yessotoxine | YES |
| DTX-1-libre | Dinophysistoxine-1 libre | |
| DTX-1-total | Dinophysistoxine-1 total | YES |
| DTX-2-libre | Dinophysistoxine-2 libre | |
| DTX-2-total | Dinophysistoxine-2 total | YES |
| GYM-A | Gymnodimine-A | YES |
| GYM-B | Gymnodimine-B | |
| Homo-YTX | Homo-Yessotoxine | YES |

| | | |
|---|---|---|
| PTX-1 | Pectenotoxine-1 | |
| PTX-2 | Pectenotoxine-2 | YES |
| PTX-6 | Pectenotoxine-6 | |
| SPX-desMe-C | Spirolide-13-desmethyl-C | YES |
| SPX-desMe-D | Spirolide-13-desmethyl-D | YES |
| SPX-A | Spirolide-A | YES |
| SPX-B | Spirolide-B | YES |
| SPX-C | Spirolide-C | |
| SPX-D | Spirolide-D | |
| YTX | Yessotoxine | YES |
| **Regulatory toxin families** | | |
| AO+DTXs+PTXs-TEFs | Sum AO + DTXs + PTXs regulated, with TEFs | YES |
| AZAs-TEFs | Sum AZAs regulated, with TEFs | YES |
| YTXs-TEFs | Sum YTXs regulated, with TEFs | YES |

The toxins constituting each of the three regulated families are as follows:

- For AO + DTXs + PTXs : AO total, DTX-1 total, DTX-2 total, PTX-1, PTX-2
- For AZAs : AZA-1, AZA-2, AZA-3
- For YTXs : YTX, Homo-YTX, 45-OH-YTX, 45-OH-homo-YTX

## Fractions

The fractions used are as follows:

- « Chair totale égouttée » = **Total flesh drained**
- « Glande digestive (hépatopancréas) » = **Digestive gland (hepatopancreas)**

For the regulatory results, the legislation having been modified during 2010, only the following fractions must be retained:

- "Total flesh drained" or "Digestive gland (hepatopancreas)" from January 1, 2010 to April 30, 2010
- **only "Total flesh drained" from May 1, 2010**

The results on the digestive gland outside these are results of study or vigilance, which should not be compared with the results on total flesh.

## Methods

Two methods followed one another:

- « CL-SM/SM toxines lipophiles 2009 - µg/kg » = **LC/-MS/-MS) lipophilic toxins 2009 - µg / kg** : in 2008-2009
- **ANSES PBM BM LSA_INS-0147 - µg/kg** : depuis 2010

The unit is always in µg/kg of shellfish flesh.

### Thresholds and limits

For the three families of regulated toxins, the different thresholds are detailed in **Table 4**. The limits of detection and quantification are given in this table for information only, since they depend on the analytical equipment and the laboratory analyst.

**Table 4. Thresholds and limits for the three families of regulated lipophilic toxins, in µg/kg of shellfish flesh**

| Toxin family | Regulatory sanitary limit | Detection limit | Quantification limit |
|---|---|---|---|
| AO + DTXs + PTXs - TEFs | 160 | between 3 et 15 | between 4.2 et 60 |
| AZAs - TEFs | 160 | between 0.2 et 10 | between 0.7 et 30 |
| YTXs - TEFs | 3750 | between 0.5 et 10 | between 5 et 30 |

## Paralytic toxin (PSP) data

Paralytic toxins have always been analyzed with a mouse bioassay, which still remains the reference method at European level. In contrast to the bioassay for lipophilic toxins, which is a qualitative test, the results of the PSP bioassay are quantitative: a calculation formula, taking into account correction and dilution factors, makes it possible to transform the survival times of the mice into a toxin concentration. The data set begins in 1988, when these toxins first appeared in France.

Three parameters are present in the dataset: TOXPSP, USPSP, PSP. Only the first one provides interpretable results.

### USPSP – Unité souris PSP = PSP mouse unit

The USPSP parameter gives intermediate results for the mouse bioassay, which cannot be interpreted without further information. **It is therefore not recommended to use them**. This parameter is no longer filled in since 2013.

### PSP – Toxines PSP = PSP toxins

This parameter concerns chemical analyses by HPLC / -FL (High Performance Liquid Chromatography / Fluorescence) carried out on about thirty samples. These are not regulatory results.

### TOXPSP – Toxicité PSP = PSP toxicity

This parameter is the only one that provides the results of the regulatory monitoring of PSP toxins.

The fraction used is always: « Chair totale égouttée » = **Total flesh drained**

Two methods followed one another:

- « Test souris PSP / quantification en équ. STX - µg/kg » = **PSP mouse test / quantification in equ. STX - µg / kg** : from 1988 to 2015
- « **ANSES LNRBM-PSP 01 - µg / kg** »: since 2015

The unit is always in µg/kg shellfish flesh (or more precisely: µg equ.STX/kg of flesh)

The sanitary threshold is equal to 800 µg/kg of shellfish flesh.

The detection threshold is variable according to the laboratories, it varies in the dataset between 280 and 400 µg/kg. Since this is a bioassay, there is no limit of quantification.

## Data on amnesic toxins (ASP)

Amnesic toxins have always been measured by HPLC / UV (High Performance Liquid Chromatography with Ultraviolet Detection) analyses, which is the reference method at the European level. The dataset begins in 1999, the date of the first research for these toxins in France in a regulatory monitoring framework.

Only one parameter is present in the data: ASP.

## ASP – Toxines ASP = Amnesic toxins

Several fractions are used in the 1999-2016 dataset for these parameters, the recommendations for use are as follows:

- « Chair totale égouttée » = **Total flesh drained:** this is the official fraction for regulatory ASP analyses
- « Chair sans glande digestive » = **Flesh without digestive gland**, « Glande digestive (hépatopancréas) » = **digestive gland (hepatopancreas)**: these two fractions are not correct or correspond to very particular studies, better not to use these data
- « Muscle + gonade », = **Muscle + gonad**, « Gonade » = **Gonad**, « Muscle » = **Muscle**: these three fractions are sometimes analyzed to report the contamination on the part consumed when the scallops are sold shucked: attention to the use of these data, which should not be compared to those on the whole flesh

Two methods followed one another:

- « CLHP/UV toxines amnésiantes - mg/kg » = **HPLC / UV amnesic toxins - mg/kg**: from 1999 to 2015
- ANSES LNRBM-ASP 01 - mg/kg: since 2014

Note that the unit is different from other toxins: mg/kg of shellfish flesh.

The sanitary threshold is equal to 20 mg/kg.

The limit of detection and the limit of quantification are variable according to the laboratories, they vary in the dataset between 0.15 and 1 mg/kg of shellfish flesh.

## Data on palytoxins and ovatoxins

Palytoxins and ovatoxins are not regulated, but they have been analyzed since 2010 on Mediterranean sea urchins in the framework of a study. The analysis is performed by CL/-SM/-SM (LC/-MS/-MS Liquid Chromatography coupled to Tandem Mass Spectrometry).

The parameters that can be measured are:

| | |
|---|---|
| PLTX | Palytoxine |
| 42-OH-PLTX | 42-OH palytoxine |
| Masca-A | Mascarenotoxine-A |
| Masca-B | Mascarenotoxine-B |
| Ostreo-D | Ostreocin-D |
| Ova-A | Ovatoxine-A |

The fraction used is usually: « Chair totale égouttée » = **Total flesh drained**. Some results exist with the fractions: « Chair sans gonade » = **Flesh without gonad** and « Gonade ».= **Gonad**.

The method is: « CL-SM/SM Palytoxines-like 2010 - µg/kg »= **LC/-MS/-MS Palytoxin-like 2010 - µg/kg.**

There is no sanitary threshold since these toxins are not regulated.

The detection limit varies with laboratories: between 9 and 25 µg/kg of shellfish flesh, in the dataset.

# Conclusion

In general, the processing of these data needs to be vigilant about all the metadata that may have impacted the data. Indeed, these thirty years of data have not always been acquired according to the same sampling strategies, these having evolved over time. Without the list being exhaustive, we can mention:

- the sampler laboratory, which should have a minor impact
- the analyst laboratory, which may have changed over time, with a more or less significant impact especially for old data
- the fraction analyzed and/or the method, which may have changed over time, with a greater or lesser impact

Consultation of comments on the sample or results, where they exist, and qualification comments for qualified data, may help in interpreting the data.

It should be noted that the more recent the data, the more they have been subjected to drastic procedures in terms of respect of analysis methods, and compliance with quality procedures. Indeed, toxin analyses have long been performed in accredited laboratories. In any case, it is recommended to contact the REPHY-REPHYTOX coordination.