



TOOLS4ENV

AiiDA : Aquatic Impact Indicators DAtabase



- Methodological guide -

TOOLS 4 ENV

AiiDA Methodological guide Version 3.00

© Tools for Environment
4, Rue de la Châtellenie
1635 La Tour-de-Trême
Switzerland









Dr. Jérôme Payet
Email: jerome.payet@cycleco.eu
Ing Odilon Hugonnot
Email: odilon.hugonnot@cycleco.eu

Date: May 2014
Language: English
File available in: PDF

Acronyms


LCA:	Life Cycle Assessment
AiiDA :	Aquatic Impact Indicators Database
AMI :	Assessment of the Mean Impact
CAS :	Chemical Abstracts Service
EC₅₀ :	Median Effective Concentration
EPA :	Environmental Protection Agency
HC₅ :	Hazardous Concentration 5 %
HC₅₀ :	Hazardous Concentration 50%
ITIS :	Integrated Taxonomic Information System
PNEC :	Predicted No Effect Concentration
PSD :	Phyla Sensitivity Distribution
QSAR :	Quantitative Structure-Activity Relationship
SSD :	Species Sensitivity Distribution
TGD :	Technical Guidance Document

Table of content

Acronyms	i
 Context	1
Background and description of the AiiDA tool	1
Traceability of the aquatic ecotoxicological indicators	2
 Objectives of the methodological guide	2
Prerequisite	3
 Aquatic Ecotoxicology	3
Presentation and definition of aquatic ecotoxicology	3
 Ecotoxicological tests	4
The primary variables which run AiiDA	4
Types of tests: Acute and Chronic	4
Tests results: outstanding values	5
Conclusion: the importance of ecotoxicological tests	6
The ecotoxicological databases	7
 The reference databases	7
AQUIRE: Aquatic ECOTOX US EPA	7
OPP: Pesticide Ecotoxicity Database	7
USGS CERC: Acute Toxicity Database	8
EAT: ECETOC Aquatic Toxicity	8
eChemPortal: REACH Database	8
 The AiiDA database	9
Creation method of the AiiDA database	9
The structure of the AiiDA database	10
Identification of the substance	10
Identification of the tested organism	11
Identification of the tests conditions and results	12
Traceability and quality of information	12
Conclusion:	13
The A.M.I. method	14
 Issue	14
The required features of an impact assessment method	14
 The principles of the A.M.I. method	14
EC ₅₀ chronic as a reference ecotoxicological value	14
Allowing the use of a maximum number of ecotoxicological values	15

Data filtration	16
Duplicates removal: grouping identical tests	17
Data verification:	17
Conclusion:	18
HC ₅₀	19
Substance-Species level	19
Substance-Phylum level	20
Substance-Impact level	21
☒ Use of QSARs	22
The QSARs methods	22
QSAR models operating mode	22
Rules and limitations of use of QSAR in AIIDA	23
Uncertainty calculation	24
HC ₅	25
☒ Calculation of HC₅ (<i>Hazardous Concentration for 5% of species</i>)	25
Method based on the Aldenberg review (2010)	25
US-EPA methodology	28
PNEC	33
☒ Calculation of the PNEC (Predicted No Effect Concentration)	33
Identification of the test and of the most sensitive species	33
Estimation of the security factor AF (Assessment Factor)	34
BIBLIOGRAPHIC RESOURCES	35

Context and objectives

 *This AiiDA user guide gathers all the information that is necessary to obtain various aquatic ecotoxicological indicators..*

Context

Background and description of the AiiDA tool

The lack and the difficulty of access to ecotoxicological data, as well as the complexity of the implementation of calculation and methods, make the use of the representativity of aquatic ecotoxicity indicators difficult

To overcome this issue, AiiDA provides more than 500 000 unique and referenced ecotoxicological tests on more than 8 000 species and 34 phyla. This global database is used to automatically calculate the different ecotoxicological indicators as well as their uncertainties according to the official Technical Guidance Document (TGD) for risk assessment of the joint research center of the European commission. AiiDA allows to cover 22 000 molecules, including 13 500 with an ecological representativity of 3 phyla or more.

The various ecotoxicological impact indicators gathered in AiiDA

- **HC₅₀ Chronic and Acute** (*Hazardous Concentration 50%*) calculated with the AMI (*Assessment of Mean Impact*) method, an outcome of Dr Jérôme Payet 2004 thesis. The risk assessment is calculated with the help of the Student table. This indicator is used by models such as USEtox to determine the characterisation factors of aquatic ecotoxicity within the frame of a LCA.
- **HC₅ and HC_{5-95%}** (*Hazardous Concentration 5%*) calculated with the Aldenberg method (2000) or the SSD (*Species Sensitivity Distribution*) extrapolation method of US-EPA (2005).
- **PNEC** (*Predicted No-Effect Concentration*), calculated with the recommendations of the Technical Guidance Document on Risk Assessment (*EU, 2003*). This indicator is used within the risk assessment approach and within the legal frame.

Traceability of the aquatic ecotoxicological indicators

AiiDA allows the traceability of the calculations and to go back to the entire set of the initial used tests. The AiiDA platform can produce SSD (Species Sensitivity Distribution) and PSD curves (Phyla Sensitivity Distribution) of the different molecules and compare their ecotoxicity.

👉 Objectives of the methodological guide

This methodological guide is intended for enterprises and, on the whole, for all AiiDA users. It will help users get acquainted with the ecotoxicological data that is provided by the AiiDA tool and acknowledge its limits. This guide summarizes the hypotheses, the choices, the steps and the methods that have been adopted during the development of the database, from the required calculation to the traceability.

Prerequisite

Minimum required knowledge to understand and use AiiDA

🔥 Aquatic Ecotoxicology

Presentation and definition of aquatic ecotoxicology

Chemicals are part of our daily life. They can be found in most consumer goods and undeniably bring significant benefits to our developed society. However, with the rapid growth of the industry, an increasing amount of new chemical substances has emerged in the environment. The number of worldwide marketed chemicals is around 6 million while the number of chemicals known by chemists (chemically synthesized products or extracted products) is currently around 22 million [1]. According to reports of the REACH regulation (*Registration, Evaluation, Authorisation and restriction of CHemicals*), the worldwide production of chemical substances has risen from 1 million tons to in 1930 to more than 400 million tons today. Close to 100 000 different substances have been inventoried on the Community market. This includes 30 000 marketed products in quantities above one ton per year [2].

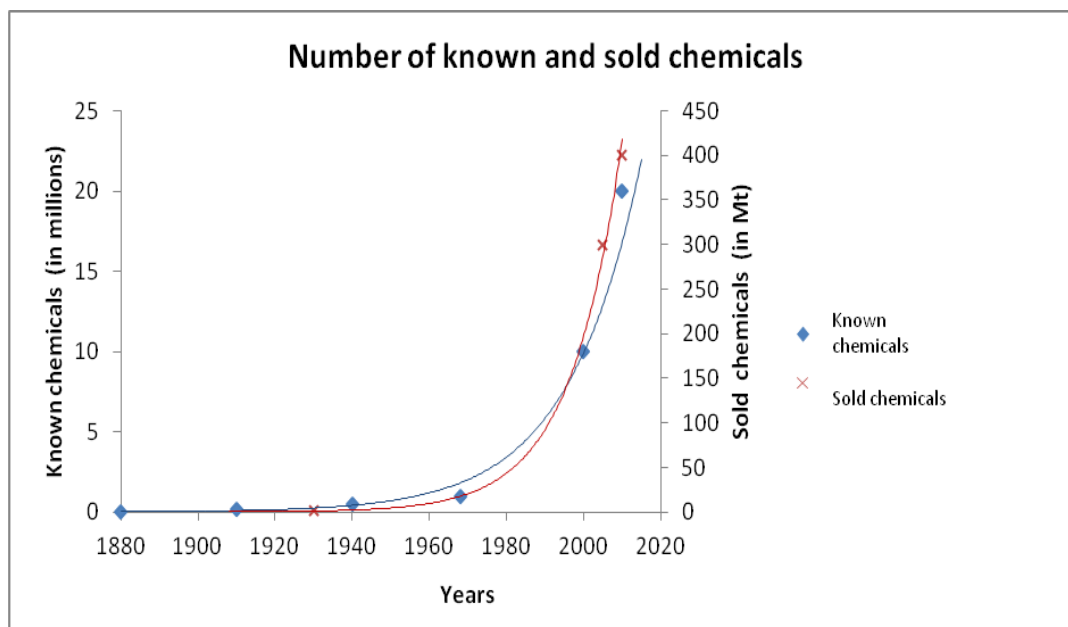


Figure 2.1: Number of known and sold chemicals. Source: REACH and CNRS [2][3]

PREREQUISITE

The proliferation of new chemical substances with unknown effects on the environment gave birth to a new science called ecotoxicology. This science can be seen as an extension of the term toxicology which studies the effects of a pollutant on an organism and its effects on the ecosystem. To make it simple, one can say that this science represents **“a scope which integrates the ecological and toxicological effects of chemicals on the population, the communities and the ecosystem (transport, transformation and degradation) with the outcome of these pollutants on the environment”** (Forbes et al. 1997) [4].

Ecotoxicological tests

The primary variables which run AiiDA

In practical terms, an ecotoxicological test is **“an experimental bioassay which determines the effect of one or several products on a selected group of organisms, in clearly defined conditions** (Keddy and al. 1994) [5]. These tests use various means to quantify the toxicity of a product. They correlate the concentration of a pollutant and its effect (**dose-response principle**). These effects, as well as their importance, at the biochemical, physiological, reproductive and behavioral level, allow us to measure toxicity. Most toxicity tests give us an estimate of the dose which affects 50% of the population. They allow AiiDA’s models to operate and can be classified in two categories.

Types of tests: Acute and Chronic

Acute Tests

The acute tests (acute) are carried out during a very short period of the life of an organism. They are fast and cheap, which is a benefit. However, because they are short, the concentration used is high. In the reality, a majority of environmental problems are due to low residual concentrations which cause long term effects that cannot be revealed by this type of test. These tests are still very commonly used and most available ecotoxicological data come from them.

Chronic Tests

The chronic and sub-chronic tests (chronic and sub-chronic) are carried out during a relatively long period of the life of an organism. They are more expensive. However they allow us to have a better idea of the long term impact of a pollutant.
AiiDA prefers this type of test and uses them for the calculation.

PREREQUISITE

There is a set of rules that allows characterizing the type of test according to the period and the various tested species. The methodology used by AiiDA to assess the tests is summarized in the chart below.

	Acute	Sub-chronic	Chronic
Vertebrates	Tests < 7 days	7 days < Tests < 32 days	Tests > 32 days
Invertebrates	Tests < 7 days	7 days < Tests < 21 days	Tests > 21 days
Plants	Tests < 7 days	-	Tests > 7 days
Algae	Tests < 3 days	-	Tests > 3 days

Table 2.1: Distinction rules between acute and chronic ecotoxicity data – from the « Assessing toxic impacts on aquatic ecosystem in Life Cycle Assessment » thesis of Jérôme PAYET [6]

Tests results: outstanding values

Each ecotoxicological test can be defined by its “end point” evaluation criteria (the effect area sought) which will focus, amongst others, on the mortality rate, the reproduction, the growth, the weight, the height or the behavior of the tested species. The tests results are concentration values specific to these endpoints. The best known values and the ones used by AiiDA are NOEC, LOEC and EC₅₀.

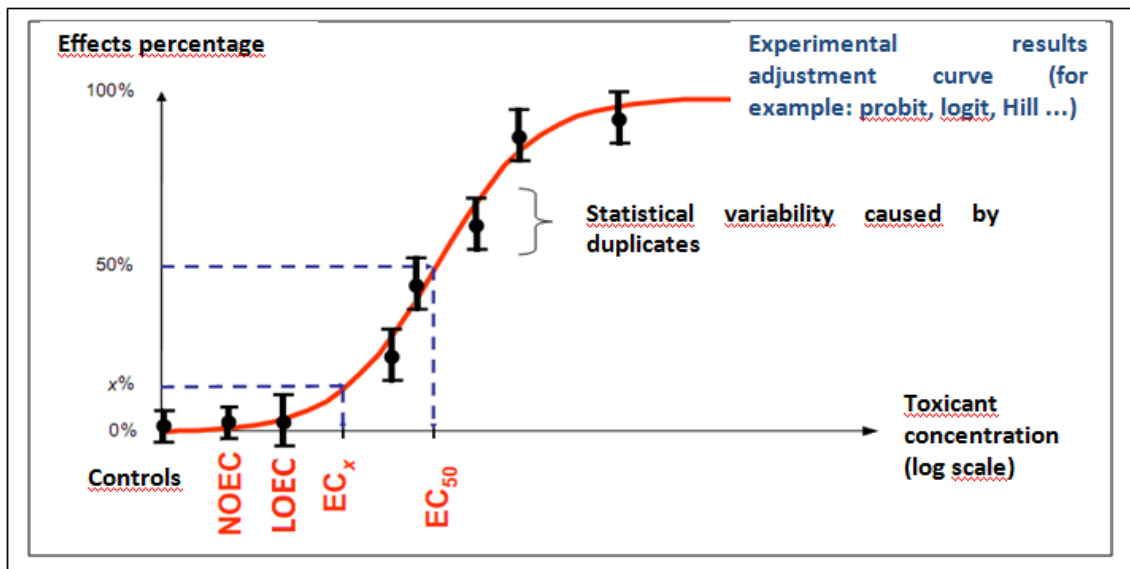


Figure 2.2: Graphic representation of some outstanding values (EC₅₀, LOEC, NOEC) on the dose-response curve of a pollutant.

The **EC₅₀** (*Effect Concentration 50%*) corresponds to the pollutant concentration which has an effect on 50% of the population, compared to the control samples not subjected to the pollutant. This value is statistically determined on the basis of a transformation of the data, then a regression or a modeling. It is a relatively stable value which poorly fluctuates whatever the statistical method is used.

The **LOEC** (*Lowest Observed Effect Concentration*) can be defined as the lowest concentration which induces an observed effect on the organisms, compared to the control samples. This value is rather variable and greatly depends on the width of the concentration sampling during the tests.

The **NOEC** (*No Observed Effect Concentration*) can be defined as the highest pollutant concentration with no observed effect on the organisms compared to the control samples. This concentration value is in general rather variable from one test to another and requires a great number of replicates (repetition of identical tests) in order to be reliable.

Conclusion: the importance of ecotoxicological tests

Ecotoxicology is an experimental science which allows to highlight toxicity and the effects of a chemical on an ecosystem according to its residual concentration. As for all experimental sciences, the values that arise from the tests are subject to strong uncertainties. However, to this day, there are not better methods to quantify this kind of complex effect. Ecotoxicological tests can be of two different types, i.e. « Acute » or « Chronic », the most reliable of them being the « Chronic » tests for the reason that they are carried out on a longer experience period. These tests give us information on specific concentration values that have more or less influence on aquatic species. The specific concentrations used by AiiDA are **LOECs**, **NOECs** and **EC50s**. These experimental data are primary variables. They allow the models to operate. The resulting aquatic ecotoxicity impacts scores will then be transcribed when doing environmental labeling for example.

The ecotoxicological databases

Origin of the ecotoxicological tests and structure of the Aiida database

The reference databases

The ecotoxicological tests in AiIDA come from either institutional ecotoxicological databases references or internal sources.

AQUIRE: Aquatic ECOTOX | US EPA

ECOTOX version 5 is an American environmental protection agency database (USEPA) which contains information on the toxicity of chemicals on aquatic and terrestrial life. It includes three independent databases (AQUIRE, PHYTOTOX and TERRETOX) from which is extracted one relevant source of data coming from peer-reviewed literature. In our context, we shall take only the AQUIRE database into account which identifies the aquatic ecotoxicological data. It gathers around 330 000 tests on more than 4 8000 chemicals and close to 3 000 different animal and plant species. These tests are classified, amongst others, according to the endpoints, the experiment period, the environment (fresh water, sea water), the phyla and the references in the scientific literature. This database is downloadable free of charge on the Internet. The data is free of access [7].

OPP: Pesticide Ecotoxicity Database

The Ecological Fate and Effects Division of the Office of Pesticide Programs (OPP), within the American Environmental Protection Agency (USEPA), examined a great number of studies on toxicity. These tests were carried out by the USEPA, the American agriculture department and the US Fish and Wildlife Service laboratories on several aquatic and terrestrial species. These studies are gathered in the Pesticide Ecotoxicity Database of the Office of Pesticide Programs (OPP *Pesticide Ecotoxicity Database, 2008*) and are considered reliable for the calculation of environmental risks [8]. This database gathers around 20 000 tests on more than 800 chemicals and close to 300 different animal and plant species. It is also downloadable free of charge and free of access on the Internet.

USGS CERC: Acute Toxicity Database

This database is the result of acute aquatic ecotoxicity tests carried out by the Columbia Environmental Research Center (*CERC*). It gathers around 5 000 tests which concern more than 400 chemicals and close to 60 fresh water animal and plant species [9]. It is also downloadable free of charge and free of access on the Internet.

EAT: ECETOC Aquatic Toxicity

The European Center of ecotoxicology and toxicology of chemicals (*ECETOC*) has compiled information from scientific publications on aquatic ecotoxicity into a database that gathers around 5 000 tests on more than 500 chemicals and close to 250 different animal and plant species. This database does not seem to be accessible on the Internet anymore. The data used for AiiDA comes from Cycleco which owns an old 2001 version of the database.

eChemPortal: REACH Database

Within the REACH regulation framework, eChemPortal [10] provides a free of charge public access to information on the chemical properties of the various classified substances, as well as to direct links towards national, regional and international level information collections. This search engine gathers a dozen independent databases, the main ones being:

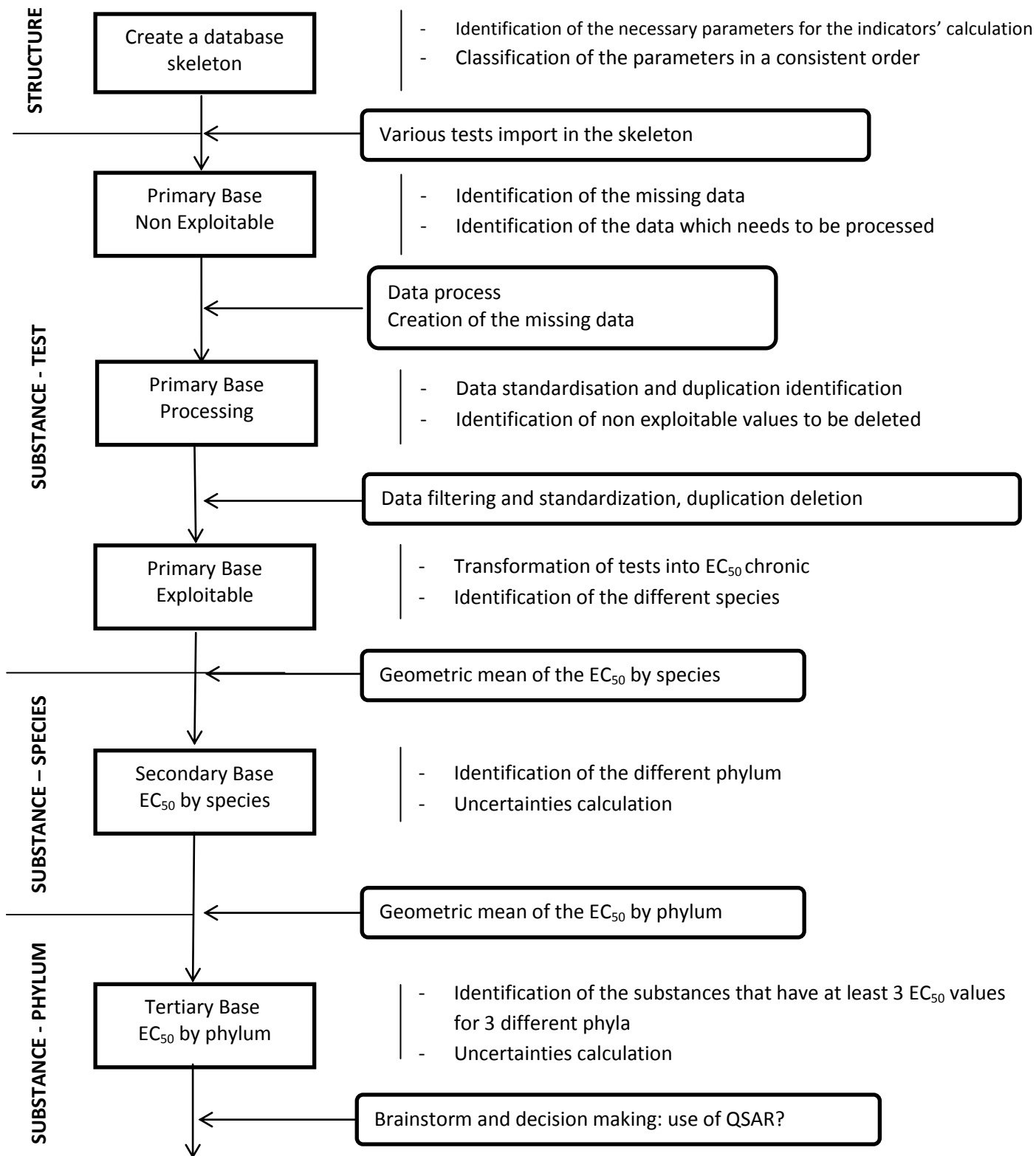
- **The Canadian Categorization Results (*CCR*):** this database contains the environmental categorization results of chemicals included in the Canadian domestic substances list. Indeed, the Canadian law regarding the protection of the environment (*CEPA 1999*) is one of the main tools used to prevent and reduce the risks of used and released substances in Canada. More than 23 000 chemicals are recorded on the « *Domestic Substances List* » and are studied so as to identify their level of ecotoxicity. The Canadian government is responsible for the content of this database which is hosted by the OCDE.

- **The European Chemicals Agency Portal (*ECHA*):** the ECHA dissemination portal provides a free of charge public access to information on the chemicals manufactured or imported into Europe. This information comes from the registration files provided by the companies to the European Chemicals Agency (*ECHA*) within the REACH regulation framework. To this day, this pooling of data has allowed to gather information on more than 4 000 substances coming from the 25 000 REACH registration file. Approximately 30 000 substances should be available on this portal by 2018.

- **The International Uniform Chemical Information Database (*IUCLID*):** the IUCLID was developed jointly with the OCDE. It is the exclusive tool which allows the collection of data on chemicals, as well as the preparation of files on substances to be evaluated. This software, which makes it possible to store, exchange and pool data on substances or mixtures of substances, plays a key role for all organizations which have to comply with the data REACH regulation.

The AiiDA database

Creation method of the AiiDA database



The structure of the AiiDA database

The AiiDA database was designed so as to meet 4 criteria needs:

- **Identification of the substance**
- **Identification of the tested organism**
- **Identification of the conditions and the results of the tests**
- **Traceability and quality of the information**

The database includes a non exhaustive extension of **28 data fields** coming from these 4 criteria needs. They are classified as follows

Source Base	Base_Location	CAS	Chemical_Name	SMILES	Weight	Solubility	M_Type
Use	Endpoint	Water_Type	Kingdom	Species_Type	Phylum	Species_Group	Name
Latin_Name	Test_Duration	Duration_Units	Test_Type	Concentration	Concentration_Units		
Author	Title	Source	Publication_Year	Measurement	Comments		

Table 3.1: *Fields layout in the primary database*

Identification of the substance

It is essential to clearly identify the substance you wish to characterize; otherwise the calculation of the indicator may be wrong. There may be more than one synonym, several conformations, variable mixtures and purity levels for a same substance. Six parameters were selected so as to clearly identify the studied substance.

CAS: a numeric field with the Chemical Abstracts Service (*CAS*) database unique registration number of the substance. It is the unique identifying number of the molecule.

Chemical_Name: a text field with the name of the substance according to the rules of the Union Internationale de la Chimie Pure et Appliquée (*UICPA*).

SMILES: a text field with the SMILES formula of the molecule. The Simplified Molecular Input Line Entry Specification (SMILES) is a symbolic language which describes the structure of the molecule with short chain ASCII characters. The SMILES chains can be imported into most molecular structure editors so as to be converted into 2D representation or 3D model.

Weight: a numeric field with the molar mass of the molecule in [g/mol]. This parameter is useful when converting the concentration and the solubility values [mol/L] into [mg/L].

Solubility: a numeric field with the water solubility of the molecule. The solubility is the maximum concentration in [mg/L] of a dissolved or disassociated chemical compound within a given temperature. The obtained chemical solution is saturated.

Molecule_Type: a text field with the type of molecule: Pesticide, Organic or Inorganic. This field is used to apply the conversation ratios.

Use: text field with the field of application of the molecule (herbicide, insecticide, surfactant, cleaning agent, etc...).

Identification of the tested organism

Six parameters were selected so as to clearly identify the tested organism. These parameters come directly from the taxonomic position methodology.

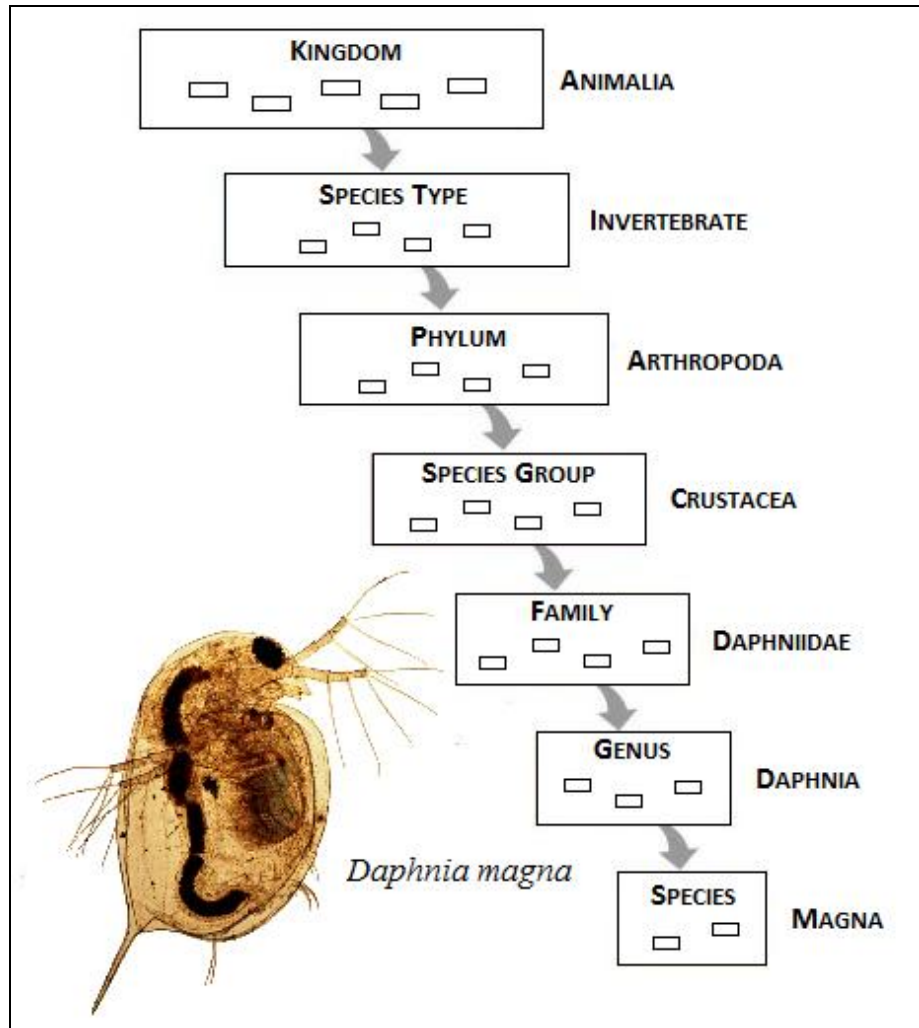


Figure 3.1: Example of taxonomic positioning of the *Daphnia magna* species

Kingdom: a text field with the species kingdom. The kingdom (or empire) in biology represents the highest and the most general taxonomic rank. It is split in several categories: the animal kingdom (*animalia*), le plant kingdom (*plantae*), the bacteria kingdom (*monera*), etc...

Species_Type: as text field with the species type. The type of species is a taxonomic rank specially created to meet the needs of the database. It allows classifying the tested species into 4 categories: **algae, plants, vertebrates and invertebrates**. This field is used to determine the type of test while making a distinction between acute and chronic ecotoxicity data.

Phylum: a text field with the species phylum. It is the second standard level of classification of living species.

Species_Group: a text field with the biological group of the tested species.

Name: a text field with the common name of the tested species.

Latin_Name: a text field with the scientific name of the species where the « *Genus* » et « *Species* » taxonomic ranks meet.

Identification of the tests conditions and results

Seven parameters were selected to describe the tests conditions and results. These parameters come directly from the ecotoxicological tests conducting methodology.

Endpoint: a text field with the endpoint, or the specific concentration of the ecotoxicological test (NOEC, LOEC, EC₅₀, etc...)

Measurement: a text field with the test's evaluation criteria (reproduction, growth, mortality, etc...)

Water_Type: a text field with the type of environment (media) in which the test takes place (two capital letters codes)

- **FW:** for *Fresh Water* Environment
- **SW:** for *Sea Water* Environment

Test_Duration: a numeric field with the time duration of the experiment.

Duration_Units: a text field with the time unit of the experiment (hours, days, weeks, years...)

Test_Type: a text field with the test category, **chronic**, **sub-chronic** or **acute**.

Concentration: a numeric field with the test's result, the average concentration of the substance responsible for the observed effect.

Concentration_Units: a text field with the measured concentration unit.

Thanks to these parameters, it is possible to accurately characterize each ecotoxicological test in the database. However, two important environmental assessment elements are missing: the traceability and the information quality control.

Traceability and quality of information

Seven parameters were selected to determine the quality of a value coming from an ecotoxicological test. These parameters are mainly linked to the source from which the information comes from.

Base_Source: a text field with the name of the original database from which the test is extracted. It ensures the information's traceability.

Base_Location: a text field with the three first letters of the original database and the unique identification number of the test (example: AQU-12345 or EAT-123). If the test identification number does not exist in the original database, it is automatically generated by numbering the tests of the database from 1 to N.

Author: a text field with the name of the person responsible for the publication of the data.

Title: a text field with the title of the publication from which the data comes from.

Source: a text field with information on the source of the data.

Publication_Year: a numeric field with the year of publication of the source.

Comments: text field with comments on the validity of the values.

Conclusion:

Dababase Name	Organisation	Update	Nb of Tests	Substances	Species	Access
Aquire	USEPA	Regular	330.000	4.800	3.000	Free
OPP	USEPA + OPP	2011	20.000	800	300	Free
USGS	CERC	1986	5.000	400	60	Free
EAT	ECETOC	2001	5.000	500	250	Private
eChemportal	ECHA + REACH	Regular	140.000	8.000	300	Free
Total tests to be covered			500.000			

Table 3.2: *Summary table of the main ecotoxicological databases*

Most of these 500.000 tests come from scientific literature. There are many redundancies, errors, not usable concentration values and end points, etc ... The goal of AiiDA is therefore to create a unique database which gathers all the reliable tests and to process the data in such a way that it can be used while still keeping a maximum of clarity and traceability.

To filter, sort and use these tests, AiiDA relies on the A.M.I. (Assessment of the Mean Impact) method principles which will be addressed in the following chapters.

The A.M.I. method

The A.M.I. method (Assessment of the Mean Impact): a method for assessing the impacts of toxic substances on the aquatic ecosystem [6].

Issue

The required features of an impact assessment method

So that it can be used within the Life Cycle Assessment framework, an ecotoxicological impacts assessment method must:

- Be compatible with the Life Cycle Inventory (LCI).
- Cover a maximum number of substances.
- Integrate the impacts through time and space, even if this information is not available in the life cycle inventory.
- Be compatible with other aquatic indicators such as acidification and eutrophication.
- Be traceable and have a confidence interval.

The A.M.I. method, which was developed during Dr. PAYET's thesis, is a method of assessment of the impacts of toxic substances on aquatic ecosystems. It was specially created to comply with the requirements of a comparative approach such as the life cycle assessment.

The principles of the A.M.I. method

EC₅₀ chronic as a reference ecotoxicological value

Because they come from statistical modeling or from linear regressions, EC₅₀ chronic are relatively stable and allow defining confidence intervals. EC₅₀ chronic are also the most frequently studied end points during ecotoxicological tests. They are therefore much better represented in terms of amount of available data in the various ecotoxicological databases. For these reasons, the A.M.I. method uses **EC₅₀ chronic** as the reference ecotoxicological values to assess ecotoxicological impacts.

Allowing the use of a maximum number of ecotoxicological values

So as to cover a maximum number of chemicals, the A.M.I. method is not restricted solely to the use of EC50 chronic. It attempts to integrate a maximum number of ecotoxicological values to evaluate the ecotoxicological impacts.

Creation of NOEC and LOEC extrapolation factors into EC₅₀ factors.

To maximize the amount of data which can be used, the A.M.I. method developed, using a statistical comparison between more than one hundred substances, factors which allow to convert NOEC and LOEC values into EC50 values. Thanks to these factors, the NOECs and the LOECs can be used during the assessment of ecotoxicological impacts.

Extrapolation	Ratio
EC ₅₀ /LOEC	2,1
EC ₅₀ acute/NOEC acute	3,3
EC ₅₀ chronic/NOEC chronic	4,8

Table 4.1: Best estimated factors for the extrapolation of NOEC and LOEC into EC₅₀ – coming from Jérôme PAYET’s « Assessing Toxic Impacts on aquatic ecosystem in LCA » thesis [6]

Creation of acute-chronic ratios (ACR)

Always with the same objective, the A.M.I. method developed, using a linear regression on a logarithmic scale on a hundred substances, ratios which allow the extrapolation of EC₅₀ acute values into EC₅₀ chronic values. Thanks to these ratios, the acute tests values can be used during the assessment of ecotoxicological impacts.

	ACR HC50 _{EC50}	ACR HC50 _{min}	ACR HC50 _{max}
R – Organic	1,9	4,2	0,8
R – Inorganic	2,8	7,4	1,1
R – Pesticides	2,2	6,1	0,8

Table 4.2: Best estimated ratios for the acute-chronic extrapolation – coming from Dr Jérôme PAYET’s « Assessing Toxic Impacts on aquatic ecosystem in LCA » thesis [6]

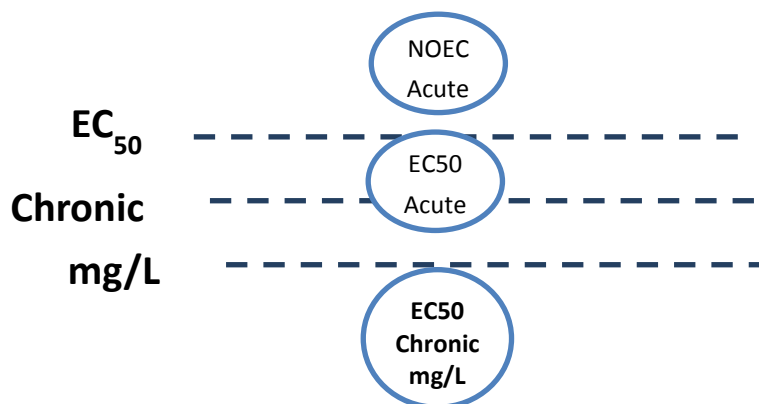


Figure 4.1: Standardization of data in EC₅₀ chronic

Data filtration

Because the calculation of indicators needs EC₅₀ chronic values in [mg/L] in the aquatic environment, a specific filter had to be created.

Selecting the tests which end points can be assimilated to an EC₅₀.

The A.M.I. method allows to extrapolate the NOECs and the LOECs into EC₅₀. All the tests which end points value cannot be assimilated to a NOEC, LOEC or a EC₅₀ will therefore be eliminated.

Can be assimilated as	EC ₅₀	NOEC	LOEC
Test which produces an effect	30 to 70% of the population	0 to 20% of the population	LETC* LOEL*

Table 4.3: Assimilation properties of a test to an EC₅₀, LOEC or NOEC
LETC: "Lethal Threshold Concentration" and LOEL: "Lowest-observable-effect-level"

The choice to consider the tests which have an effect on 0 to 20% of the population as NOEC, and not as of LOEC, is based on the fact that below 20%, the effect cannot be considered as representative compared to the variability of the control samples.

Filter of selection of the tests which can be assimilated as chronic

According to the A.M.I. method, it is possible to extrapolate acute tests values into chronic tests values. All the tests which cannot be identified as acute, chronic or sub-chronic and that do not give an indication on the experiment duration will therefore be eliminated.

Filter of selection of aquatic tests

All the tests which value (Water_Type field) does not correspond to "Sea Water" or "Fresh Water" will be eliminated for the reason that they cannot be identified as aquatic tests.

Filter of selection of the tests which concentrations cannot be converted into [mg/L]

All the tests which concentration value cannot be directly converted into [mg/L] will be eliminated.

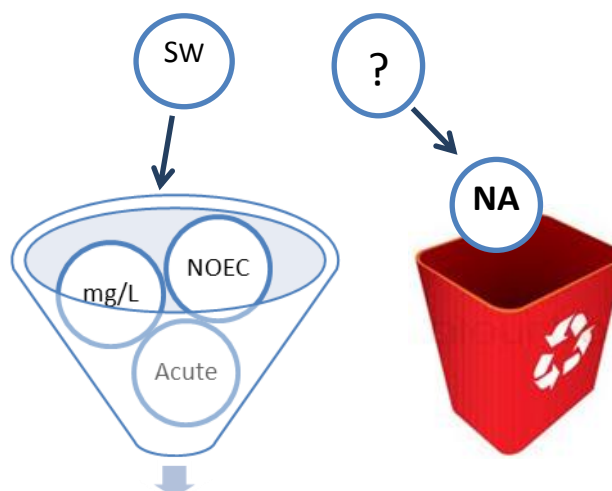


Figure 4.2: Filtering of the usable data

Duplicates removal: grouping identical tests

After a quick overview of the different sources databases, it seems that a lot of data are duplicated between the databases. In order not to bias the averaged EC_{50} by using several times the same value, it is necessary to erase the duplicates. As it is not possible to carry out this task manually, a script allowing to group the different data has been developed.

The principle of the script is to merge in one value all the tests for which there are duplicates in only one concentration value for the same molecule and tested species. Thus, duplicates are erased. For other fields that are not grouped together, the final value to use will be calculated using the decision tree in figure 4.1.

This decision tree has been created using a worst-case scenario. For tests identified as duplicates, we obtain the same concentration value for different endpoints, the used data will only be the one with the highest toxicity, EC_{50} . The “fresh water” data will also be used, because the toxicity indicator is based on the impact in freshwater and the longest experience time closed to real environments. Then, at the source level, the most recent publication will be used because of its highest reliability.

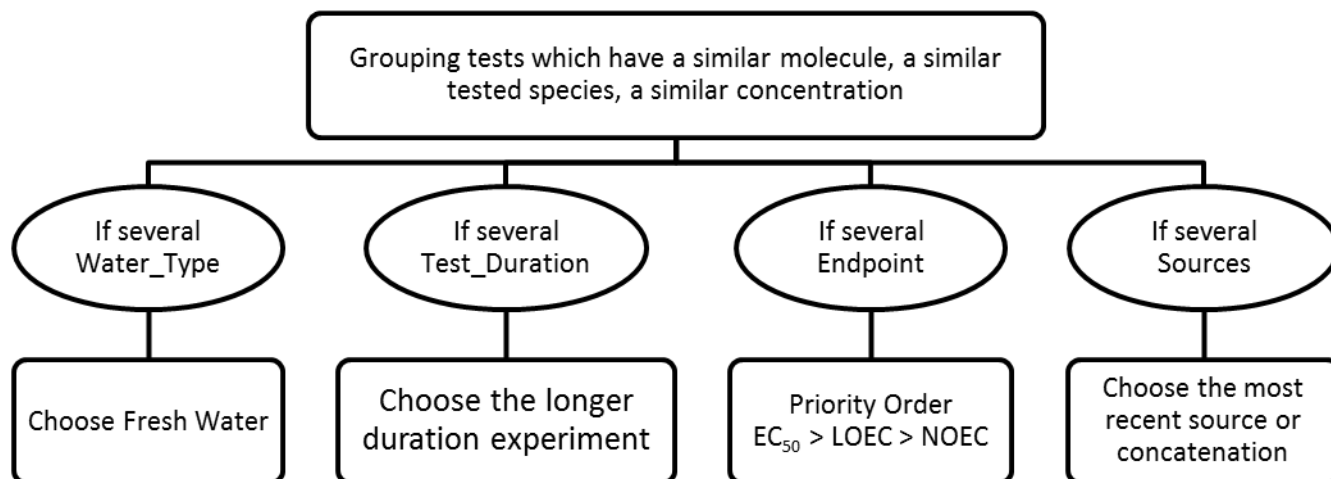


Figure 4.3: Decision tree while grouping identical tests

Data verification:

An automatic verification script is launched so as to avoid important errors. This script goes through the following information:

- **Check that the CAS number is valid:** a calculation algorithm allows to make sure that the CAS number is right.
- **Maximum concentration of 50 g/L:** if the concentration of a test exceeds 50 g/L, it is not taken into account. It is either a unit of measurement error or a non toxic molecule.

THE A.M.I. METHOD

- **Minimum concentration of 0,1 ng/L:** if the concentration of a test is below 0,1 ng/L, it is not taken into account. It is probably a unit of measurement error being that no dosage method can give such a precise result.
- **A pollutant's concentration must be inferior to its maximal water solubility:** the fact being that the dissolved concentration cannot exceed its water saturation level. The tests which do not fall into this category will have a comment in the "Comments" field.

Conclusion:

The A.M.I. method used by AiiDA to calculate the various aquatic toxicity indicators, is the most comprehensive and realistic method to evaluate the environmental impacts of aquatic ecosystems. Its main benefit is to make it possible to use almost all the available ecotoxicological data and therefore to maximize the coverage of chemicals. Unlike the other methods, it makes it possible to characterize the aquatic toxicity at the phylum level and not at the species level, making it even more realistic.

This method was developed within the IMPACT 2002+ framework and was retained during the creation of the USEtox model as a reference method for the calculation of the effect factor. It was also validated by the UNEP-SETAC.

HC₅₀

HC₅₀ indicator and uncertainty calculation method

HC₅₀ (Hazardous Concentration for 50% of species) calculation

As the distribution of the toxicity of a chemical within a multiple species ecosystem follows a log-normal law, AMI uses the principle of the HC₅₀ method. This method is based on the use of the SSD curves so as to calculate the HC₅₀ (average concentration affecting 50% of the species at a level superior to their EC₅₀) by working at three taxonomic levels.

Substance-Species level

To calculate an HC₅₀, AiiDA selects all the available tests in the database for a specific substance. It then brings them together according to the various species.

Prioritizing EC₅₀ chronic tests and extrapolations

For each species, AiiDA splits the tests into two groups: chronic versus acute. If chronic tests exist for a species, the acute tests are put aside. In the absence of chronic tests, the acute tests are selected and extrapolated into chronic tests.

Within the extrapolated acute or chronic group of data, AiiDA then groups the ecotoxicity tests according to their endpoints, EC₅₀, NOEC and LOEC. If EC₅₀ exist, then the NOEC and the LOEC tests are ignored, in the absence of EC₅₀ all the NOEC and LOEC are used and extrapolated into EC₅₀.

Finally, if several values of chronic EC₅₀ exist (extrapolated or not extrapolated) for a same species, the geometric mean of the tests is used.

$$\log(EC_{50s}) = \frac{1}{n} \sum_n \log(EC_{50})$$

EC_{50s} : Geometric mean of the EC₅₀ of different tests within the same species.

n : Number of tests per species

Substance-Phylum level

In order to improve its ecological realism, the AMI method recommends that the HC_{50} calculation is done at the phyla level. Indeed, some phyla are much more sensitive to some pollutants. They may be over-represented at the level of the number of tested species and therefore corrupt the ecological realism of the SSD distribution.

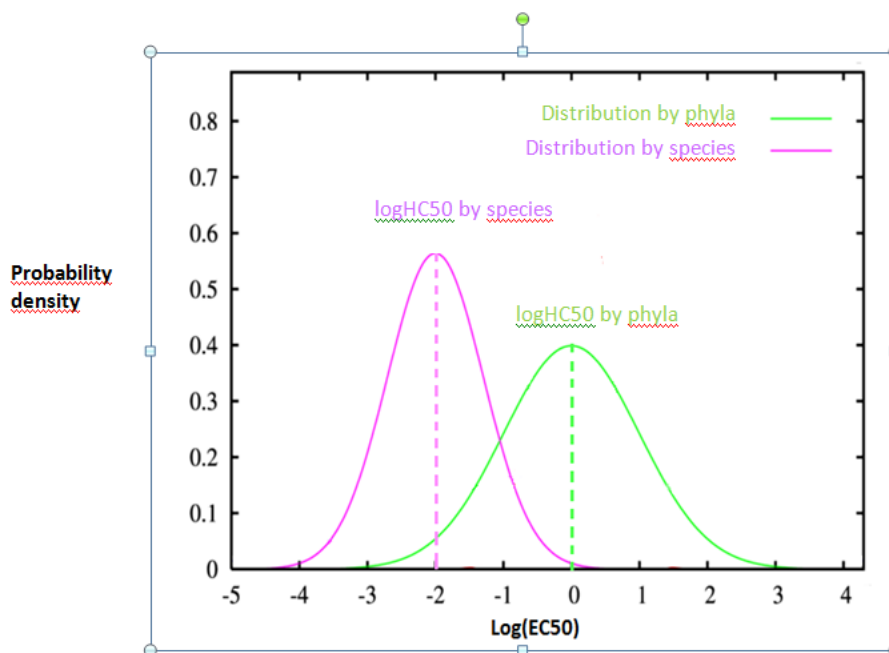


Figure 5.1: Example of the difference between an HC_{50} at the species and phyla level when a small number of phyla sensitive to chemicals but over-represented exist at the species level. $HC_{50\text{ species}} < HC_{50\text{ phyla}}$

When several values of EC_{50} exist for a same phyla, the geometric mean of the EC_{50} is selected.

$$\log(EC_{50p}) = \frac{1}{n} \sum_n \log(EC_{50s})$$

With, for each substance:

EC_{50p} : geometric mean of EC_{50s} of the different species within a same phyla

EC_{50s} : geometric mean of EC_{50s} of the different tests within a same species

n : number of species per phyla

Substance-Impact level

When at least 3 values of EC_{50p} exist for a same substance, the geometric mean of EC_{50p} is used for the calculation of the HC_{50} . In the absence of data on 3 phyla, AiiDA uses the QSARs models in order to determine the values of EC_{50p} missing.

$$\log(HC_{50}) = \frac{1}{n_p} \sum_{n_p} \log(HC_{50p})$$

HC_{50} : geometric mean of EC_{50p} of the different phyla

HC_{50p} : geometric mean of EC_{50s} of the different species within a same phylum

n_p : number of different phyla and $np \geq 3$ in order to have a result.

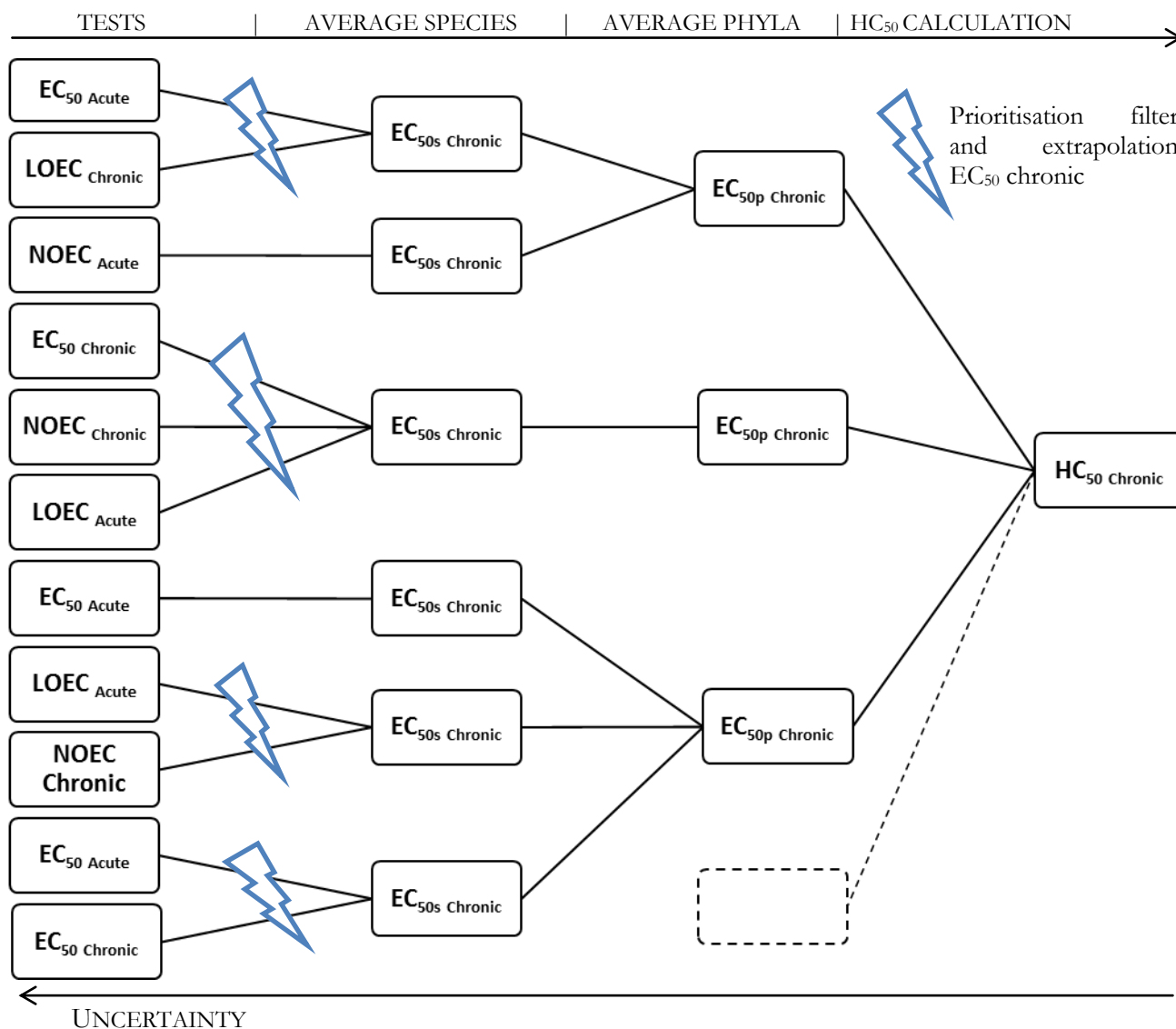


Figure 5.2: Principle of the AMI method for the calculation of HC_{50}

Use of QSARs

The QSARs methods

A Quantitative Structure-Activity Relationship (or QSAR) is a process where a chemical is correlated to a specific effect. This method is used in order to predict the toxicity of a substance by comparing the similarity of its chemical structure to other substances which toxicity has already been measured.

The QSARs have been used since 1981 by the US EPA in order to predict the aquatic toxicity of new industrial chemicals in the context of the absence of toxicity data. These models are generally used in order to develop priority lists for ecotoxicological tests by identifying the substances with the highest risk for the environment. The role of QSAR has clearly been defined in the technical support published by the European directive 93/67/EEC. This document provides in the general context of risk assessment, the situations in which QSAR can be used. The use of QSAR is recommended for:

- The evaluation of ecotoxicological data
- Contribution to the decision-making process (new tests, priority substances)
- The identification of the effects that can be the source of potential preoccupation and for which ecotoxicological tests data is not available

However, the use of QSAR, in the context of the absence of data, may cause important variabilities compared to the reality, it is thus recommended that you use caution when calculating the HC50.

QSAR models operating mode

The main statistical tools used in the QSAR models are methods of linear and multilinear regression. These methods link the octanol/water partition coefficient of the substance to its toxicity on a logarithmic scale and for each chemical class. You then just need to enter the CAS or the SMILE formula of a substance so that its chemical class(es) is automatically identified and its log K_{ow} is estimated. According to the chemical classes used, one or several QSAR methods are applied allowing the prediction of acute and chronic EC_{50} in [mg/l] on different species.

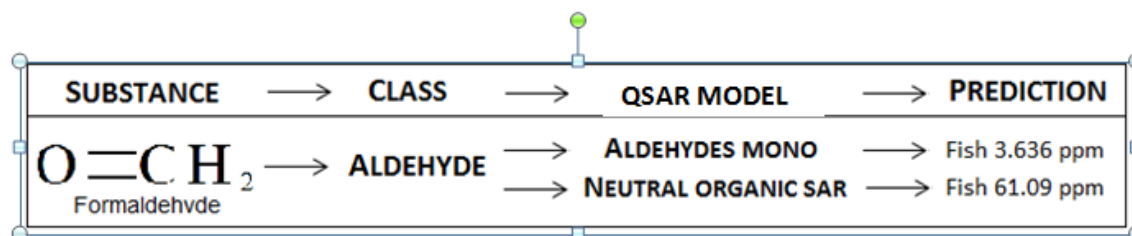


Figure 5.3: Operating principle of the ECOSAR software for the prediction of toxicity values

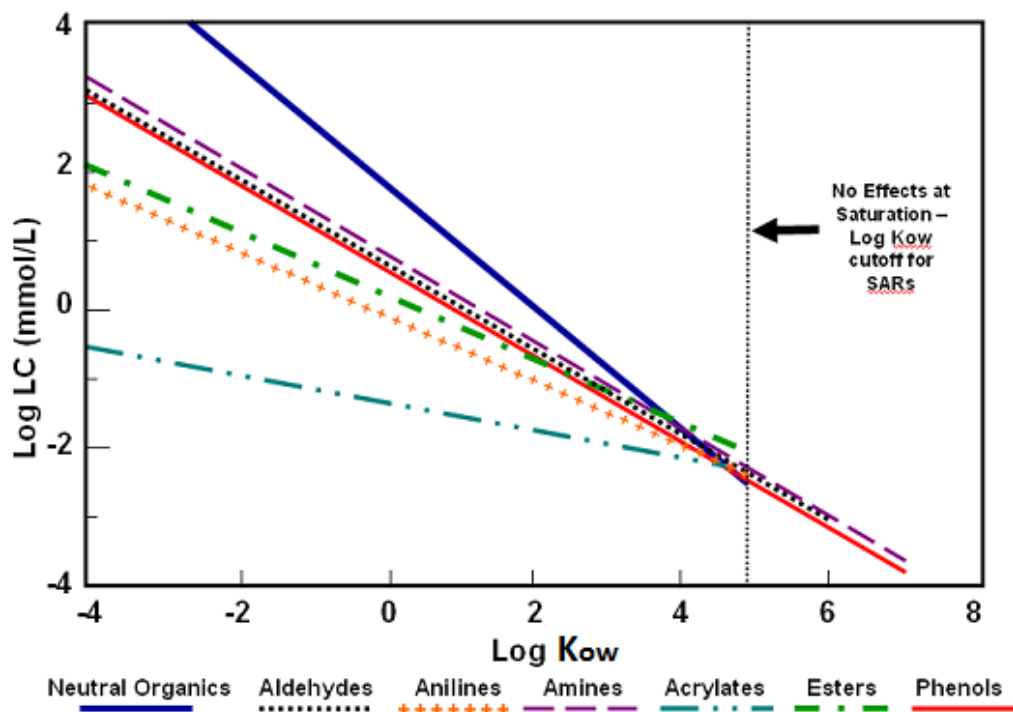


Figure 5.4: Linear regressions of the aquatic toxicity on fish with several chemical classes according to the octanol/water coefficient

Rules and limitations of use of QSAR in AiIDA

The QSARs cannot be used for all chemicals. The application domain **mainly covers only organics**. Inorganics, organometallics, polymers and the substances which have a molar mass superior to 1000g/mol are not represented.

Each QSAR model **has a domain of validity** according to the $\log K_{ow}$. The values, which come from predictions, are reliable only if the $\log K_{ow}$ of the substance lies within the validity domain. Outside of the validity domain, predictions may be totally wrong, they are therefore not taken into account in AiIDA. AiIDA uses the “CHV” models which make it possible to predict the chronic aquatic toxicity on various species. For these models, the validity domain is: $-3 < \log K_{ow} < 8$

When AiIDA identifies several chemical classes for a same substance, no rules allowing the identification of the best QSAR model to use exist to this day. In this case, the “Worst Case” principle is then applied and **the value with the highest toxicity (lowest concentration) is then selected**.

Moreover, it is also important to keep in mind that QSAR estimations tend to reduce the data variability and therefore tend to under-estimate the uncertainties during the calculation of the HC₅₀.

Uncertainty calculation

In Aide, a confidence interval is associated to the HC₅₀. Indeed, during a comparative evaluation using a life cycle assessment approach, it is crucial to identify if chemicals are different in terms of toxicity. This is the reason why the AMI method proposes to automatically calculate the confidence interval at 95% by using the Student table. [6]

$$\text{Log}(HC_{50}) \pm \frac{1}{\sqrt{n}} \times t_{n-1}^{0.05} \times SDev(\text{Log}((EC_{50p})))$$

With, for each substance:

HC_{50p}: geometric mean of the EC_{50s} of the different species within a same phylum

EC_{50p}: geometric mean of the EC_{50s} of the different tests within a same species

n: number of tested phyla

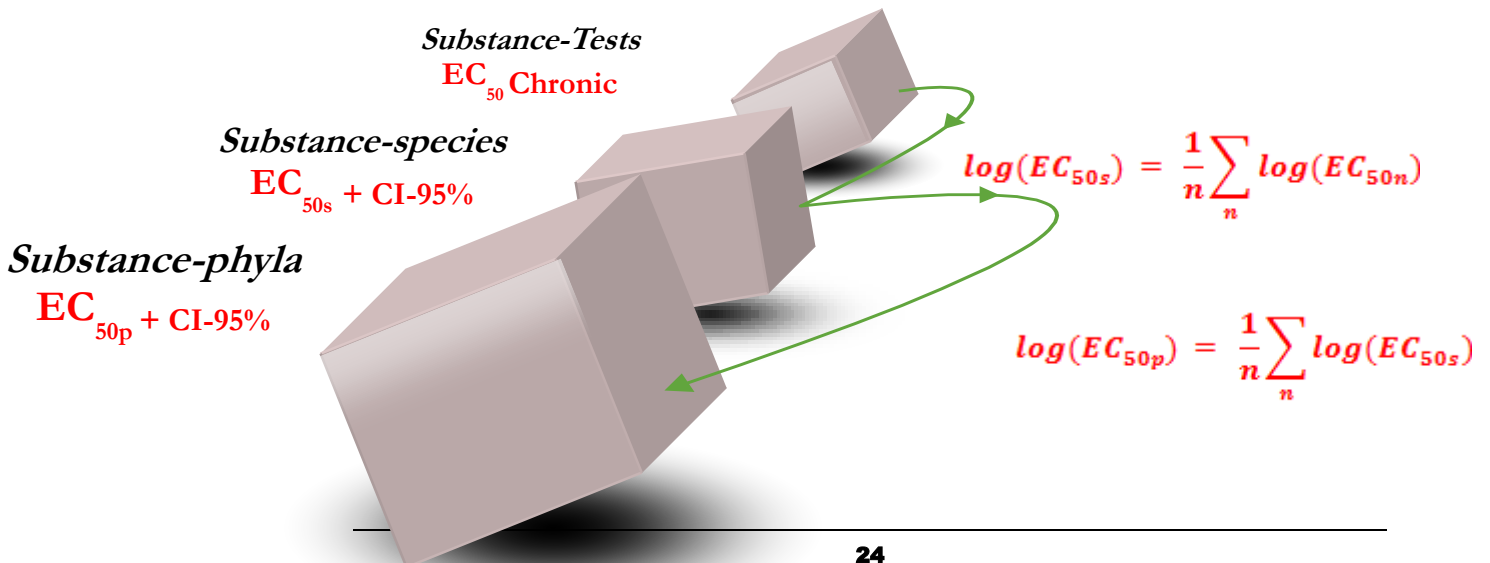
t_{n-1}^{0.05}: Student table value for a confidence interval of 95% with n-1 freedom degree

SDev: standard deviation of the logs of the various EC_{50p}

The confidence interval is directly correlated to the number of phyla considered in the calculation. The confidence interval for a chemical can therefore be reduced by the inclusion of data on new species and new phyla.

In AiiDA, these uncertainties are proposed for each calculation level of the HC₅₀:

- Confidence interval at 95% for EC_{50p} at the species level
- Confidence interval at 95% for EC_{50s} at the phyla level
- Confidence interval at 95% for HC₅₀ at the ecological impact level.



HC₅

Calculation method of the HC₅ and its uncertainty

Calculation of HC₅ (Hazardous Concentration for 5% of species)

The HC₅ are used in order to provide environmental quality objectives (reference value in the Netherlands regulation). Indeed, if the concentration of a compound in the environment is inferior to its HC₅, then 95% of the biological species will not be affected by its presence. Both concepts, Hazardous Concentration and Potentially Affected Fraction (PAF), are of fundamental importance for the probabilistic ecotoxicological risk assessment.

Method based on the Aldenberg review (2010)

In the probabilistic and statistical theory, the normal law is one of the most adapted laws to model natural phenomenon of random events. Let's suppose that the sensibility of the different biological species to a toxic substance, expressed in log(EC₅₀), follows a distribution with parameters of the normal law: μ and σ .

Then, log (HC_p) for p% of the species population can therefore be calculated according to the following formula:

$$\text{Log}(\text{HC}_p) = \mu - k_p \cdot \sigma$$

With:

HC_p: Hazardous Concentration for p% of the species population

k_p: fractile of p% of the standard normal law

p: percentage of the species population

μ: mean of the distribution

σ: standard deviation of the distribution

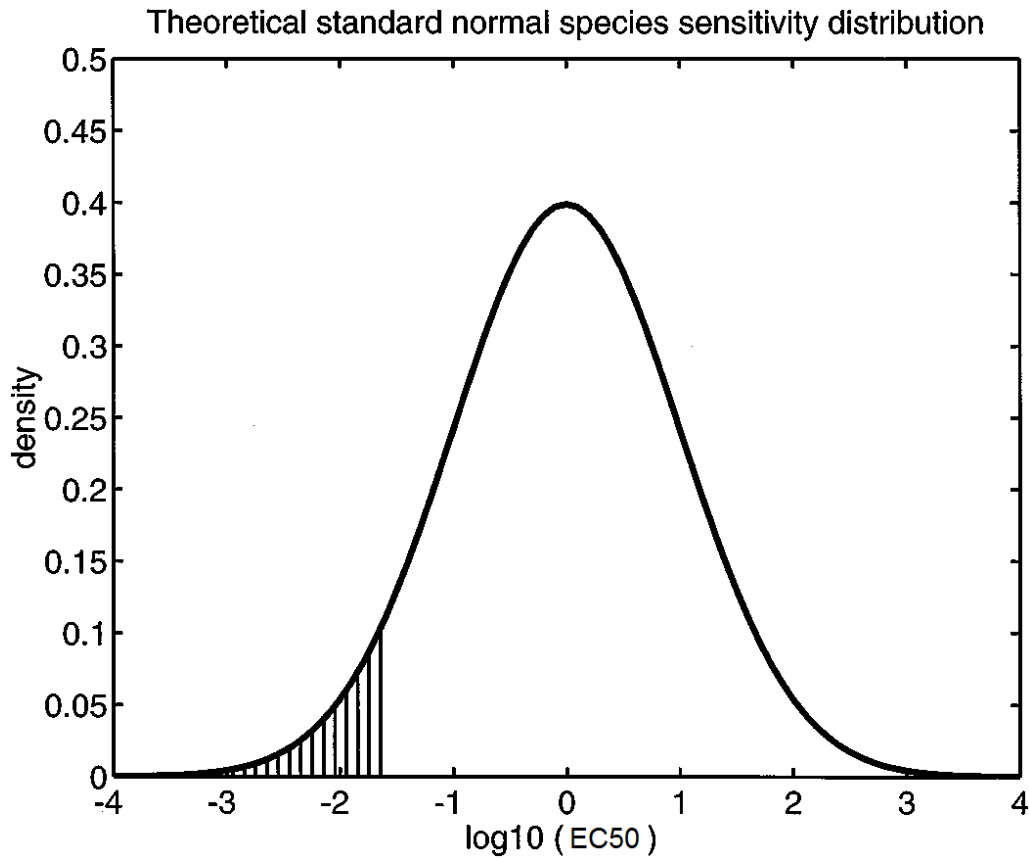


Figure 6.1: Illustration of a theoretical distribution of species sensitivity (SSD) according to a normal distribution

SSD (Species Sensitivity Distribution) extrapolation factors [11]

According to the Aldenberg review, it is possible, for a given toxic substance, to calculate the value of HC₅ and its confidence interval for the partial knowledge of the species sensitivity, for example, by the intermediate of a small sample of ecotoxicological tests.

For this, it is necessary to calculate the mean (μ) and the standard deviation (σ) of the full set of ecotoxicity data grouped by species after logarithmic transformation. You then just need to apply one or several extrapolation factors according to the following formula:

$$\mathbf{Log(HC_5)} = \mu - k_{s/Median}(n) \cdot \sigma$$

With:

HC₅: Hazardous concentration affecting 5% of the species population

k_s: Aldenberg extrapolation factor (see table 6.1) that directly depends on the number of studied species

n: number of species represented in the whole toxicity dataset

Aldenberg and Slob (1993) provide a distribution of extrapolation factors for the estimation of log (HC₅) based on the hypothesis of a Species Sensitivity Distribution according to the log-normal distribution. [12]

Sample size	FA = 1%			FA = 5%		
	Lower	Median	Upper	Lower	Median	Upper
2	37.0936	3.3760	0.9538	26.2597	2.3387	0.4748
3	10.5527	2.7645	1.1297	7.6559	1.9384	0.6391
4	7.0424	2.6008	1.2462	5.1439	1.8295	0.7433
5	5.7411	2.5258	1.3309	4.2027	1.7793	0.8178
6	5.0620	2.4828	1.3964	3.7077	1.7505	0.8748
7	4.6417	2.4551	1.4492	3.3995	1.7318	0.9204
8	4.3539	2.4357	1.4931	3.1873	1.7187	0.9580
9	4.1430	2.4213	1.5303	3.0312	1.7091	0.9899
10	3.9811	2.4103	1.5625	2.9110	1.7016	1.0173
11	3.8523	2.4016	1.5908	2.8150	1.6957	1.0413
12	3.7471	2.3945	1.6158	2.7363	1.6910	1.0625
13	3.6592	2.3886	1.6382	2.6705	1.6870	1.0814
14	3.5845	2.3837	1.6585	2.6144	1.6837	1.0985
15	3.5201	2.3795	1.6769	2.5660	1.6808	1.1140
20	3.2952	2.3652	1.7492	2.3960	1.6712	1.1746
30	3.0639	2.3516	1.8397	2.2198	1.6620	1.2498
50	2.8624	2.3412	1.9362	2.0650	1.6549	1.3294
100	2.6840	2.3337	2.0401	1.9265	1.6498	1.4143
200	2.5697	2.3300	2.1182	1.8372	1.6473	1.4778
500	2.4750	2.3280	2.1910	1.7630	1.6458	1.5367
Inf	2.3263	2.3263	2.3263	1.6449	1.6449	1.6449

Table 6.1: Extrapolation factors, for log HC_{FA} with three levels of confidence interval (95, 50, and 5%) according to the toxicity data sample's size [12]

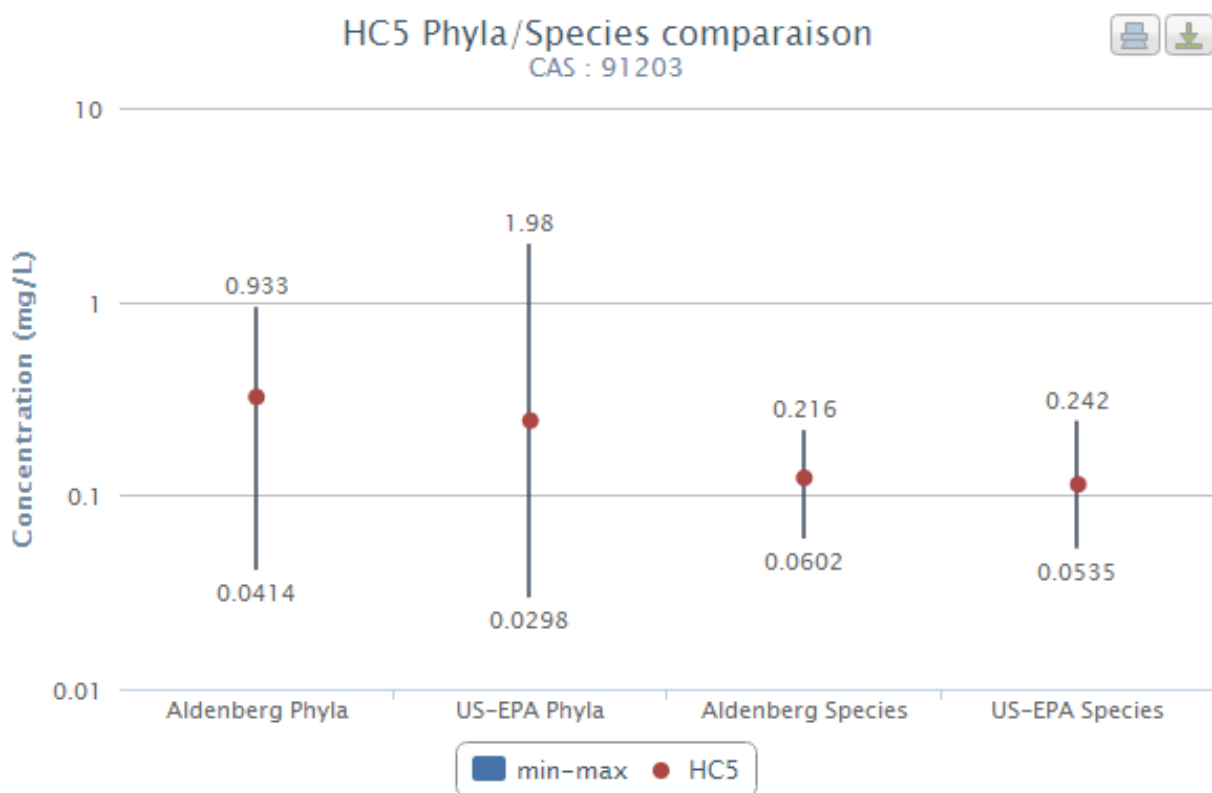
The Lower and Upper factors allow to define the confidence interval at 95%:

$$\text{Log}(HC_{5min}) = \mu - k_{s/Lower}(n) \cdot \sigma \qquad \text{Log}(HC_{5max}) = \mu - k_{s/Upper}(n) \cdot \sigma$$

PSD (Phyla Sensitivity Distribution) extrapolation factors

AiiDA allows the automatic calculation of the HC₅ and its confidence interval (as seen previously) according to the species as well as according to the phyla. The principle is exactly the same, with the main difference being that the toxicity data are averaged per species, then per phyla before being processed with the Aldenberg method [11]. It is possible to see that HC₅ Phyla, even if they are ecologically realistic, sometimes have important confidence intervals due to the low number of phyla represented compared to the species.

Method	Phyla	Species	Min _{95%}	HC ₅	Max _{95%}	Units
Aldenberg Phyla	8		0.0414	0.322	0.933	mg/L
US-EPA Phylum	8		0.0298	0.243	1.98	mg/L
Aldenberg Species		58	0.0602	0.123	0.216	mg/L
US-EPA Species		58	0.0535	0.114	0.242	mg/L



Source AiiDa v1.0 © Tools4env

Figure 6.2: Difference between HC₅ phyla and HC₅ species in AiiDa for Naphthalene

US-EPA methodology

The method recommended by the US-EPA [13],[14], [15], when the number of ecotoxicity data is sufficient for a statistical exploitation (EC, 2003), is also the SSD method (Species Sensitivity Distribution, Posthuma and al, 2002). As for the Aldenberg method, it consists in describing the variation in species sensitivity considered as representative of a population or an ecosystem, with its cumulative curve frequency according to the exposure conditions. This curve is obtained with the adjustment of a statistical law (log-probit) to the statistical distribution of a given effect criterion: the chronic EC₅₀ in AiiDA. The steps for the creation of the SSD and PSD curves, according to the US-EPA method in Aide, are as follows:

Linear Regression of Probit = f (Log EC_{50s}) to obtain the central tendency

1. Calculation of the means of the log (EC₅₀) for each species/phyla (axis X). Then, classification by sensitivity increasing order so as to obtain a sensitivity rank for each species/phylum between 1 and n (n being the total number of species and phyla)

$$\log(EC_{50s}) = \frac{1}{n_t} \sum_{n_t} \log(EC_{50})$$

EC_{50s}: geometric mean of EC₅₀ for different tests within a same species/phylum

n_t: number of tests per species/phylum

2. Conversion of the sensitivity rank into species/phyla affected proportion

$$Prop = \frac{rang - 0,5}{n}$$

Prop: affected Species/Phyla proportion

n: total number of species/phyla

3. Transformation of the proportions in Probit. The Probit function is defined as the reciprocal of the distribution function of the Standard Normal Law with a mean of 5 and a standard deviation of 1. A mean of 5 was chosen so as to ensure that all the Probit values are positive and different from 0.

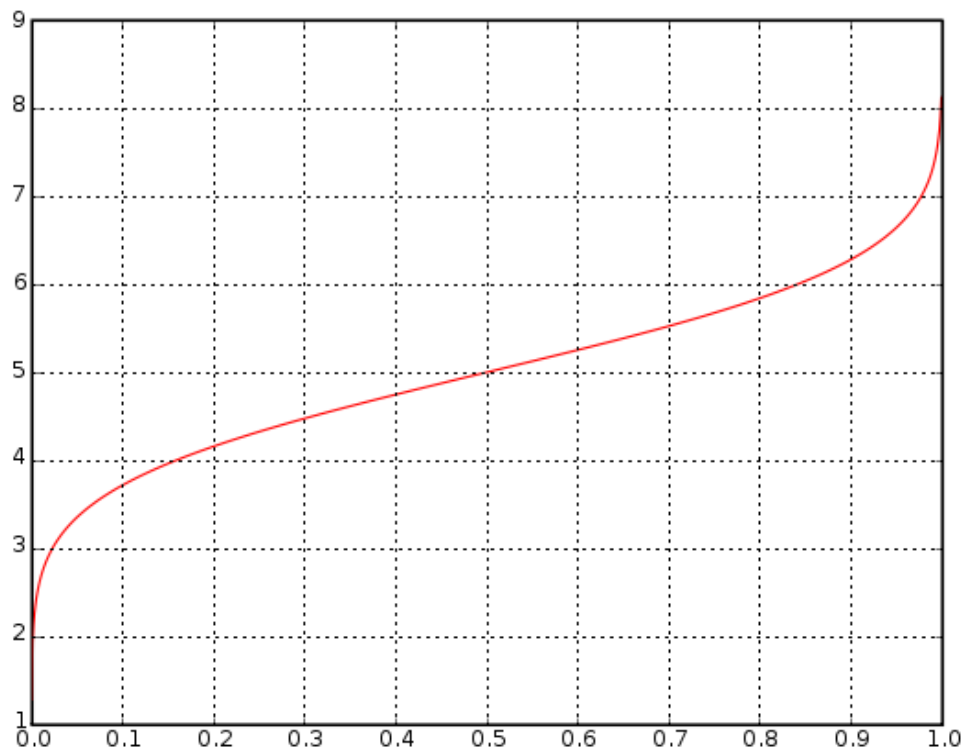


Figure 6.3: Probit function with a mean of 5 and a standard deviation of 1

- Obtaining the distribution of Probits according to $\log(\text{EC}_{50s})$ (see figure 6.4). AiiDA calculates the **slope** and the **origin ordinate** of the linear regression so as to define the central tendency of this distribution.

Phyla	Daphnia	Mollusca	Algae	Chordata
EC _{50, mean mg/L}	0,0034	0,0098	0,012	0,054
log(EC50s)	-2,468	-2,008	-1,920	-1,26
Rang	1	2	3	4
Proportion	0,125	0,375	0,625	0,875
Probit	3,849	4,681	5,318	6,150

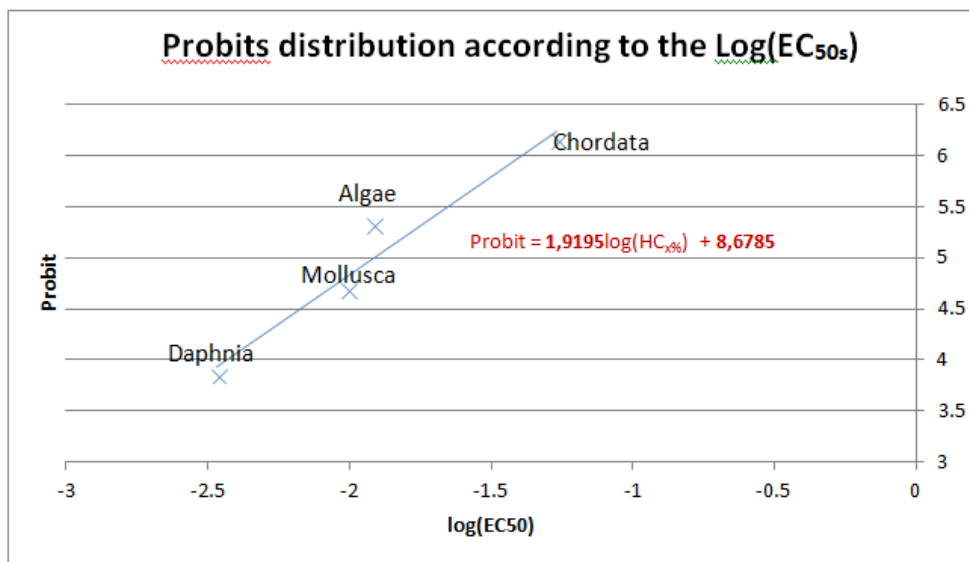


Figure 6.4: Example of the Probit curve for 4 phyla

- The central tendency allows to produce the SSD/PSD curve and to calculate the HC₅ with the help of the linear regression formula.

$$\text{Log}(\text{HC}_{x\%}) = \frac{\text{Probit}(x\%) - \text{Origin ordinate}}{\text{Slope}}$$

$$\text{Log}(\text{HC}_5) = \frac{\text{Probit}(5\%) - \text{Origin ordinate}}{\text{Slope}}$$

Log(HC_{x%}): Central tendency of the concentration affecting x% of the species/phyla

Probit(x%): Probit number for x%

Origin ordinate: origin ordinate of the regression line (Probit, $\log EC_{50s}$)

Slope: coefficient of the regression line (Probit, $\log EC_{50s}$)

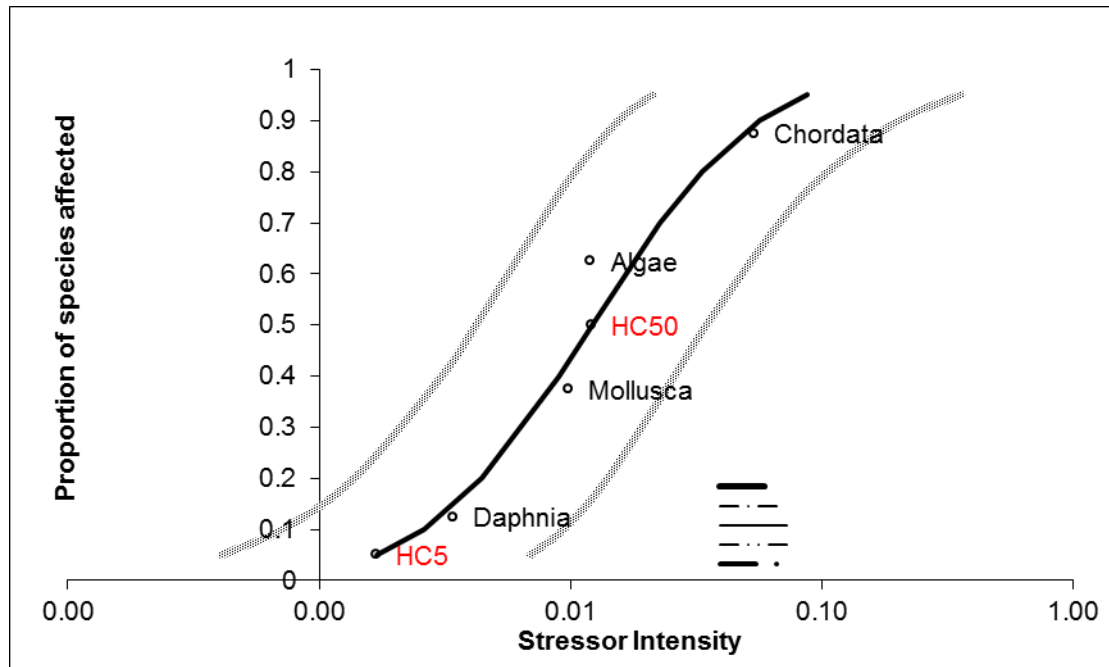


Figure 6.5: SSD curve for 4 phyla with HC_5 and HC_{50}

Calculation of the confidence interval (Neter and al. 1990) [14]

6. Calculation of the MSE: the average quadratic error is quite useful when comparing several estimators, notably when one of them is biased. For each species/phylum, it is necessary to subtract the observed Probit value from the central tendency Probit value. Increase this difference to the square, sum these values for all species/phyla, then divide by $n-2$ (n being the total number of species and phyla).

$$MSE = \frac{\sum(\text{Probit} - \text{Probit}_{TC})^2}{n - 2}$$

$$\text{Probit}_{TC} = \text{Slope} \times \log(EC_{50s}) + \text{origin ordinate}$$

With

Probit_{TC}: Probit value estimated thanks to the central tendency

7. Calculation of the sum of the square (CSSQ): for each species/phyla, calculate the square of each log-concentration, then add up to obtain the sum of the squares. Then calculate the sum of the log-concentration, calculate the squares and divide by the total number of species/phyla. Subtract the two terms in order to obtain the sum of the corrected squares.

$$CSSQ = \sum \log(EC_{50s})^2 - \frac{(\sum \log(EC_{50s}))^2}{n}$$

8. Calculate the general mean: Mean of all the log values (exposure)

$$Mean = \frac{1}{n} \sum \log(EC_{50s})$$

EC_{50s}: geometric mean of EC₅₀ of the different tests within a same species

n: total number of phyla/species

9. Calculation of the relative error at each point (SSQ)

$$SSQ = \frac{MSE}{Slope^2} \left(1 + \frac{1}{n} + \frac{(\log(HC_{x\%}) - Mean)^2}{CSSQ} \right)$$

log(HC_{x%}): central tendency of the concentration affecting x% of species/phyla

10. Calculation of the prediction interval (PI) thanks to the t critical value from the Student table

$$PI = \log(HC_{x\%}) \pm t_{n-2}^{0.05} \times \sqrt{SSQ}$$

With:

t_{n-2}^{0.05}: Student table value for a confidence interval of 95% with an n-2 degree of freedom

n: number of tested phyla/species

Phyla	Daphnia	Mollusca	Algae	Chordata
EC _{50mean} mg/L	0,0034	0,0098	0,012	0,054
log(EC _{50s})	-2,469	-2,009	-1,921	-1,268
Rank	1	2	3	4
Proportion	0,125	0,375	0,625	0,875
Probit	3,849	4,681	5,318	6,15
			Slope	1,919
			Origin ordinate	8,678
Probit _{TC}	3,941	4,824	4,993	6,26
			MSE	0,074
			CSSQ	0,735
		t	2,92	Mean
Log(HC _{x%})	-2,516	-2,083	-1,751	-1,317
SSQ	0,0350	0,0259	0,0259	0,0349
PI Up	-1,970	-1,613	-1,281	-0,772
PI Down	-3,062	-2,553	-2,221	-1,863

Tableau 6.2: Example of calculation of the prediction interval for 4 phyla

PNEC

PNEC calculation method and its security factor

Calculation of the PNEC (Predicted No Effect Concentration)

The PNEC (*Predicted No Effect Concentration*) is based on the hypothesis that the ecosystem's sensitivity depends on the most sensitive species. It therefore allows to determine the highest concentration with no risk for the environment by studying the most sensitive species to the pollutant.

To determine the PNEC from the results of laboratory tests, AiiDA uses the Risk Assessment methodology which is described in the **Technical Guidance Document (TGD, 2003)** [16]

$$PNEC = [Concentration]_{Most\ sensitive} \times AF$$

With:

[Concentration]_{Most sensitive} : concentration value coming from the most sensitive test

AF: Security Factor which depends on the number and the quality of the available tests

Identification of the test and of the most sensitive species

AiiDA provides all the available ecotoxicity tests for a substance and selects as a priority the results of the chronic toxicity tests: indeed, these tests assess the long term effects of the substance on living organisms and therefore have a better ecological realism. In the absence of chronic tests, AiiDA selects the whole of the available acute tests.

Within the selected tests (NOEC, LOEC, EC₅₀) AiiDA identifies the most sensitive test, i.e. the test with the lowest effective concentration for an observed effect (with the species and phyla for this test).

Estimation of the security factor AF (Assessment Factor)

The PNEC is obtained by applying a security factor to the values from the tests carried out on organisms. This security factor takes into account the uncertainty degree that results from the extrapolation to a real environment from laboratory tests on a limited number of species. Consequently, the degree of uncertainty and the security factor are reduced when there are lots and long ecotoxicity tests.

Available data set	Security Factor
1 acute test	10000
2 acute tests on at least 3 phyla	5000
3 acute tests on at least 3 different phyla	1000
1 chronic test	100
2 chronic tests on at least 2 phyla	50
3 chronic tests on at least 3 phyla	10

Tableau 7.1: Security Factors (Aquatic PNEC) [16]

You will find in table 7.1 the security factor proposed by the technical guidance published by the European Commission for aquatic freshwater organisms. These factors are applied to the lowest ecotoxicity data obtained in the laboratory tests.

PNEC and Assessment Factor

PNEC : (mg/L)	AF	Most Sensitive Species	Phylum	Acute			Chronic		
				Tests	Species	Phyla	Tests	Species	Phyla
5.1E-5	10	Stephanodiscus hantzschii	Bacillariophyta	1694	202	16	174	41	12

Data Source PNEC

Base	Endpoint	Water Type	Test Duration	Conc. (mg/L)	Source	Author
Aquire_5	EC50	FW	96 h	0.00051	Rep.No.CL81/100, Natl.Inst.Public Health Environ.Hyg.:107 p.	Adema,D.M.M., J.H. Canton, W. Slooff, and A.O. Hanstveit

Figure 7.1: AiiDA interface for the PNEC indicator

BIBLIOGRAPHIC RESOURCES

- [1] **[Magazine]** Le journal de la délégation Centre-Auvergne-Limousin du CNRS - Hors-série > Microscop / Numéro 12, octobre 2003, <http://www.univ-orleans.fr/icoa/presentation/modelisation.pdf>
- [2] **[Publication]** Musu T. (2004), REACH au travail - Les bénéfices potentiels de la nouvelle politique européenne sur les agents chimiques pour les travailleurs
http://www.unep.org/labour_environment/TUAssembly/ref_docs/REACH-FR.pdf
- [3] **[Rapport]** Harpet Cyrille, Séminaire CNRS (2009), Chimie et DD, prof. INSA Lyon
http://www.cnrs.fr/inee/recherche/fichiers/ANGDChimieCargese/HarpetCargese10_09.pdf
- [4] **[Livre]** Valéry E. Forbes, Thomas L. (1997) Écotoxicologie: théorie et applications - Editions Quae
http://books.google.fr/books/about/%C3%89cotoxicologie.html?hl=fr&id=FGbpWYH2LDcC&redir_esc=y
- [5] **[Site internet]** Définition du dictionnaire environnement et développement durable : Test Ecotoxicologique
http://www.dictionnaire-environnement.com/test_ecotoxicologique_ID2365.html
- [6] **[Thèse]** PAYET Jérôme (2004), « *Assessing toxic impacts on aquatic ecosystem in Life cycle assessment* » - Ecole polytechnique fédérale de Lausanne
- [7] **[Site internet]** Site officiel de l'EPA : Téléchargement de AQUIRE
<http://cfpub.epa.gov/ecotox/>
- [8] **[Site internet]** Site Officiel de OPP : Pesticide Ecotoxicity Database
<http://www.ipmcenters.org/Ecotox/index.cfm>
- [9] **[Site internet]** Site Officiel de l'USGS CERC : Acute Ecotoxicity Database
<http://www.cerc.usgs.gov/data/acute/acute.html>
- [10] **[Site internet]** Site Officiel eChemPortal : moteur de recherche de données de toxicité
<http://www.echemportal.org/>
- [11] **[Publication]** Aldenberg, T., Jaworska, J. S., 2000. *Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicology and Environmental Safety.* 46, 1-18.
- [12] **[Publication]** Aldenberg, T., Slob, W., 1993. *Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicology and Environmental Safety.* 25, 48-63.
- [13] **[Site internet]** U.S. Environmental Protection Agency (EPA). 2005. Methods/indicators for determining when metals are the cause of biological impairments of rivers and streams: species sensitivity distributions and chronic exposure-response relationships from laboratory data. Cincinnati, Ohio, U.S. EPA, Office of Research and Development, National Center for Environmental Assessment.
http://www.epa.gov/caddis/da_software_ssdmacro.html
http://www.epa.gov/caddis/downloads/SSD_Generator_V1.xlt

BIBLIOGRAPHIE

[14] **[Publication]** Neter, J., W. Wasserman and M.H. Kutner. 1990. *Applied Linear Statistical Models, 3rd ed.* Irwin, Boston, MA. 1184 pp.

[15] **[Publication]** Posthuma, L., G.W. Suter II, and TP Traas. 2002. *Species Sensitivity Distributions in Ecotoxicology.* Lewis Publishers, Boca Raton, FL. 587 pp.

[16] **[Guide]** Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances; Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances; Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market
http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/doc/tgd