ASSESSING TOXIC IMPACTS ON AQUATIC ECOSYSTEMS IN LIFE CYCLE ASSESSMENT (LCA)

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Abstract

In the last decade, several Life Cycle Assessment (LCA) methods for assessing impact of products on living resources have been developed. Beyond the quantified assessments of impacts on living systems, it also checked the feasibility of the impact assessment on human health and ecosystems quality and helps to identify the limits of such methods. Among the different impact categories, that of toxic substances on ecosystems occupies an important place. The extent of these impacts has been stressed on many occasions and the necessity of preserving ecological areas and biodiversity has become a major issue on an international level.
By focusing on aquatic ecosystems, this thesis aims at identifying constraints connected with assessment of the impact of chemical substances on ecosystems in LCA and setting up a method for assessing impacts of toxic substances on aquatic ecosystems which meets the requirements of a comparative approach like Life Cycle Assessment. The overall purpose of the thesis is to propose a comparative method for the Life Cycle Impact Assessment of toxics on aquatic ecosystems. With that aim, the dissertation is going throughout 6 major issues:
1- The feasibility of the comparative impact assessment on ecosystems and the identification of associated constraints.
2- The development of a statistical method for comparing impact on ecosystems;
3- The review of the data availability for calculation of Effect Factors.
4- The choice of the most relevant ecotoxicity measure (ECxs[1], NOECs[2] and LOEC[3]s) for a comparative purpose.
5- The development of best-estimate extrapolation factors for assessing chronic effects based on acute data.
6- The analysis of the ecological realism of the comparative assessment method.
These points are analysed throughout the 7 chapters of the thesis.

Chapter 1 aims at introducing the thesis. A general presentation of Life Cycle Assessment is proposed, following by a detailed description of the Life Cycle Impact Assessment on ecosystems. This description covers the

[1] ECx: concentration of susbtance that affects 50% of the individuals tested for a given effect.
[2] NOEC: No Observable Effect Concentration
[3] LOEC: Lowest Observable Effect Concentration

state of the art of researches and identifies the development needed. Therefore the scope of the thesis and the main points that must be addressed by this research are presented.

Chapter 2 starts with a review of existing methods for Life Cycle Impact Assessment on ecosystems (LCIA), the chapter presents the parametric version of the AMI method (Assessment of the Mean Impact), which has been developed during the PhD for the assessment of impact on aquatic ecosystems. For this purpose, a framework and the main requirements for the development of this method are presented. For a comparative assessment, the Hazardous Concentration of a toxic affecting 50% of the species over their chronic EC50 (Effect Concentration affecting 50% of tested individuals), also called $HC50_{EC50}$, is selected for the calculation of Effect Factors to be implemented in current LCIA methods. The Confidence Interval on the $HC50_{EC50}$ is provided, enabling comparison between the impact values obtained as results of a Life Cycle Assessment study. The choice of EC50s is based on review of the main ecotoxicological databases, and analysis of the availability and reliability of test results. Moreover, bearing in mind that mostly acute data are available, while LCA deals mainly with chronic exposure, best-estimate extrapolation factors for the $HC50_{EC50}$ and the associated uncertainty are provided for inorganics, non-pesticide organics, and pesticide organics. Concerning the method itself, in order to find the best methods for calculation of a toxicity indicator, several statistical estimators, parametric and non-parametric approaches are compared, identifying their properties and respective strengths for a comparative method. The analysis relates to both the reliability of the estimator and its Confidence Interval, especially in terms of statistical robustness and Effect Factor stability. Based on these findings, the AMI method is described in detail, and an example of application comparing two wheat crop scenarios differing by the pesticides used is presented.

Chapter 3 presents in detail the four methods currently used for the development of Effect Factors for the Life Cycle Impact Assessment (LCIA) on Ecosystems: the parametric version of AMI (Assessment of the Mean Impact) based on $HC50_{EC50}$s; the Eco-Indicator based on $HC50_{NOEC}$s; USES-LCA based on both $HC5_{NOEC}$s and the Most sensitive species, and the PNEC (Predicted No- Effect Concentration) based on the Most sensitive species. After presentation of the LCIA framework and its main divergences from

Environmental Risk Assessment for chemical regulation, the four methods are detailed and applied for the calculation of Effect Factors for 83 substances, covering inorganics, non-pesticide organics, and pesticide organics. Each method is therefore analysed concerning three key points: applicability in the LCA framework, environmental relevance, and statistical reliability.
Particular attention is paid to possible bias and the uncertainty, highlighting the following findings: (1) $HC5_{NOEC}$s are on average 50 times higher than the most sensitive species, and this difference in conservatism introduces a bias in the analyses for the method mixing $HC5_{NOEC}$s and most sensitive species. (2) Effect Factors based on the most sensitive species increase the relative weight of the most toxic chemicals by two orders of magnitude, depending on whether the study is based on US or European ecotoxicity databases. (3) the methods based on $HC50_{EC50}$s and $HC5_{NOEC}$s are the only ones able to provide a Confidence Interval on the Effect Factor, but the Confidence Interval on the $HC5_{NOEC}$s can be more than 10 orders of magnitude greater than that of the $HC50_{EC50}$s. (4) compared with the Confidence Interval on the $HC50_{EC50}$s, the most sensitive species cannot be distinguished from $HC50_{EC50}$s for chemicals characterised by fewer than 5 species, and the $HC5_{NOEC}$s cannot be distinguished from the $HC50_{EC50}$s for chemicals characterised by fewer than 8 species.

Chapter 4 compares two statistical estimators, aiming at calculate the average toxicity of substances on biological species. The two methods provide an estimation of the $HC50_{EC50}$ and the associated Confidence Interval. On the one hand, parametric method using the geometric mean and a calculation of the confidence interval with Student is considered. On the other hand, a distribution-free method calculates the $HC50_{EC50}$ based on the median response of species and the confidence interval based on bootstrap. In order to facilitate the use of the non-parametric method, a table linking the number of species tested and the size of the confidence interval is provided for samples from 5 to 500 species. The comparison is based on actual data concerning 191 substances covering inorganics, non-Pesticide organics, and Pesticide organics. The mean and width of the chronic EC50s samples for all the substances are presented. The Shapiro-Wilk test is performed for the 191 EC50s samples and the assumption of log-normality of the distribution failed in more than 20% of the cases. Two causes of this non Log-normality are identified; (1) the skewness, which is shown to be an

important issue for the assessment of the average toxicity of chemicals while (2) the multi-modal distributions, which are not likely to influence considerably the final result. A detailed application of the two methods is done with the comparison of two herbicides, the Sulfosulfuron and the Prosulfuron, where the distribution-free method appears to be more powerful than the parametric for a substance-to-substance comparison. Nevertheless, the distribution-free method requires a minimum of 5 chronic EC50s, that cannot be satisfied in most cases.

Chapter 5 aims at illustrating the previous chapter in using the non-parametric version of the AMI method for the comparative assessment of the impacts of metals on aquatic ecosystems. This chapter briefly describes the method, then it focuses on the comparative analysis of 9 metals sometimes tested with different salts and speciations. Two interesting results can be highlighted: (1) the toxicity of metals covers the whole range of toxicity of chemicals; (2) the confidence interval of the $HC50_{EC50}$ for metals is on average twice as great for metals compared with other chemicals. This increase in the variability of ecotoxicological responses from species is likely to be due to the change in bioavailability of metals associated with a change of test conditions (e.g. pH, or Organic Matter).

Chapter 6 reviews and analyses the reliability of existing aquatic toxicity databases which can be used for the calculation of Effect Factors for Life Cycle Impact Assessment (LCIA). For that purpose, the main LCIA methods are presented focusing on their data requirement. It concerns: EDIP[4] (based on the PNEC); AMI[5] (based on parametric $HC50_{EC50}$); Eco-Indicator (based on the $HC50_{NOEC}$); USES-LCA (based on the $HC5_{NOEC}$). Moreover 6 ecotoxicity databases available in an electronic format are analysed: Aquire; Pesticide Ecotoxicity Database (PED); IUCLID; Acute Toxicity Database (ATD); Fathead Minnow database (FMD); and ECETOC Aquatic Toxicity Database (EAT). The analysis especially focuses on the identification of the substances and organisms, the definition of the tests conditions, and the control procedure of the database. A selection of tests is done, retaining a dataset of 128,864 tests results, acute, sub-chronic and chronic.

[4] EDIP: Environmental Design for Industrial Product
[5] AMI : Assessment of the Mean Impact

A description of the data availability on the basis of the selected test is performed, considering the available EC50s (Effect Concentration affecting 50% of the individuals tested), LOECs and NOECs (Lowest or No Observed Effect Concentration). The number of covered substances is also analysed regarding the number of species or phyla considered. On that basis, an estimation of the maximum number of possible Effect Factors is performed. The results highlight the discrepancy between the large number of test results available (128,864), and the relatively restricted number of Effect Factors (betweeen 34 and 4959 depending on the method)) that can be calculated for a comparative purpose like LCIA.

Chapter 7 provides a conclusion to the work, answering the points underlined in introduction. Then, the fey features of the AMI method are restated and the perspectives and output of the work are presented.

In the last part of the thesis, the AMI $HC50_{EC50}$ database is presented. It provides acute and chronic $HC50_{EC50}$ data calculated with the parametric version of AMI (geometric mean of the EC50s and confidence interval based on Student) for 522 susbtances.

Résumé

Ce travail de recherche se concentre sur l'évaluation des impacts sur les écosystèmes aquatiques en Analyse de Cycle de Vie (ACV), et vise à identifier les contraintes relatives à l'évaluation de l'impact des substances chimiques sur les écosystèmes en ACV et à mettre en place une *méthode d'évaluation des impacts des substances toxiques sur les écosystèmes aquatiques* qui satisfasse aux exigences d'une approche comparative comme l'Analyse de Cycle de Vie.

L'objectif général de cette thèse est de permettre l'évaluation comparative des substances toxiques sur les écosystèmes dans le cadre de l'ACV. A cette fin, le travail vise à explorer les points suivants :

1- La faisabilité d'une évaluation comparative des impacts sur les écosystèmes et l'identification des contraintes afférentes.
2- Le développement d'une méthode statistique permettant la comparaison des impacts des substances toxiques sur les écosystèmes.
3- La revue des données écotoxicologiques disponibles nécessaires au calcul des facteurs d'effet sur les écosystèmes.
4- La sélection des données écotoxicologiques les plus adaptées (EC50[6], NOEC[7] ou LOEC[8]) pour une analyse comparative.
5- La mise en place de facteurs d'extrapolation non biaisés permettant l'évaluation des effets chroniques sur la base de données de toxicité aigues.
6- L'analyse de la validité écologique de l'évaluation comparative des impacts des substances toxiques sur les écosystèmes aquatiques.

Les points mentionnés ci-dessus sont traités au cours des sept chapitres de la thèse.

Le chapitre 1 introduit ce travail en présentant tout d'abord l'Analyse de Cycle de Vie de façon générale, suivit d'une description plus détaillée de l'évaluation des impacts des substances toxiques sur les écosystèmes. Cette description fait l'état de l'art des méthodes d'évaluation de l'impact des substances toxiques sur les écosystèmes et d'autre part présentent les

[6] EC50 : Concentration d'une substance affectant 50% des individus testés
[7] NOEC : Concentration sans effet observable
[8] LOEC : Plus petite concentration induisant un effet

principaux besoins dans ce domaine. L'objectif de la thèse ainsi que les principaux points explorés sont alors abordés.

Le chapitre 2 commence par une revue des méthodes existantes d'évaluation des impacts sur les écosystèmes en ACV. L'effort est alors mis sur la présentation de la version paramétrique de la méthode AMI (Assessment of the Mean Impact) qui a été développée dans le cadre du travail de thèse pour l'évaluation des impacts des substances chimiques sur les écosystèmes. Dans ce but, les exigences méthodologiques relatives au développement de la méthode sont présentées. Dans le cadre d'une comparaison des impacts, la $HC50_{EC50}$ qui est la concentration en toxique affectant 50% des espèces du milieu aquatique au-delà de leur EC50 a été retenue pour le calcul des facteurs d'effet des substances toxiques en ACV. De plus, l'intervalle de confiance sur la $HC50_{EC50}$ est également calculé afin de permettre la comparaison des impacts obtenus lors des études ACV. Le choix de la EC50 est fait suite à une revue des données écotoxicologiques disponibles, après analyse de la disponibilité et de la fiabilité des données. pouvant permettre le calcul des facteurs d'effet en ACV. Par ailleurs, gardant à l'esprit que la plupart des données écotoxicologiques sont des données aigues alors que les effets observés sont généralement chroniques, des facteurs d'extrapolation non biaisés pour l'évaluation de $HC50_{EC50}$ chronique à partir de données aiguës sont calculés pour les substances inorganiques, les substances organiques non pesticides, ainsi que les substances organiques pesticides. Concernant la méthode elle-même, les propriétés de divers estimateurs statistiques (paramétrique ou non) sont analysées au regard de leur applicabilité dans le cadre d'une approche comparative. L'analyse porte à la fois sur l'estimateur lui-même et sur l'intervalle de confiance qui lui est associé, et couvre tout particulièrement la robustesse de l'estimateur ainsi que la stabilité des facteurs d'effet qui en découlent. Sur la base de ces arguments, la version paramétrique de la méthode AMI est présentée. A titre d'illustration, la méthode est mise en application dans la comparaison des impacts de deux fongicides utilisés pour la culture du blé et substituables l'un à l'autre.

Le chapitre 3 présente en détail les quatre méthodes actuellement utilisées pour l'évaluation de l'impact des substances chimiques sur les

écosystèmes : la version paramétrique de la méthode AMI[9], basée sur la $HC50_{EC50}$; la méthode Eco-Indicator, basée sur la $HC50_{NOEC}$; la méthode USES-LCA utilisant à la fois la $HC5_{NOEC}$ est l'espèce la plus sensible, et enfin la méthode EDIP[10] basée sur l'espèce la plus sensible.
Une courte présentation de l'évaluation des impacts sur les écosystèmes en ACV et des principales divergences avec l'Analyse de Risque Environnemental pour la régulation des substances chimiques est proposée. Après cela, les quatre méthodes sont utilisées pour le calcul des facteurs d'effet de 83 substances, couvrant des substances organiques non pesticides, des organiques pesticides, ainsi que des substances inorganiques. Ainsi chaque méthode peut être analysée sur la base de trois aspects clé : l'applicabilité dans le cadre des Analyses de Cycle de Vie ; le réalisme écologique ; et la fiabilité statistique. La question des biais méthodologiques est tout particulièrement explorée, et permet de souligner les points suivants : (1) En moyenne, les valeurs de PNEC[11] basées sur les $HC5_{NOEC}$ sont 50 fois plus haute que leur équivalent basé sur l'espèce la plus sensible. Cette différence introduit un biais lorsque les deux approches sont utilisées en même temps dans une étude ACV. (2) Les facteurs d'effets basés sur l'espèce la plus sensible ont tendance à surestimer de deux ordres de grandeur le poids relatif des substances les plus toxiques, selon que l'étude utilise des bases sur des données écotoxicologiques américaines ou européennes. (3) Seules les méthodes basées sur les valeurs de $HC50_{EC50}$ et de $HC5_{NOEC}$ sont à même de permettre le calcul d'intervalles de confiance sur les facteurs d'effet. Néanmoins, les intervalles de confiances relatifs à la $HC5_{NOEC}$ peuvent être jusqu'à 10 ordres de grandeur plus important que leur équivalent relatif à la $HC50_{EC50}$. (4) au regard de l'intervalle de confiance de la réponse moyenne des espèces ($HC50_{EC50}$), la valeur minimum des EC50 (espèce la plus sensible) ne peut pas être distinguée de la $HC50_{EC50}$ si moins de 5 espèces sont testées ; le nombre de EC50 requis est porté à 8 pour que la $HC5_{NOEC}$ se distingue de la $HC50_{EC50}$.

Le chapitre 4 compare deux estimateurs statistiques qui ont été développés durant cette thèse afin de calculer la toxicité moyenne des

[9] AMI, Assessment of the Mean Impact: évaluation de l'impact moyen des substances toxiques
[10] EDIP: Environmental Design for Industrial Product
[11] PNEC, Predicted No Effect Concentration : seuil de concentration d'une substance dans l'environnement en deça duquel aucun effet n'est prévu.

substances chimiques sur les espèces vivantes. Les deux méthodes permettent le calcul de la $HC50_{EC50}$ et de son intervalle de confiance : d'une part une méthode paramétrique basée sur le calcul de la moyenne géométrique et son intervalle de confiance calculé avec la méthode de Student et d'autre part, une méthode non-paramétrique se base sur le calcul de la médiane et de son intervalle de confiance calculé par bootstrap. Afin de faciliter l'application de la méthode non-paramétrique, une table reliant l'intervalle de confiance à la taille de l'échantillon est proposée pour les échantillons de 5 à 500 substances. La comparaison des méthodes est faîte à partir de données réelles concernant 191 substances intégrant des substances inorganiques, des pesticides organiques, et des substances organiques non-pesticides. La moyenne et la dispersion des EC50 sont présentées pour l'ensemble des substances. Par ailleurs, un test de Shapiro-Wilk est appliqué aux 191 substances afin de contrôler l'hypothèse de distribution log-normale. L'hypothèse est rejetée dans 20% des cas. Ceci est imputable à deux causes qui sont analysées dans ce chapitre : (1) les distributions présentent une dissymétrie importante susceptible d'affecter le profil environnemental des substances. (2) Les distributions sont multimodales, mais cela ne semble pas influencer considérablement les facteurs d'effet.
Afin de mieux comprendre l'influence respective des méthodes sur l'estimation de la toxicité des substances, une comparaison de deux herbicides est réalisée, le Sulfosulfuron et le Prosulfuron. La méthode non-paramétrique semble plus performante que son équivalent paramétrique pour une comparaison entre deux substances. Néanmoins, la méthode non-paramétrique nécessite un minimum de 5 EC50 chroniques, ce qui limite sérieusement les possibilités d'application. De ce fait, il apparaît judicieux d'utiliser au premier chef la méthode paramétrique, et de réaliser une étude de sensibilité en utilisant les facteurs d'effet non-paramétrique afin de tester la robustesse des résultats au regard de l'hypothèse de distribution log-normale.

Le chapitre 5 vise à illustrer le chapitre précédent par la mise en application de la version non-paramétrique de la méthode AMI dans le cadre de l'évaluation comparative des impacts des métaux sur les écosystèmes aquatiques. La méthode est décrite rapidement au début du chapitre puis une analyse comparative considérant 9 métaux, parfois testés avec différents sels ou différentes spéciations, est proposée. Deux

résultats illustrent particulièrement l'intérêt de ce travail. Tout d'abord, il est montré que la toxicité moyenne des métaux (sur la base des $HC50_{EC50}$) varie considérablement d'une substance à une autre, couvrant le même spectre de toxicité que les substances organiques. Ensuite, l'intervalle de confiance sur la $HC50_{EC50}$ est en moyenne deux fois plus important pour les métaux que pour les autres substances. Cette augmentation de l'intervalle de confiance ne semble pas provenir du nombre de EC50 disponibles mais plutôt des variations de la biodisponibilité des métaux associées à des changements de conditions de milieu lors des tests (par exemple le pH ou la matière organique).

Le chapitre 6 propose une revue des bases de données d'écotoxicité aquatique, et leur analyse en terme de fiabilité des données. Dans ce but, les principales méthodes sont présentées en mettant l'accent sur leurs exigences en terme de données. Cette analyse concerne la méthode EDIP (basée sur la PNEC), la méthode AMI dans sa version paramétrique (basée sur la $HC50_{EC50}$), la méthode Eco-Indicator (basée sur la $HC50_{NOEC}$); et enfin la méthode USES-LCA (basée sur la $HC5_{NOEC}$). Par ailleurs 6 bases de données disponibles en format électronique sont analysées : « Aquire »; « Pesticide Ecotoxicity Database » (PED); IUCLID; « Acute Toxicity Database » (ATD); « Fathead Minnow database » (FMD); and ECETOC « Aquatic Toxicity Database » (EAT). L'analyse se concentre sur l'identification des substances et des organismes testés, la définition des conditions des tests et la procédure de contrôle de qualité des bases de données. A l'issue de cette analyse, une sélection de tests est réalisée, retenant un set de données de 128 864 résultats de tests aigus, chronic et sub chronic. Les données sélectionnées servent de support à l'évaluation de la disponibilité des EC50, LOEC et NOEC. Le nombre de substances disponibles est également évalué au regard du nombre d'espèces testées ou de phyla testés par substance. Sur cette base, l'estimation du nombre maximum de facteurs d'effet pouvant être calculé est réalisée. Le résultat souligne l'énorme différence entre le nombre considérable de résultat de tests (128 864) et le nombre limité de facteurs d'effet pouvant être calculé (entre 34 et 4959 selon la méthode considérée) par les méthodes d'ACV.

Le chapitre 7 conclut la thèse, en répondant de façon précise et détaillée aux points importants mentionnés en introduction. Puis les paramètres

principaux de la méthode AMI sont rappelés. Enfin, les futurs dévelopements envisageables pour l'évaluation comparative des substances toxiques en ACV sont présentés, et les principales mises en application de la méthode AMI au plan international sont finalement mentionnées.

En dernière partie, la base de données de la méthode AMI est présentée. Elle permet à ce jour de calculer les facteurs d'effet aigus et chronique ainsi que leur intervalle de confiance sur la base des $HC50_{EC50}$ (version paramétrique) pour 522 substances.

CHAPTER 1

General Introduction*

*The introduction is partially based on:

Pennington, D. W., J. Payet, M. Hauschild, O. Jolliet. (2004). "Multiple Species Ecotoxicological Measures in Life Cycle Impact Assessment." Environmental Toxicology and Chemistry **23**(7) ; pp.1796–1807

Jolliet, O., M. Margni, R. Charles, S. Humbert, J.Payet, G.Rebitzer. (2003) : "Impact 2002+: A New Life Cycle Impact Assessment Methodology." International Journal of LCA **8**(6); pp. 324-330.

Foreword

In the early seventies, the growing realisation that the availability of energy resources was limited led to the emergence of the Life Cycle Assessment (LCA) tool. The objective of Life Cycle Assessment was to allow the optimisation of energy resources for a given service (a Functional Unit in LCA terminology). This tool was subsequently used more and more extensively in the decision-making process, enabling the calculation of energy balances concerning products or services (e.g.: comparison of different packaging) and covering all the product's life stages: production, use and end of life (Jolliet, Saade et al. 2004).

While this development was taking place, ecological and epidemiological research revealed the impact of human activities on living organisms (Carson 1962; Bouguerra 1997; EEA 2001). As for damage caused to ecosystems, emphasis was placed on improving knowledge concerning chemical substances. The information obtained regarding the most problematic substances would allow the calculation of a maximum acceptable concentration in ecosystems for each substance (US-EPA 1984; EU-Commission 1994). The setting up of an acceptability threshold allowed industrial and agricultural development and aimed at ensuring the integrity of ecosystems.

In the eighties, increased knowledge regarding environmental mechanisms demonstrated that the chief limitation was the absorption capacity of the environment. From then on, consideration of impact on living resources in Life Cycle Assessment became essential and new approaches added methods of protecting ecosystems and human health to the Life Cycle Assessment tool (Heijungs, Guinée et al. 1992; Wenzel, Hauschild et al. 1998). This phase enabled quantified assessments of impacts on living systems to be carried out in LCA, but also – and especially – to check the feasibility and to identify the limits of such an improvment. Henceforth it was no longer the energy efficiency of products which had to be optimised but environmental efficiency as a whole, by covering impact categories as diverse as depletion of resources, climatic changes, effects on human health or alterations in the quality of ecosystems.

Nevertheless, at this stage, each impact category became a speciality in itself, and it was therefore necessary to integrate the specificities of each domain

(radiative forcing, chemical fate, etc) into the general Life Cycle Assessment methodology. Among the different impact categories, that of toxic substances on ecosystems occupies an important place. The extent of these impacts has been stressed on many occasions and the necessity of preserving ecological areas and biodiversity has become a major issue on an international level (United Nations 1992). The quantification of impacts of toxic substances on ecosystems in Life Cycle Assessment requires the use of a method for comparing impacts based on a reliable indicator.

By focusing on aquatic ecosystems, this thesis aims at identifying constraints connected with assessment of the impact of chemical substances on ecosystems in LCA and setting up a *method for assessing impacts of toxic substances on aquatic ecosystems* that meets the requirements of a comparative approach like Life Cycle Assessment.

Problem Setting

Life Cycle Assessment (LCA)

Life Cycle Assessment (LCA) is a tool for assessing the environmental impact of a product, or more precisely, of a system required for a particular unit of function (Guinée, Heijungs et al. 1996). LCA provides a systematic framework, which helps to identify, quantify, interpret and evaluate the environmental impacts of a product, function, or service from its cradle to its grave.

This tool can assist in (ISO 1998):

- identifying the opportunities to improve the environmental aspects of products at various points in their Life Cycle;
- decision making in industry, governmental or non-governmental organizations;
- selection of relevant indicators of environmental performance;
- marketing for example in comparative assertions, environmental product declaration, ecolabels etc.

The LCA method can be described as a four-stage procedure (Figure 1):

- Definition of scope and goal: provides a description of the product system, sets systems boundaries and defines the function and functional unit of the product or service.
- Life Cycle Inventory (LCI): Quantifies the resource consumption and emissions into air, water and soil at all the stages in a product's life cycle. These emissions are likely to occur at multiple sites and different times.
- Life Cycle Impact Assessment (LCIA) aims at improving the understanding of the inventory results providing an aggregation of the inventory data in order to quantify the potential environmental impact.
- Life Cycle Interpretation: occurs at every stage of the LCA and aims at drawing lessons and conclusions from the results of the study, and improving the reliability of the results.

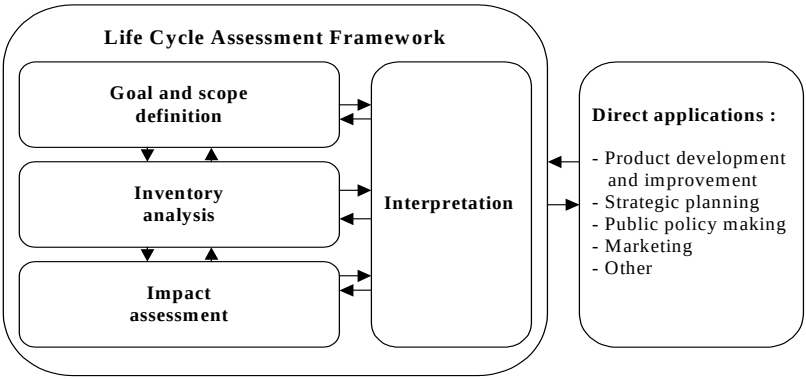

Figure 1: Phases of an LCA (according to ISO 14040, 1998)

The inventory analysis provides a matrix of emission related to the considered product. For that purpose, all the emissions occurring in different places at different times are added up per substance. It is then determined which emission contributes to each impact category, in a stage called "classification". The impact categories (also called Midpoint categories in Impact 2002+) are presented in Figure 2.

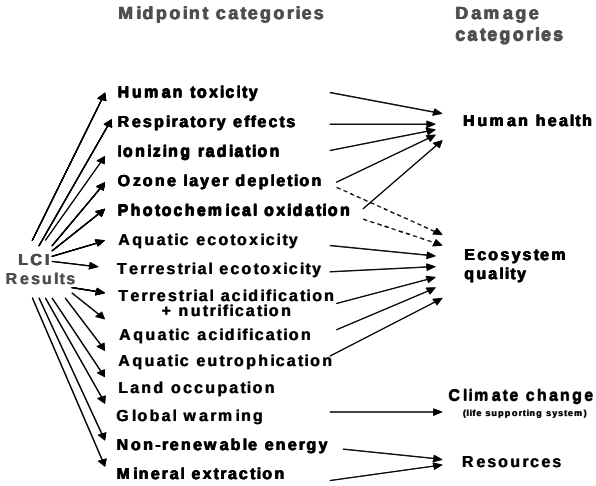

Figure 2 : Overall scheme of the IMPACT 2002+ framework, linking LCI results via the midpoint categories to damage (Jolliet, Margni et al. 2003)

After each substance has been linked to one or more categories, the emissions inventory data are multiplied by characterisation factors to provide indicators in the context of various impact categories (like the mid-point categories presented in Figure 2). Characterisation factors therefore express the relative importance of emissions in the context of a specific environmental impact category (Margni 2003).

Nevertheless, as mentioned by Pennington et al, (Pennington, Jolliet et al. 2004), LCA is a comparative assessment methodology. Inconsistencies in the assessment can introduce unintentional bias. Direct adoption of regulatory methodology and data is not always appropriate. Regulatory methods and data, again particularly in toxicological risk assessment, are not always developed for use in a comparative context. A conservative estimate of the ecotoxic effect of a substance is unwanted in this relative comparison context. Best-estimates are desirable in LCA, with the need to account for uncertainties when making distinction amongst results.

Environmental Risk Assessment (ERA)

Environmental Risk Assessment is the examination of the risks due to environmental changes (for example emission of pollutants) that affect living systems (humans, animals, plants).
Ecological Risk Assessment is the part of Environmental Risk Assessment (ERA) that addresses impacts on ecosystems. EcoRA aims at estimating the risk associated with the release of a substance in an environmental compartment. This risk depends on the level of hazard of the substance, and in the level of exposure of living organisms. The level of hazard is generally estimated on the basis on ecotoxicological testing by relating levels of adverse effects to concentration of substances. The level of exposure can be measured or estimated by modelling the fate of the substance from the emission to the studied environmental medium.
Nevertheless several discrepancies can be observed between EcoRA and LCIA on ecosystems.
1) As mentioned above, EcoRA often aims at a conservative estimate of the toxic effects of a substance while the LCIA aims at the best estimate for a comparative assessment.
2) Risk assessment is generally performed in a regulatory context where the purpose can be to help ensure that there is not unacceptable risk to the environment from a given emission at a given site. LCIA, on the other hand, attempts to address all relevant environmental impacts due to a product not necessarily considering the time and localisation of the emissions (Hauschild and Pennington 2003).
3) EcoRA focuses strictly on the potential impact of one toxic or a mixture of toxics on the ecosystem. LCIA, on the other hand, has to ensure the compatibility of its the estimate of toxic impact with the other classes of impact on the ecosystems (eutrophication, acidification, etc).

Life Cycle Impact Assessment of Toxic Substances on Ecosystems

At the interface between industries (economy) and ecosystems (ecology), the method translating emissions from the inventory in an impact on aquatic ecosystems is under focus. The overall purpose of the work is to assess and eventually to reduce or limit the anthropogenic pressure on animals and plants in the natural environment.

Framework

Figure 3 presents the impact assessment of toxics in LCA. It can be divided into four parts. Fate modelling relates the emission in the Life Cycle Inventory with the increase in concentration in a given medium. The impact model includes three parts, the exposure model which quantifies the amount of substances absorbed by the organism depending on the concentration in the different media, the impact model that relates the amount absorbed to the effect on the organism, and the damage model translating the effect on the organisms in a change integrated over time and space for a group of organisms (humans or biodiversity).

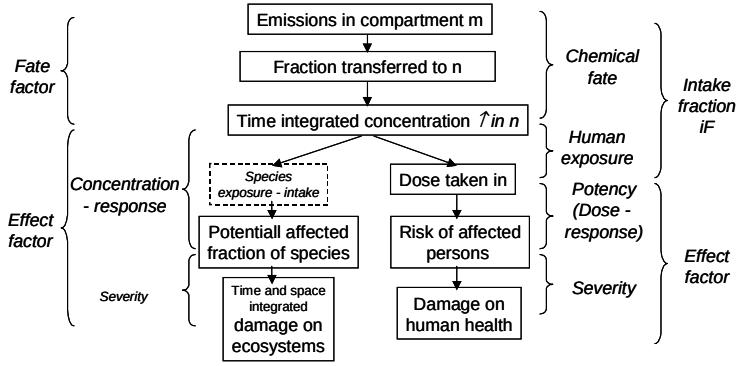

Figure 3: General scheme of the Impact pathway for human toxicity and ecotoxicity (Jolliet, Pennington et al. 2004)

This impact modelling has to satisfy several requirements. The main one concerns the comparative assessment of LCA, but the development of a method for the impact assessment of toxics on ecosystems in the framework of LCA must also be compatible with the following key feature.
Compatibility with Life Cycle Inventory data requires (1) coverage of a large number of substances; (2) integrating the impact over time and space since most of the LCI data are not spatially and temporally differentiated. This leads to the use in LCIA of a fate model based on the assumption of steady state in the LCIA method. To be compatible with the effect model, the fate model must translate chemical emissions calculated in the Life Cycle Inventory into an increase in concentration in the relevant medium for a defined time period. As highlighted by Pennington et al (Pennington, Payet et al. 2004), the available characterisation factors account for chemicals fate in the environment and species exposure, as well as for differences in exposure response, as presented in the equation of the characterisation factor below.

$$\frac{\text{effect}}{\text{emission}} = \frac{\text{fate}}{\text{emission}} \cdot \frac{\text{exposure}}{\text{fate}} \cdot \frac{\mathbf{effect}}{\mathbf{exposure}} \qquad \mathbf{(1)}$$

For aquatic toxicological effects, the effect factor is often expressed in terms of concentration-response while the fate factor quantifies the increase in concentration in the aquatic medium. Therefore, the effect factor (bold characters in Equation 1) for aquatic ecosystems integrates both the exposure model and the effect model. This assumes that the main route of exposure of the aquatic organisms is the concentration in water.

The compatibility of the different impact categories is an important issue in LCIA. For example, several midpoint categories are gathered under the endpoint category “Ecosystem Quality”, and a final impact value for Ecosystem Quality is required. It means that the assessment of effect factors both for terrestrial and aquatic ecosystems is needed. Furthermore, toxic effect also has to be compatible with other ecosystems stressors such as eutrophication, acidification, etc. A possible way to ensure the compatibility is to express all the results in terms of impact on biodiversity.

Research concerning terrestrial ecosystems, multiple stressors, and biodiversity modelling are beyond the scope of this work, but some promising tracks are

now explored that raised these issues (Jolliet, Margni et al. 2003; Payet, Larsen et al. 2003; Payet, Margand et al. 2004).

State of the art

An overview of Life Cycle Impact Assessment (LCIA) methods for ecosystems analysed several models, which can be used for calculation of the ecotoxicity effect factors in LCA (Schulze, Jödicke et al. 2001; Hauschild and Pennington 2003).

The PNEC (Predicted No Effect Concentration) based on most sensitive species is currently used by the EDIP method (Hauschild and Wenzel 1998; Wenzel, Hauschild et al. 1998). This method is directly based on the raw data, using as basis for calculation of the Effect Factor the lowest toxicological data. Depending on data quality, the PNEC is assessed applying a safety factor on the lowest EC50 or the lowest NOEC (or LOEC). The use of the PNEC in the context of LCA requires the assumption of a linear extrapolation from PNEC down to 0, where the intensity of the effect is proportional to the slope.
A possible alternative to the PNEC based on the most sensitive species is to use the $HC5_{NOEC}$ (Hazardous Concentration affecting 5% of species aboce the NOEC) value instead. The $HC5_{NOEC}$ is calculated on the basis of an SSD (Species Sensitivity Distributions) (Kooijman 1987; Sloof 1992; Aldenberg and Slob 1993; Hauschild and Wenzel 1998; Wenzel, Hauschild et al. 1998; Stephan 2001), assessing the concentration affecting 5% of species over the NOEC. This method has been adapted for LCIA (Huijbregts, Thissen et al. 2000) with the USES-LCA. Like the PNEC, the method is based on a linear extrapolation from the HC5 down to 0. The method has been further improved with an assessment of the potential influence of mixtures, comparing effect-additive and concentration-additive models (Huijbregts, VandeMeent et al. 2002). Furthermore, the need for a chronic NOEC-based SSD has been often considered as a restriction, since many data are required. In order to solve this problem, an extrapolation method has been proposed enabling the calculation of a chronic SSD using acute EC50s (DeZwart 2002; Roelofs, Huijbregts et al. 2003). Two attempts have been made for considering mixtures and acute ecotoxicity data (Roelofs, Huijbregts et al. 2003) in USES-LCA but characterisation factors have not been provided yet.

The Combi-PAF method proposed Eco-Indicator (Goedkoop, Effting et al. 2000) is based on SSD. This method aims at calculating the Fraction of Affected Species (PAF) due to a change in toxic concentration. This method has several specificities. Effect Factors are calculated using a theoretical SSD curve of mixture. A reference value is chosen on the Combi-PAF curve representing the current ambient level of toxic stress (working point) and the marginal change in Fraction of Affected Species at the working point is used as basis for impact calculation (current level of toxic stress is estimated at 24% of affected species). Toxics arriving in a given medium are converted into Hazard Units and added up, following the indications of a concentration additive mixture model (Hamers, Aldenberg et al. 1996). The Effect Factor is based on the marginal variation of the Fraction of Affected Species due to a change in Hazard Unit in an environmental medium.

Aim of the Thesis

The overall purpose of the thesis is to explore the feasibility of develop and test a comparative method for the Life Cycle Impact Assessment of toxics on aquatic ecosystems. With that aim, the dissertation addresses 6 major issues:

- Explore the feasibility of comparative impact assessment on ecosystems with special emphasis on: (1) The choice of the most relevant ecotoxicity mesure (ECx, NOECs and LOECs) for a comparative purpose; (2) The development of best-estimate extrapolation factors for assessing chronic effects based on acute data.
- Compare the developed method for comparative assessment with the main existing LCIA methods for aquatic ecosystems;
- Analyse and develop a statistical estimator for comparing toxic impacts on ecosystems.
- Apply and test the developed method for the assessment of metal impacts on aquatic ecosystems.
- Review the data availability for calculation of Effect Factors;
- Analyse the ecological realism of the comparative assessment method.

To address these 6 points, the thesis is structured as follows:

Chapter 2 presents the method we have developed for the comparative assessment of toxics on ecosystems. This method, called AMI [Assessment of the Mean Impact] can be based on parametric or non-parametric statistics, and the parametric version is presented in this chapter. Thus, we present how a comparative assessment of toxic impact can be performed on the basis of existing data.
For this purpose, several issues are detailed: (1) Which is the most suitable toxicological measure (EC50 or NOEC) for comparative assessment, also considering acute and chronic data availability? (2) Which indicator (most sensitive species, mean, median, geometric mean) is least sensitive to the selection of tested species and discriminates most between chemicals, and would therefore be appropriate as a toxicity indicator? (3) How is it possible to assess the uncertainty of this indicator? (4) How can the extrapolation procedure from acute to chronic data be improved?
These questions are addressed throughout Chapter 2 and a final presentation of an application of the parametric version of AMI is presented at the end of the chapter.

Chapter 3 explores the underlying assumptions of LCIA on ecosystems by comparing the AMI method with the mostly used assessment method for Life Cycle Toxic Impact on aquatic ecosystems. It starts in highlighting the discrepancy between Environmental Risk Assessment for chemical regulation and Life Cycle Impact Assessment. Afterwards, the existing methods for LCIA on ecosystems are presented in detail and then compared to each other. The comparison considers the EDIP method (Wenzel, Hauschild et al. 1998), the Eco-Indicator method (Goedkoop, Effting et al. 2000), the USES-LCA method (Huijbregts, Thissen et al. 2000) and the AMI method in its parametric version. For comparing methods, chronic Effect Factors for 83 substances are calculated and results are analysed throughout a list of criteria that has to be fulfilled by LCIA on ecosystems. These criteria are defined following three axes:
- the applicability of the method to the LCA framework;
- environmental relevance;
- statistical reliability.

In doing so, the chapter answers to several important questions: (1) How far can the methods be applied for a large number of chemicals? (2) Are there any sources of bias in the Effect Factors? (3) How stable are the Effect Factors, regarding the selection of tested species, addition of new data, or change in databases? (4) To what extent can the methods rank the chemicals on the basis

of toxicity, associating a Confidence Interval with the Effect Factor? (5) How relevant are the results regarding environmental conditions?

Chapter 4 focuses on the statistical aspects of the comparative assessment. Indeed a second version of AMI based on non-parametric statistics has been developed. This method is presented in detail in the chapter and then, calculation of Effect factors concerning 191 substances covering inorganics, non-pesticide organics, and pesticide organics is performed with both the distribution-free method and the parametric one. The two versions of the AMI method are compared on the basis of these factors, and the comparison raises several questions: (1) the calculation of the Effect Factors is based on ecotoxicity data that do not necessarily fit a log Normal Distribution, and the question is therefore *to know if better Effect factors are obtained with the underlying assumption of a Normal Distribution or if non-parametric methods would be more relevant.* (2) The second observation is the scarcity of ecotoxicity data compared to the huge number of substances used daily in industrial processes, and the question addressed concerns the *applicability of a parametric or non parametric estimator to small samples* (three or four EC50s). (3) Still related to the lack of chronic data, the question also concerns the *compatibility of the estimator with an extrapolation from an acute to a chronic* $HC50_{EC50}$. Considering these points, the chapter aims at defining under which conditions better Effect Factors are calculated using $HC50_{EC50}$ based on distribution-free techniques. It also looks at how the two approaches can complement themselves.

Chapter 5 presents an illustration of the use of the non-parametric version of AMI with the quantification of metals toxicity for aquatic species. The main components of the non-parametric version of AMI are presented, and the comparison is performed for quantifying the average toxicity of metals and comparing it to the toxicity of organic substances. The comparison considers both the median toxicity and its confidence interval based on bootstrap. Considering 9 metals tested with different salts and speciations, this chapter aims a clarifying both the strength and the limits of the distribution-free method for the calculation of Effect Factors; and at identifying the lack in the estimation of the toxicity of metals in Life Cycle Assessment.

Chapter 6 explores the data availability for the calculation of Effect Factors for LCIA on Ecosystems. Existing databases for aquatic toxicity data are presented.

Then databases are analysed regarding their content and their level of reliability, especially focusing on the identification of the substances considered; the identification of the organism tested; the description of the test conditions; and the quality control procedure. This analysis covers the following questions: (1) Which databases can be used for the development of LCIA effect factors on ecosystems? (2) What is the content of these databases?
After this analysis, the reliable data of the databases are gathered in one dataset containing 128,864 tests results covering EC50s, LOECs and NOECs for 4,959 substances. This dataset is therefore used as a basis for answering a third key question: (3) How many factors (Acute and chronic) can be potentially calculated for each method?
Considering the four main methods of LCIA on aquatic ecosystems, an estimate of the maximum number of Effect Factors that can be calculated using existing databases is provided considering both acute and chronic toxicity data.

As presented above, this dissertation focuses on a core method for the quantification of toxic impacts in Life Cycle Assessment for aquatic assessment. Other ecosystems and other LCA issues related to ecosystems are not considered here, but are seen as perspectives of extension of the method in the concluding Chapter 7. It concerns for example issues like the impact assessment on terrestrial ecosystems, or the link between stressors affecting ecosystems. The perspectives associated to these issues are considered in the conclusion.

As this thesis is based on a collection of stand-alone chapters that have been or are being submitted to peer-reviewed journals , the literature review is performed at the beginning of each chapter. In each introduction, specific objectives related to the overall thesis topic are expanded.

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CHAPTER 2

Life Cycle Impact Assessment on Aquatic Ecosystems: The AMI Method [Assessment of the Mean Impact]*

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Abstract

After a review of existing methods for Life Cycle Impact Assessment on ecosystems (LCIA), the chapter presents a new method called AMI (Assessment of the Mean Impact) for the assessment of impact on aquatic ecosystems. For this purpose, a framework and the main requirements for the development of this method are presented. For a comparative assessment, the Hazardous Concentration of a toxic affecting 50% of the species over their chronic EC50 (Effect Concentration affecting 50% of tested individuals), also called $HC50_{EC50}$, is selected for the calculation of Effect Factors to be implemented in current LCIA methods. The Confidence Interval on the $HC50_{EC50}$ is provided, enabling comparison between the impact values obtained as results of a Life Cycle Assessment (LCA) study. The choice of EC50 values is based on review of the main ecotoxicological databases, and analysis of the availability and reliability of test results. Moreover, bearing in mind that mostly acute data are available, while LCA deals mainly with chronic exposure, best-estimate extrapolation factors for the $HC50_{EC50}$ and the associated uncertainty are provided for inorganics, non-pesticide organics, and pesticide organics. Concerning the method itself, in order to find the best methods for calculation of a toxicity indicator, several statistical estimators, parametric and non-parametric approaches are compared, identifying their properties and respective strengths for a comparative method. The analysis relates to both the reliability of the estimator and its Confidence Interval, especially in terms of statistical robustness and Effect Factor stability. Based on these findings, the AMI method is described in detail, and an example of application comparing two wheat crop scenarios differing by the pesticides used is presented.

Key words: Life Cycle Assessment; Life cycle impact assessment; aquatic ecosystems; uncertainty; extrapolation factor.

Introduction

The purpose of Life Cycle Assessment (LCA) is to evaluate the large number of chemicals potentially emitted in the environment during a product's whole life cycle. How the impact of these emissions on aquatic ecosystems can be compared among products is the key issue of this research. The idea has emerged that this comparison can be made by assigning weighting factors to the emission. Each weighting factor is the combination of a Fate Factor and an Effect Factor (Figure 4). The Fate Factor quantifies the time-integrated mass of the substance in a given environmental medium, while the Effect Factor, measuring the toxicity of a unit mass of chemical, quantifies the environmental impact due to this mass of substance in the fresh water environment.

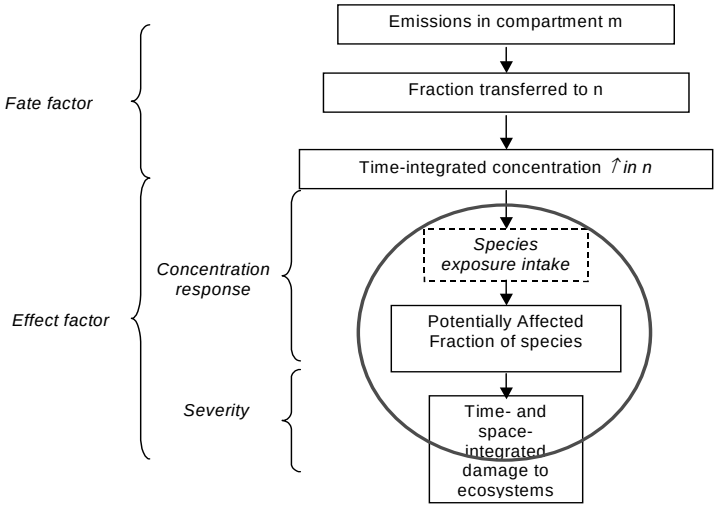

Figure 4: Description of the LCIA procedure including the Effect model .

An overview of Life Cycle Impact Assessment (LCIA) methods for ecosystems analysed several models, which can be used for calculation of the ecotoxicity

Effect Factors in LCA (Schulze, Jödicke et al. 2001; Hauschild and Pennington 2003).

The PNEC-based method (Predicted No Effect Concentration) (Heijungs, Guinée et al. 1992; Hauschild, Wenzel et al. 1998), assesses the no-effect level from the Most sensitive species tested (US-EPA 1984; EU-Commission 1994; EU-Commission 1996) . This method is applied for example in the EDIP (Wenzel, Hauschild et al. 1998) or CML methods (Guinée, Heijungs et al. 1996) for deriving Effect Factors. A characteristic of PNEC-based methods using the most sensitive species is their aim to protect all species of the ecosystem. This approach can however be more or less conservative depending on whether it is based on OECD, US-EPA or European guidelines. Applied in the LCA context, effects are considered proportional to the PEC (Predicted Effect Concentration)/PNEC ratio. This also means that no threshold is considered in the LCA application of PEC/PNEC and that an implicit linear extrapolation is made from 1/PNEC down to zero. Furthermore, the PNEC calculation is finally based on only one EC50 or NOEC value (the lowest), which is not necessarily representative for entire ecosystems. Another problem with the PNEC approach is outlined by Forbes and Forbes, mentioning that as data for more species are accumulated for a particular chemical, the lowest value of the lot can only get lower (Forbes and Forbes 1993). Thus increased knowledge concerning a chemical will systematically lead to diminution of the PNEC value – never an increase, which is counter-intuitive since more information could lead to smaller safety margins, not larger. Also, considering LCIA's purpose, as it is based on the most sensitive species, it is highly dependent on the choice of species tested and therefore discriminates poorly between substances.

Aimed at improving the method's environmental relevance (i.e. ease of interpretation in terms of damage to natural ecosystems), further developments of the PNEC approach in LCIA, including a statistical extrapolation method, were introduced by Huijbregts (Huijbregts 1999; Huijbregts, VandeMeent et al. 2002). This method aims at assessing the level at which 95% of the species are regarded as protected, defining a concentration of chemical which should affect 5% of the species present in the ecosystem. The concentration of pollutant that aims at protecting 95% of the species of the ecosystem is commonly called HC5 (also referred to as $PNEC_{0.05}$). According to whether we use the HC5 or PNEC method for calculation of an effect indicator, the final result will greatly depend on the data selected for comparison. While the PNEC using the most sensitive species can be based on all kinds of ecotoxicological endpoints (acute or chronic, ECx or NOEC) using different extrapolation factors, the HC5 for

regulatory purposes is currently preferably based on chronic NOECs. Since the PNEC based on the Most sensitive species is strongly dependent on extrapolation factors - varying from 1 to 1,000 according to the endpoint and method used (US-EPA 1984; OECD 1992; EU-Commission 1994) - the final differences between results can attain several orders of magnitude. For these reasons, several authors have stressed that for comparative assessment, it would not be appropriate to mix the PNEC based on the most sensitive species and the PNEC based on statistical extrapolation in one LCA study (Heijungs, Guinée et al. 1992; Emans, VandePlassche et al. 1993; Guinée and Heijungs 1993; VanderZande-Guinée, Slangen et al. 1999).
Another method based on statistical extrapolation is the Combi-PAF method, proposed by Goedkoop and Spriensma (Goedkoop, Effting et al. 2000) and further extended to the impact assessment of chemical mixture on aquatic ecosystems (Huijbregts et al, 2002). This method was especially developed for Life Cycle Assessment (LCA), and is presented as more suitable for comparative assessment than PNEC methods since it takes into consideration the fraction of species affected by background concentration of chemicals, for the moment in Dutch ecosystems only. For extension to the European scale, however, the method requires substantial improvement of the quantification of the current level of affected species in European ecosystems, and a large number of chronic ecotoxicity data for elaboration of acceptable Species Sensitivity Distribution (SSD) curves for each chemical. Assessment for a large number of chemicals, as required in LCA, does not therefore seem currently feasible without crudely simplifying assumptions.

Ways of improving LCIA methods for ecosystems are described in recent articles (Hauschild and Pennington 2003; Pennington, Payet et al. 2004). Following these indications, this chapter presents a methodology, the AMI method (Assessment of the Mean Impact), intended to provide a quantitative measure of toxicological impact on aquatic communities complying with the needs of comparative Life Cycle Assessment. In the framework of a Life Cycle Impact Assessment, methods have to provide a best estimate of the damage, avoiding bias, with respect to environmental relevance. At the same time, the method has to provide factors for many chemicals and use of most of the existing ecotoxicological databases is thus essential. It is then a question of developing a method providing a reliable comparative estimate of impact, with the possibility of assessing Effect Factors for many chemicals using a reliable statistical estimator.

For this purpose, several issues must first be addressed: (1) Which is the most suitable toxicological measure (EC50 or NOEC) for comparative assessment, also considering acute and chronic data availability? (2) Which indicator (most sensitive species, mean, median, geometric mean) is least sensitive to the selection of tested species and discriminates most between chemicals, and would therefore be appropriate as toxicity indicator? (3) How is it possible to assess the uncertainty of this indicator? (4) How can the extrapolation procedure from acute to chronic data be improved?
These questions will be addressed successively in the following sections: Section 2 studies ecotoxicological measures, while Section 3 addresses mathematical issues analysing the most appropriate statistical estimator and its associated uncertainty. Section 4 makes proposals for extrapolation from acute to chronic, and a review of data availability is provided in Section 5. Based on these outputs, the AMI method, presented in Section 6, offers the possibility of addressing diversity of exposed organisms and at the same time including a high number of chemicals. The AMI method is then described, detailing calculation of effect indicators and their Confidence Intervals, and an example of application of the AMI method comparing two fungicide treatment of a wheat crop is then presented.

Ecotoxicological measures

In assessment of chemical toxicity, test results are modelled in order to obtain a concentration-effect curve. This curve indicates the sensitivity of a species to a chemical. Several points on the curve are generally used as indicators, the most common being the EC50 and NOEC (No Observed Effect Concentration), but the EC5, EC10 or LOEC can also be used.

Several criticisms of the NOEC pointed out that the result is strongly dependent on the experimental design (Laskowski 1995; OECD 1998). Depending on whether the number of concentrations tested is high or low, the NOEC value - the highest concentration at which no effects are observed - may vary. The same remark can be applied to the LOEC (Lowest Observed Effect Concentration) - the lowest concentration at which effect occurs. In addition, for LCA it does not make sense to characterise the magnitude of an effect using a No Effect Concentration.

The EC5 and EC10 are less dependent on environmental design than the NOEC, but these effect levels cannot generally be distinguished from the test control, and these measurements are therefore below the level of observable effect in many cases (Isnard, Flammarion et al. 2001). Consequently, these data are mostly estimated via extrapolation and not confirmed experimentally.
Basing the effect indicator on acute or chronic EC50 data has a number of advantages in a comparative approach like LCIA: For most ecotoxicological studies, use of EC50 modelling recommends interpolating the EC50 level among concentrations tested. Consequently, the dose-effect ratio presents minimum variability at the 50% or mean effects level or close to that level of effect (Forbes and Forbes 1993; Riviere 1998).
As presented in Table 1, the EC50 value is the most frequently reported ecotoxicological endpoint for vertebrates, invertebrates and plants.
In LCIA, it is particularly relevant to explicitly link an impact like ecotoxicity to the damage it causes to exposed ecosystems, to enable comparison with damage caused by other impacts considered in LCA, such as land use or eutrophication. The link with different damage can be established through measuring the reduction of biodiversity (e.g. quantification of disappeared species). It is for example possible to define a connection between an ECx value and probability of disappearance (Tanaka and Nakanishi 2000). This link could not be established if the endpoint were a no-effect level like the NEC (No Effect Concentration) or NOEC.

Therefore acute and chronic EC50s are retained as the relevant measure for the new method developed.

Table 1 : Review of data availability from several databases (ECETOC, 2002; EU-Commission 2000; US-EPA 2001) for three taxonomic groups and three ecotoxicological measures.

		EC50	LOEC	NOEC	Total
Acute	Vertebrates	62219	753	2116	65088
	Invertebrates	42513	590	1718	44821
	Plants & Algae	5108	232	904	6244
	Total	**109840**	**1575**	**4738**	**116153**
Sub-ch. & chronic	Vertebrates	2419	1569	1955	5943
	Invertebrates	2908	1343	2052	6303
	Plants &Algae	3986	498	1205	5689
	Total	**9313**	**3410**	**5212**	**17935**

Statistical estimator and uncertainty

Toxicity test results can be converted into an Effect Factor using several methods. The oldest is based on the most sensitive species (EU-Commission 1996) and meets the requirements of the regulation concerning identification of a hazard threshold for each chemical in the environment. A possible alternative based on an SSD curve has been developed over the last two decades (Stephan, Hansen et al. 1985; Kooijman 1987; Aldenberg and Slob 1993; Aldenberg, Jaworska et al. 2002). Current SSDs use the 5^{th} percentile of the cumulative frequency distributions of chronic NOECs as basis for calculation of the effect indicator. Attempts have been made to adapt an impact assessment based on SSD to several compounds (Hamers, Aldenberg et al. 1996; Kleeper, Bakker et al. 1998; Huijbregts, VandeMeent et al. 2002).

A possible alternative is the use of a chronic $HC50_{EC50}$ (Hazardous Concentration affecting 50% of the species over their EC50 chronic level) as basis for the Effect Factor. The $HC50_{EC50}$ can be calculated on the mean, the median and the geometric mean of EC50 test results. The mean of the EC50 is not relevant in this framework since most of the data are log-normally distributed and the mean is therefore strongly influenced by the highest EC50s. The use of the median has been explored (Payet and Jolliet 2004), but this is a breakdown point estimator sensitive to multi-modal distributions. Since EC50s generally fit a log-normal distribution, the geometric mean appears the most

appropriate statistical estimator of the $HC50_{EC50}$. Furthermore, even in the case of multi-modal - distribution, this estimator is the most robust.

Furthermore, the statistical estimator developed in the LCIA framework for impact assessment on ecosystems has to meet several requirements, especially regarding data availability, statistical aspects, calculation of uncertainty and ecological realism. The PNEC, $HC5_{NOEC}$ and $HC50_{EC50}$ methods are analysed below, based on these requirements.

- *Data availability:* The estimator's applicability within the framework of LCA must also be addressed. An $HC50_{EC50}$-based method presents several advantages at this level. Using acute, chronic, and QSAR EC50s, it becomes possible to calculate Effect Factors for thousands of substances. This is an important point since Life Cycle Inventory (LCI) results typically cover several hundred substances, and a new method must ensure large coverage of the emissions. This would not be the case for an HC5 based on chronic data for example. The minimum requirement of 8 (Host, Regal et al. 1991) to 10 chronic toxicity data covering 8 different phyla (EU-Commission 2002) per substance can be met only for a few priority chemicals.

- *Statistical properties:* For comparative assessment, a stable indicator is required An Effect Factor based on the most sensitive species can vary considerably between databases, depending on whether a very sensitive species is included in the database, giving unstable Effect Factors. It is also necessary to prefer a best-estimate indicator like the geometric mean rather than an indicator like the PNEC using arbitrary extrapolation factors. In terms of statistical robustness, the $HC50_{EC50}$ based on the geometric mean is the most robust indicator since it is less sensitive to deviation from statistical assumptions than other HCxs.

- *Uncertainty calculation:* Furthermore, no considerable gain of information is made through assessment of the HC5 value compared to the HC50 value, while the discriminating power will decrease with assessment of the HC5 since the 5^{th} percentile has a larger Confidence Interval than the 50^{th} percentile (as presented in chapter 3). A key aspect of the method based on the $HC50_{EC50}$, in AMI is to provide an uncertainty estimate of the Effect Factor. In terms of interpretability, it is important for a decision-support tool like LCA to quantify result reliability. New LCA methods will address more and more uncertainty, both in LCI and

LCIA. With the AMI method, an attempt is made to provide an uncertainty estimate of the Effect Factor based on the $HC50_{EC50}$. The uncertainty is based on the Confidence Interval of the geometric mean. It is therefore possible to first compare the toxicity of different chemicals based on the average response of species, and it is also possible to compare the final results of an LCA study. For the assessment of a Confidence Interval on the $HC50_{EC50}$, two methods have been explored. A non-parametric estimate of the Confidence Interval (CI) around the geometric median using bootstrapping has first been explored (Payet and Jolliet 2004), and an alternative based on the geometric mean and a CI based on Student have been tested. Strengthes and weaknesses have been identified in both cases. Both methods quantify the 95% CI of the mean (or median), and in both cases, the size of the CI decreases when the number of data increases. Several divergences have been identified. First of all, there is no distribution assumption for the CI based on a non-parametric estimate, while those based on Student assume a log-normal distribution of the dataset. Secondly, the Student-based CI is less sensitive to outliers, while the non-parametric CI is more influenced by the very sensitive species, due to respect of the asymmetry of distribution. Furthermore, the Student-based CI can be calculated with three EC50s only (minimum data required by the AMI method), while the non-parametric CI based on bootstrap needs at least 5 data (unless we make an assumption concerning tail distribution). In conclusion, for the comparison the CI based on bootstrap seems adapted for specifically acting chemicals when several chronic EC50s are available, while the CI based on Student seems relevant for small samples or when EC50 data are log-normally distributed. Nevertheless, a Student- based CI involves the risk of excluding from the CI all data concerning a phylum if numerous data are available for a chemical. In order to avoid this problem, it is possible to substitute the average response of the most sensitive phylum to the lower limit if the phylum is outside the CI (this can concern pesticides, for example).

- Ecological realism: In terms of ecological realism, it makes sense to use the 50 percentile of affected species since the actual level of affected species in nature is likely to be between 20 and 50% (depending on the considered area, such as forest, agricultural, urban) (Hamers, Aldenberg et al. 1996). The effect slope of the linear model does not vary much between 20 and 50 %. This also means that even if we focus on toxic stress, field exposure concentrations are not relevant for LCIA. Indeed, species are exposed to several stressors at the same time and toxics act on the same biological species as other stressors so it is

therefore not relevant to consider this source of stress separately from others. For example, species already exposed to a lack of dissolved oxygen in water will be more sensitive to toxic stress (Stuijfzand, Helms et al. 2000).

The main aspects of the above analyses are summarised in Table 2, comparing the indicators (most sensitive species, $HC5_{NOEC}$ and $HC50_{EC50}$) on the basis of the main requirements for LCIA.

Table 2: Comparison of main indicators used for calculation of an Effect Factor.

	Most sensitive species	**$HC5_{NOEC}$**	**$HC50_{EC50}$**
Type of exposure	Acute or chronic	Chronic	Acute or chronic
Measure	EC50 and NOEC	NOEC	EC50
Range	Minimum value	5 %	50 %
Statistical robustness	-	low	high
Sensitivity to species addition	Very high	High	Low
Minimum Data requirement	1 EC50 or NOEC	10 chronic NOEC from 8 different phyla	3 EC50 from 3 phyla
Number of chem. expected	4500 without QSAR	<100	2000 without QSAR
Relation to damage	Not feasible	Not feasible	Possible link using a damage model
Ecological realism	Low	Unknown	High with multiple stressors
Uncertainty	Not feasible	Feasible	Available

Comparison of the different statistical estimators with LCIA requirements highlights the strength of the $HC50_{EC50}$ for comparative assessment. The $HC50_{EC50}$ based on the geometric mean has been selected for linking the change in concentration of a substance and the corresponding effect in the AMI method. This relationship is based on an average linear model ($HC50_{EC50}$ down to 0), which is the most common model when no assumption can be made concerning curve shape (Udo de Haes, Finnveden et al. 2003). For the uncertainty of the

$HC50_{EC50}$ value, lower and upper limits of the standard error on the mean (based on Student) are selected as best suitable for LCIA.

Extrapolation from acute to chronic

Analysis of ecotoxicological databases indicates that available data are mainly based on acute tests, with about 100 times more acute data than chronic data (ECETOC 1993; EU-Commission 2000; US-EPA 2001). For chronic Characterisation Factors, it is therefore important to define the best way to assess chronic toxicity on the basis of acute data.
Traditionally, extrapolation has been performed for individual tests. For a comparative approach like LCIA, best-estimate extrapolation factors are appropriate. As described by Roex et al. (Roex, VanGestel et al. 2000), analysing the relation between the chronic NOEC and the acute EC50, the Acute/Chronic Ratios (ACRs) depends on the type of chemicals, and this publication suggests the use of extrapolation factors of 2.6 for narcosis, 9.8 for polar narcosis, 17.3 for specific mode of action, and 15.3 for metals. These factors are coherent with results of other articles (e.g. Margni, M. et al. 2002).
This however led to a relatively high variability between acute and chronic test results, making comparative assessment difficult. With the aim of reducing variability and improving the quality of the relationship between acute and chronic, a more stable extrapolation procedure calculating a chronic $HC50_{EC50}$ directly on the basis of an acute $HC50_{EC50}$ has been developed. De Zwart (DeZwart 2002) indicates that it could provide better factors than the species taken individually. In addition it is not only the $HC50_{EC50}$ but also the lower and upper limits of the standard error on the mean that need to be extrapolated between acute and chronic, since the size of the Confidence Interval around the mean can be different between the acute and chronic $HC50_{EC50}$. Using data from the main databases (ECETOC 2002; EU-Commission 2000; US-EPA 2001), we have compared the acute and chronic $HC50_{EC50}$s of 92 chemicals. The $HC50_{EC50}$s for this analysis are presented in Appendix 1. The acute and chronic data have been selected in accordance with the indications in Appendix 2. The acute data are compared to both chronic and sub-chronic data gathered in a common group. Several groups of chemicals are covered by this study, including inorganics, non-pesticide organics , and pesticide organics.

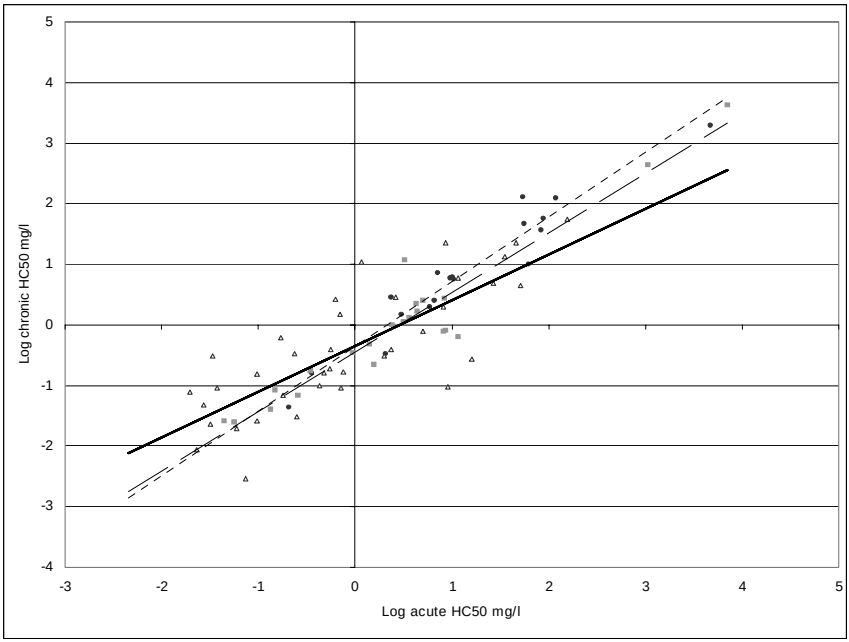

Figure 5 : Comparison between acute and chronic $HC50_{EC50}$s for 92 chemicals. ● : Non-pesticide organics (N=18); ■ : Inorganics (N=22); △: Pesticide organics except carbamates and organotins (N=37).Regression lines: Organics (…..): $HC50_{chronique}$ =-0.35+1.06$HC50_{acute}$ [intercept ± 0.22; slope ± 0.14]; Inorganics (------): $HC50_{chronique}$=-0.45+0.98 $HC50_{acute}$ [intercept ± 0.16; slope ± 0.13]; Pesticides except carb & organotins (_____).:$HC50_{chronique}$ =-0.35+0.75 $HC50_{acute}$[intercept ± 0.21; slope ± 0.19]

As presented in Figure 5, the ACRs for most of the chemicals are between a factor 1 and 10. The ACRs for $HC50_{EC50}$s are sometimes lower than one, which can be due to two reasons: species represented in the dataset can vary greatly between the acute and chronic $HC50_{EC50}$s; many of the chronic data are in fact sub-chronic and sometimes quite close to acute data. In spite of these limitations, the relation obtained between acute and chronic $HC50_{EC50}$s is very

good for non-pesticide organics and inorganics with an R^2 of 0.93 and 0.91 respectively. For pesticide organics (except carbamates and organotins), the relation is not so good with an R^2 = 0.64. For carbamate and organotin pesticides, the relation between acute and chronic $HC50_{EC50}$s presents considerable uncertainty with an R^2 of less than 0.3. Based on the data for these 92 chemicals, we have calculated a set of best-estimate extrapolation factors for the different groups of chemicals. Extrapolation factors concerning the lower and upper limits of the Confidence Interval of the geometric mean are also proposed. Results are presented in Table 3, and enable the use of both acute and chronic data in calculation of Effect Factors in the AMI method. Since the Confidence Interval on the slope include 1, a simple acute-chronic ratio instead of an equation linking the acute and the chronic data.

Table 3 : Best-estimate acute-chronic $HC50_{EC50}$ ratios (ACR) of non-pesticide organics, inorganics, and pesticide organics with ratios for the standard error on the mean based on Student.

	ACR $HC50_{EC50}$	ACR $HC50_{min}$	ACR $HC50_{max}$
ACR organics	1.9	4.2	0.8
ACR inorganics	2.8	7.4	1.1
ACR pesticides (except carbamates and organotins)	2.2	6.1	0.8

Data availability and selection

In terms of data availability, the trade-off between low data requirements and a reliable indicator is an important issue. A new method for the Life Cycle Impact of toxic effects on ecosystems requires a large number of reliable Effect Factors whereas there is only a very limited amount of data available and great variability between data for both acute and chronic effects. To help resolve this problem, indications for data selection are proposed. These indications are based on analysis of three databases and the indications of the European Technical Guidance Document (ECETOC 1993; EU-Commission 2000; US-EPA 2001; EU-Commission 2002).

Type of data

Several types of data can be used to represent the toxicity of substances. The most accessible are QSAR data (Quantitative Structure Activity Relationship), estimating the toxicity of a chemical from its molecular or chemical properties, typically the K_{ow} partitioning coefficient (Bradbury 1995; EU-Commission 1996; Posthumus and Sloof 2001). Acute toxicity data from laboratory tests are less accessible but in most cases more reliable. Chronic data is the most relevant but also scarce. Therefore, the method must allow the use of acute or chronic EC50s with a preference for chronic when available. For $HC50_{EC50}$s based on acute data, the extrapolation rules presented in Section 5 can be used for estimation of chronic $HC50_{EC50}$s. For experimental data, selection must also consider the reliability of test conditions; for example when the test result is greater than the solubility limit of the substance, or when the test duration is too short and the steady state between species and test solution may not be reached.

Distinction between acute and chronic

A recurrent problem in environmental risk assessment is the distinction between acute, chronic, and sub-chronic data. There is no consensus between scientists on this issue concerning animals, and the problem is even more complex when plants are considered, since the life cycle of unicellular algae can be very short and thus incompatible with the long-term toxicity tests required in chronic toxicity assessment. In these conditions, we made a choice based on a review of existing articles and standardised tests. The results of this analysis are presented in Appendix 2.

Minimum data requirement

There is also a need to ensure a good representation of several phyla (arthropods, chordates, etc.) to avoid artificial reduction of EC50 variability. In order to check the reliability of HC50EC50 based on small dataset (1, 2 or 3 phyla), we have compared, in Figure 6, $HC50_{EC50}$ values based on 1 to 5 phyla from small European and US databases (Mayer and Ellersieck 1986; EU-Commission 2000; ECETOC 2002) with $HC50_{EC50}$ reference values based on a

large database (maximum number of phyla available) (Mayer and Ellersieck 1986; EU-Commission 2000; US-EPA 2001; ECETOC 2002; US-EPA 2002).

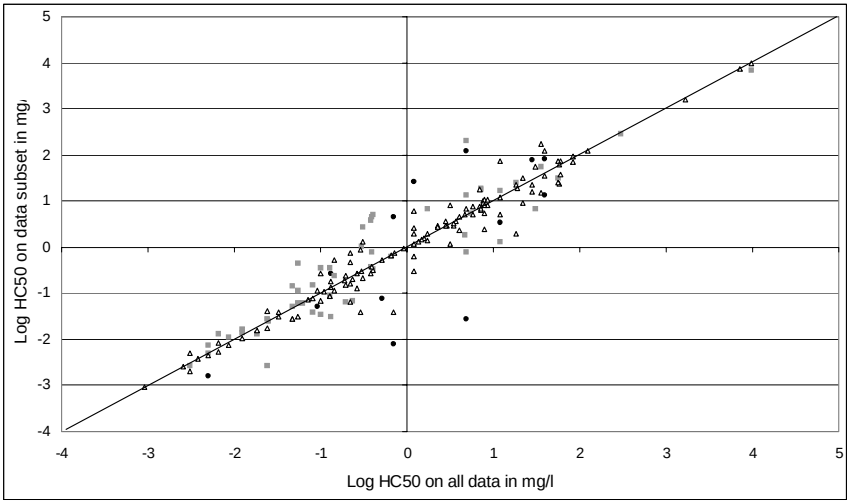

Figure 6 : Analysis of the reliability of the $HC50_{EC50}$ value according to the number of phyla used in the $HC50_{EC50}$ calculation. ● : $HC50_{EC50}$ on 1 phylum (N=13; R^2=0.56); ■ : $HC50_{EC50}$ on 2 phyla (N=52; R^2=0.86); △ : $HC50_{EC50}$ on 3 phyla and more (N=119; R^2=0.95).

Regression analysis indicates that confidence limits on the slope always include the slope of 1, and the origin is always included in the confidence limit on the intercept. Nevertheless, the R^2 can vary greatly depending on whether we are working with only 1 phylum or 3 to 5 phyla. Table 4 presents the main results of comparison between $HC50_{EC50}$s based on 1 to 5 phyla, and the reference $HC50_{EC50}$ with the variability of its 95% CI, and the corresponding R^2. From 3 phyla upwards, the Confidence Interval is about one order of magnitude and the R^2 is good at 0.95. Thus if the $HC50_{EC50}$ is based on EC50s from 1 or 2 phyla only, the value is too uncertatin to be used for calculating Effect Factors. Using

these results, the Effect Factor in the AMI method is therefore based on at least three acute or chronic toxicity data covering at least three taxa.

Table 4 : Average ratio between the $HC50_{EC50}$ based on 1 to 5 phyla with the reference $HC50_{EC50}$ and the 95% Confidence Interval with the corresponding R^2.

	Average	CL 95%	R^2
1 phylum	1.64	[0.01 ; 246.93]	0.56
2 phyla	0.76	[0.07; 8.12]	0.86
3 to 5 phyla	1.03	[0.28; 3.88]	0.95

Description of the AMI method

Based on the above results, the AMI method has been designed for use in comparative assessments, and consists of several steps: first calculation of the acute or chronic $HC50_{EC50}$ and its Confidence Interval, and then conversion of the results into an Effect Factor which can be multiplied by a Fate Factor for calculation of the Characterisation Factor.

Calculation of the $HC50_{EC50}$ (acute or chronic) and its uncertainty

Calculation of the Effect Factor for a chemical requires first a selection of the ecotoxicological data. All the EC50s, NOECs, and LOECs concerning the given chemical are collected. In order to normalise data, the ecotoxicological data are modified with a Log_{10} transformation. A list of test results covering several species is then available for the chemical, covering all sorts of effect and all test durations available. The next stage consists of distinguishing between acute and chronic/sub-chronic data on the basis of the indications in Appendix 2.

For a given chemical, the Log ($HC50_{EC50}$) is calculated as the mean of the Log (EC50) values. The EC50 values must cover at least three different taxa. The taxon can be animal phyla (for example arthropods, chordates) or the plant taxon (including algae). Tests based on bacteria are not used since they present

a very large variability depending on the genome of the organism. For a given chemical, if several EC50 values are available for the same species, the geometric mean of the EC50 is calculated to represent this species.

When data are lacking, the EC50 can be extrapolated from NOEC or LOEC data. In order to avoid bias in the extrapolation, we have made a comparison between acute EC50s and acute NOECs in Figure 7; chronic EC50s and chronic NOECs in Figure 8 and EC50s versus LOECs (both acute and chronic) on Figure 9.

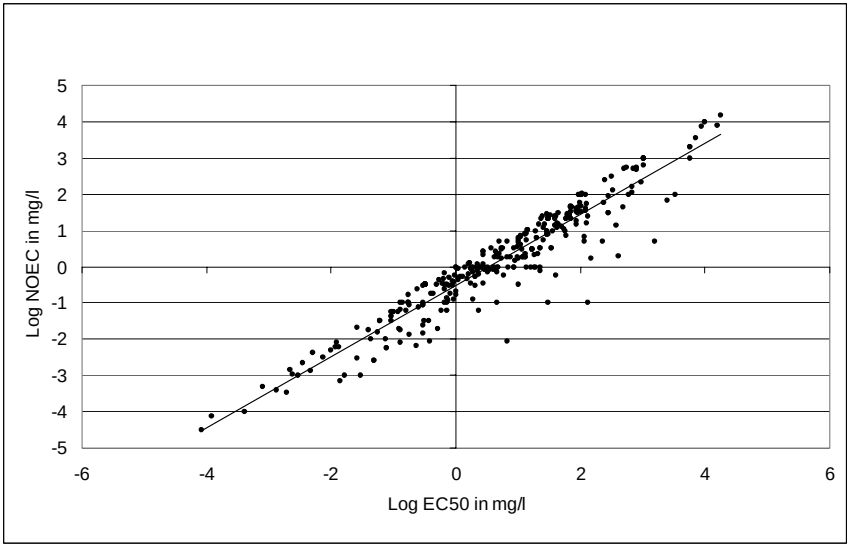

Figure 7 : Comparison between $EC50_{acute}$ data versus $NOEC_{acute}$ (N=329).

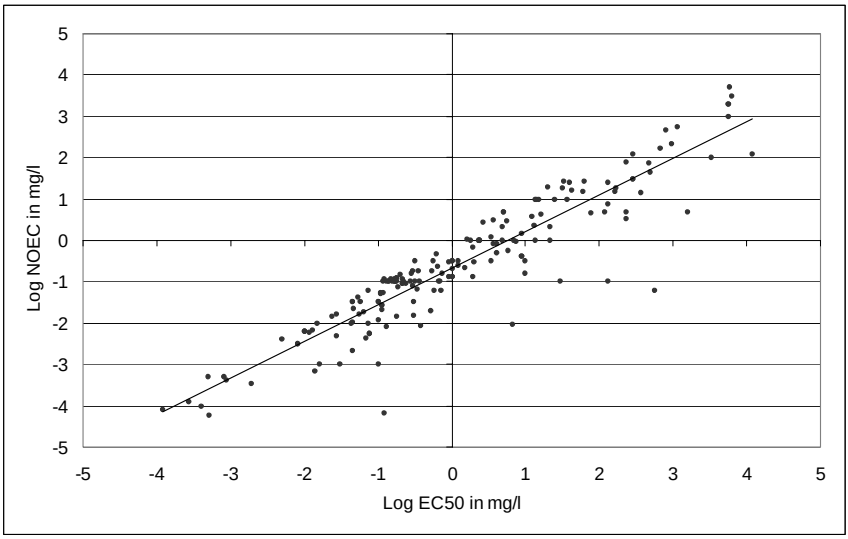

Figure 8 : Comparison between EC50chronic data versus $NOEC_{chronic}$ (N=182).

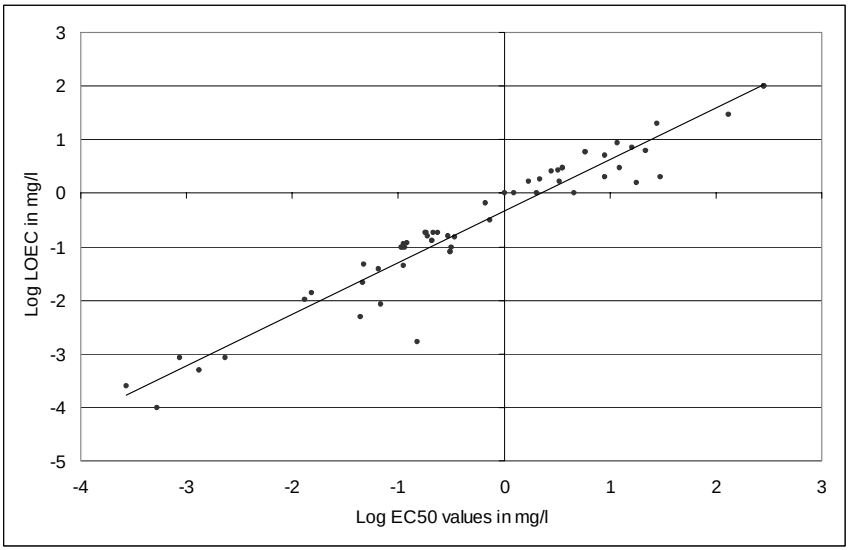

Figure 9 : Comparison between EC50 data versus LOEC (N=60).

Best-estimate extrapolation factors have been calculated on a dataset of 329 NOECs/EC50s for acute data, 182 NOECs/EC50s for chronic and sub-chronic data, and 60 LOECs/EC50s ratios (including both acute and chronic data) taken from three databases (ECETOC 1993; EU-Commission 2000; US-EPA 2001). The figures above confirms that EC50s are highly correlated to NOECs and LOECs both for acute and chronic data. The three regression lines are very close to each other, and the relation between EC50 and NOEC or LOEC is very good with R^2=0.90 for acute NOEC/EC; R^2=0.85 for Chronic NOEC /EC, and R^2=0.93 for LOEC/EC. For the LOEC, acute and chronic data have been put together since the data are closely interconnected. For the NOEC, a Student test comparing the acute and chronic NOECs indicates that the two groups are not similar [Ho: $\mu_1=\mu_2$ rejected with $T_{calc}.(11.8) > T_{0.05}(1.96)$]. Therefore, the two groups have been kept separate. Relations between EC50s and NOECs or LOECs are presented in Table 5. Nevertheless, the 95% Confidence Intervals on the slopes of the three regressions do not indicate a clear discrimination with a

slope of 1. This suggests that a simple ratio between NOECs or LOECs and EC50s is relevant in this situation. These ratios are presented in Table 6.

Table 5 : Regression results between the LogNOEC or the LogLOEC and the LogEC50, with the Confidence Limit (CL) on the parameters.

	Regression	CL intercept	CL slope
Log EC50 versus LogLOEC	Log LOEC= 0.30+0.97 EC50	± 0.10	± 0.07
Log $EC50_{acute}$ vs Log $NOEC_{acute}$	$LogNOEC_{acute}$= 0.54+0.91 $LogEC50_{acute}$	± 0.05	± 0.03
Log $EC50_{chronic}$ vs Log $NOEC_{chronic}$	$LogNOEC_{chron.}$= 0.66+0.96 $LogEC50_{chron.}$	± 0.10	± 0.06

Table 6 : Best-estimate ratios for extrapolation of the EC50 on the basis of a NOEC or a LOEC

Extrapolation	Ratio
EC50/LOEC	2.1
EC50 acute/NOEC acute	3.3
EC50 chronic/NOEC chronic	4.8

When chronic data do not cover three different taxa, calculation of chronic $HC50_{EC50}$s must be based on acute data. The procedure based on acute EC50s is similar to that based on chronic but the acute $HC50_{EC50}$ is finally divided by the extrapolation factors presented in Table 3 for extrapolating a chronic $HC50_{EC50}$.

To complete missing EC50 data, it is possible to include QSAR data in the assessment. Nevertheless, due to variability of QSAR data, we suggest using it carefully in the calculation of Effect Factors (Posthumus and Sloof 2001). Furthermore, it is also important to bear in mind that QSAR estimates tend to reduce data variability, and therefore tend to underestimate the uncertainty of the $HC50_{EC50}$s.

A Confidence Interval is associated with the acute or chronic $HC50_{EC50}$. Indeed, in a comparative assessment, as required in LCIA, it is crucial to identify whether chemicals present a difference in terms of toxicity. The AMI method associates uncertainty with the Effect Factor. For this purpose, the 95% Confidence Interval on the $HC50_{EC50}$ is calculated using the Student method:

$$Log(HC50_{EC50}) \pm \frac{1}{\sqrt{n}} \times t_{n-1}^{0.05} \times SDev(Log(EC50)) \quad (1)$$

$HC50_{EC50}$ Hazardous concentration affecting 50% of the species over their EC50

n Number of species tested

$t_{n-1}^{0.05}$ t value from the Student table for a 95% Confidence Interval with n-1 degree of freedom

Sdev Standard deviation of the EC50

The Confidence Interval is dependent on the number of species considered in the calculation. The size of the Confidence Interval for a chemical can be reduced by the inclusion of data concerning new species. For specifically acting chemicals, the Confidence Interval can be underestimated; therefore the average sensitivity of the most sensitive phyla can sometimes be regarded as the lower limit of the Confidence Interval. For that reason, AMI Effect Factors database also provides the average response of the most sensitive phyla when this value is not included in the Student Confidence Limit.

Calculation of the Effect Factor

The Effect Factor in LCIA for ecosystems must represent most of the species potentially affected by the chemical. For this reason, the PAF_{EC50}, which is the fraction of species affected over their chronic EC50, has been selected for AMI. The relationship between variation of substance concentration and corresponding effect is based on an average linear model (equation below), which is the most appropriate model when no assumption can be made concerning curve shape.

The following equation is used for calculation of the Effect Factor based on the acute or chronic $HC50_{EC50}$:

$$EF = \frac{\Delta PAF}{\Delta C} = \frac{0.5}{HC50_{EC50}} \qquad (2)$$

EF Change in the *Potentially Affected Fraction* of species that experiences an *Increase* in stress for a change in contaminant concentration [m^3 kg^{-1}]
C Exposure concentration [kg m^{-3}]
$HC50_{EC50}$ Geometric mean of the hazardous concentration affecting 50% of the species tested above their EC50
PAF Potentially Affected Fraction of species due to exposure to the chemical for which an EF is derived.

This equation describes the Effect Factor, which is defined by the slope of the HC50 down to 0 and has the following properties: (1) it increases with greater toxicity (lower $HC50_{EC50}$); (2) it can be interpreted as the change in PAF due to a unit increase in concentration.

The procedure for calculation of Effect Factors is summarised in Figure 10.

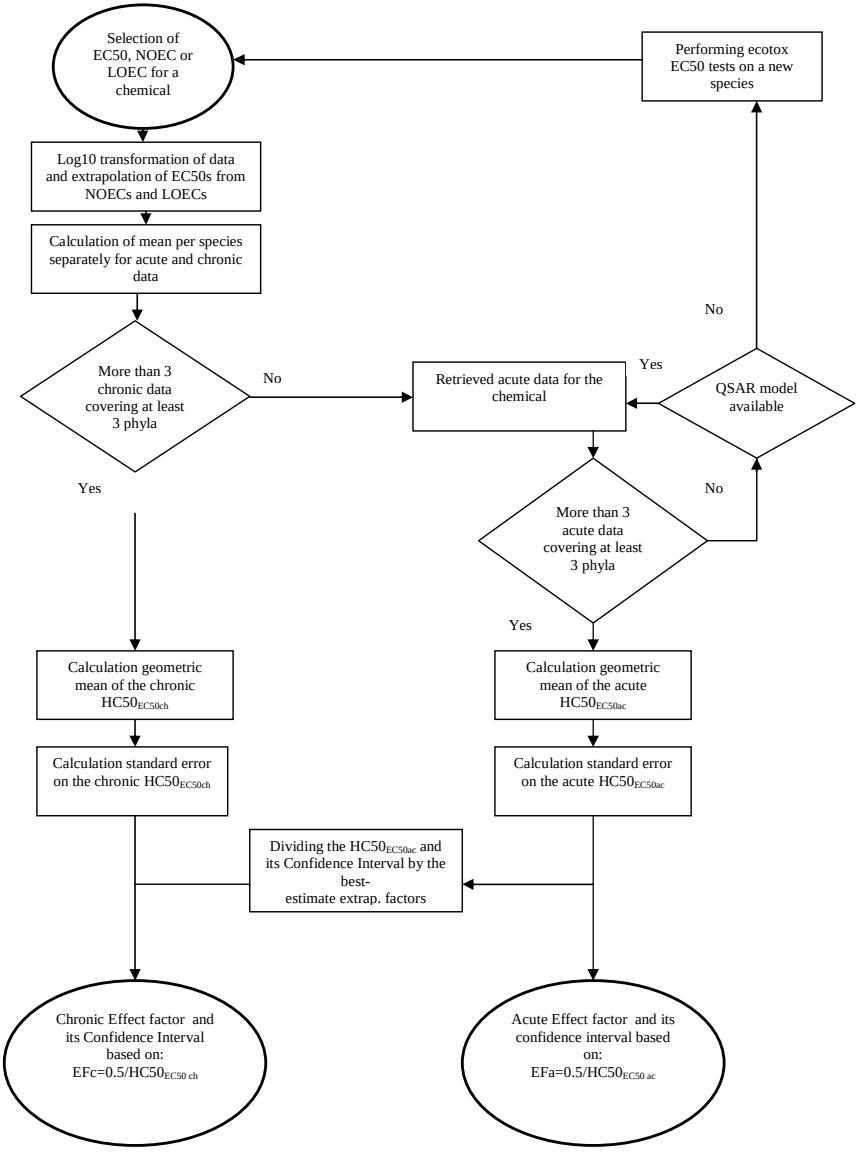

Figure 10 : Procedure for calculation of AMI Effect Factors

For application in the LCIA framework, assessment of the impact of a pollutant emitted in an aquatic ecosystem is obtained by multiplying the change in concentration of the pollutant in the ecosystem by the Effect Factor. This change in concentration can be measured in the water medium, or estimated using fate modelling. In this case, the potential hazard of each chemical can be described by a Characterisation Factor, product of a Fate Factor multiplied by the Effect Factor. The equation expressing the fate and effect of the chemical in terms of impact is described by Jolliet et al (2003) and presented below:

$$CFa_i = F_i^{mw} \cdot \theta_i^{w} \cdot EF_i \qquad (3)$$

CFa_i is the charcterisation factor for aquatic ecosystem due to a substance i and expressed in PAF·m^3·year/kg

F_i^{mw} is the dimensionless fraction of the emission of substance i in compartment m transferred to fresh water

θ_i^{w}, in years, is the equivalent residence time of substance i in water.

On the basis of this equation, it is possible to express an emission in a given compartment in terms of fraction of affected species in the aquatic ecosystem. This link is feasible with most fate modelling provided that it translates chemical emissions calculated in the Life Cycle Inventory into an increase in concentration in the relevant medium for a defined time period.

Example of application: comparison of two fungicides treatment

A simplified application comparing two agricultural alternatives for a wheat crop is presented as an illustration of the AMI method. The example is based on the impact assessment of substitutable fungicides, Chlorothalonil (1897-45-6) and propiconazole (60207-90-1).

The two scenarios compared differ regarding the amount and type of pesticides applied during one year in one hectare of agricultural soil. The fraction transferred to water directly and via air and soil is calculated with the fate model IMPACT 2002 fate model (Jolliet, Margni et al. 2003). IMPACT 2002 translates the amount applied in the agricultural soil during one year into an amount transferred in water integrated over time and space. Therefore, the

impact is quantified on the basis of an average increasing concentration of water in the ecosystem for a given chemical, due to the emission in the agricultural field during one year.

The amount of Chlorothalonil applied in 1 hectare of crop during one year is 4.5 kg while the amount of Propiconazole required for the same conditions would be 0.3 kg. Running the IMPACT 2002 fate model, we calculate the total mass of chlorotalonil and Propiconazole in the water compartment after a transfer via air and soil of respectively 3.16 kg and 0.07 kg (with a transfer fraction from the application to water of 0.048 for Chlorothalonil and 0.0094 for Propiconazole).

At the same time, we calculate Effect Factors based on the chronic toxicity data presented in Appendix 3. The final Effect Factors and the Confidence Interval are presented in Table 7.

Table 7 : $HC50_{EC50}$s (in mg/l) and chronic Effect Factors (in m^3 kg^{-1}) for Propiconazole and the Chlorothalonil with their Confidence Limit

	$HC50_{EC50}$	Effect Factors
Chlorothalonil	0.0425 [0.0009; 2.062]	11773.5 [242.5; 571647.5]
Propiconazole	1.16 [0.37; 3.66]	429.8 [136.6; 1352.0]

We therefore calculate the increase in concentration in the freshwater compartment for the European fresh water volume (2.10^{12} m^3). The impact in the freshwater ecosystem is therefore calculated by multiplying the change in concentration by the Effect Factor of the substance. The fraction of freshwater species potentially affected by the use of fungicides in wheat crops is $1.86.10^{-8}$ PAF.m3.year/Ha treated for Chlorothalonil with Confidence Limit of [$3.82.10^{-10}$; $9.03.10^{-7}$] and $1.5.10^{-11}$ PAF.m3.year/Ha for Propiconazole with Confidence Limit of [$4.76.10^{-12}$; $4.73.10^{-11}$].

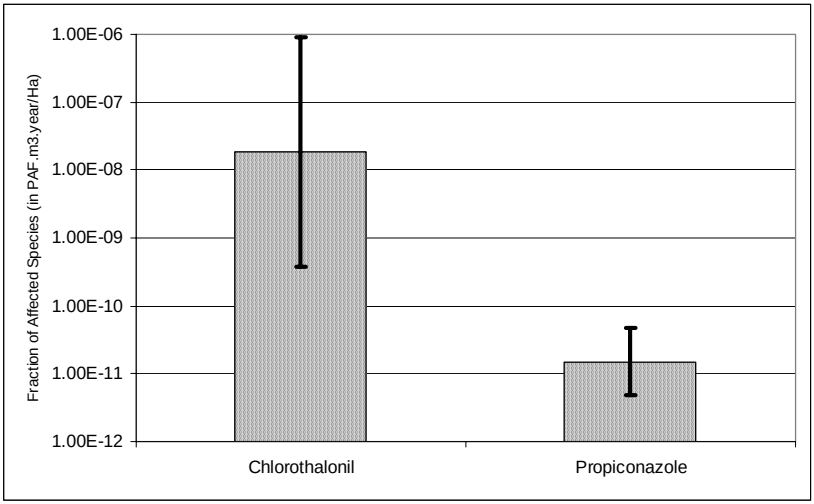

Figure 11 : Comparison of the impact on freshwater ecosystems of two substitutable fungicide treatments applied on wheat crops

Propiconazole presents an impact significantly smaller than Chlorothalonil with a difference between the two scenarios of more than three orders of magnitude. Considering the Effect Factors, the Confidence Interval of Chlorothalonil (tested with four species) is quite large, with more than three orders of magnitude, while that of Propiconazole (tested with 13 species) is only one order of magnitude. In order to reduce the uncertainty, more species could be tested for Chlorothalonil.

As presented with the example, the AMI method offers the possibility of comparing results in terms of fraction of potentially affected species for the aquatic ecosystems, taking into account the Confidence Interval of the Effect Factor. First, it helps to decide between the alternatives by considering the reliability of the Effect Factor. Second, it helps to prioritise data collection for the improvement of Effect Factors. A limitation remains however concerning calculation of the uncertainty of inventory data and Fate Factor.

Conclusions

The AMI method enables the assessment of numerous substances with an indicator based on several species, and providing a Confidence Interval on the Effect Factor. The method avoids bias due to a conservative approach and distortion caused by the use of indicators based on different sorts of PNEC. At the same time, the AMI method is already implemented in the IMPACT 2002 model for the assessment of toxicological impacts on human and ecosystems in Life Cycle Assessment, giving satisfying results while fulfilling LCA requirements (linear effect models, integration of impacts over time and space, comparative assessment).

Compared to former methods, AMI improves the comparison between potential effects of chemicals, associating a Confidence Interval with the geometric mean of the response of species in order to facilitate the interpretation of results in terms of predicted impact on ecosystems.

The key features of the AMI method are :

- Regarding data selection, the choice of EC50s as the ecotoxicological measure for the indicator avoids certain risky assumptions concerning an expected linear relationship between concentration and effect under the no-effect-concentration, as current approaches suggest.
- Regarding the limited availability of ecotoxicological data and the requirement for estimator stability, $HC50_{EC50}$s (i.e. the geometric mean of EC50s) offer considerable improvement in comparison with the most sensitive species.
- The assessment of a Confidence Interval based on the standard error on the $HC50_{EC50}$ using the Student method also provides acceptable results for small samples of species and is helpful for result interpretation. Nevertheless, such a method for assessment of the Confidence Interval assumes a log-normal distribution of the EC50 for all chemicals. This method is the most robust but must be corrected if it leads to the exclusion of the most sensitive phylum from the Confidence Interval for chemicals with a particular Toxic Mode of Action.
- The development of best-estimate acute-to-chronic extrapolation factors, both for the geometric mean response of species and its Confidence Interval, gives a more reliable relationship between acute and chronic

data than species-by-species extrapolation. At the same time, the calculation of best-estimate factors for extrapolation from the NOEC or LOEC to the EC50 offers the opportunity of using most of the available data in ecotoxicology.

- The Effect Factors based on $HC50_{EC50}$s offer the possibility of expressing the Potentially Affected Fraction of species (PAF) in terms of biodiversity losses, creating a possible link with other ecosystem stressors considered in LCIA.

Nevertheless, some limitations remain which require further research concerning several important aspects: (1) The link with impact on terrestrial ecosystems; only aquatic ecosystems are currently covered by AMI; (2) A distinction between freshwater and marine ecosystems is necessary for a better estimate of the impact and is currently not addressed in AMI; (3) A better consideration of the bio-availability of metals: current Effect Factors are based on an average estimate of metal toxicity which is not relevant in the field since metal toxicity is determined by bio-availability due to environmental parameters; (4) Biodiversity modelling is necessary for weighting the impact of toxics with the other stressors (eutrophication, acidification, etc). An endpoint indicator like biodiversity should also provide a better picture of the whole level of impact on ecosystems; (5) For calculation of the uncertainty, the current calculation approach, based on Student, presents some weaknesses with the exclusion of some phyla when many EC50s are available. Non-parametric estimate of the Confidence Interval has to be explored further since it could be more relevant for specifically-acting chemicals. Concerning the uncertainty, its use is currently limited since it concerns only the Effect Factor, but the LCA interpretation will be facilitated when the uncertainty is also associated with the inventory results and fate model.

In spite of these limitations, the AMI method nonetheless provides an Effect Factor database for the comparative assessment of aquatic toxicity for more than 500 chemicals. This database, including the geometric mean ($HC50_{EC50}$) of the toxicity and the standard error on the geometric mean, presented in the last part of the thesis, makes the method applicable for Life Cycle Assessment studies.
An updated version can be downloaded from [http://gecos.epfl.ch/lcsystem].

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Appendix 1: Acute and chronic $HC50_{EC50}$s of the 92 chemicals used for ACR calculation

		Acute			Chronic		
Chemical name	**Class**	**N species**	**Log HC50 (EC50)**	**Standard deviation**	**N species**	**Log HC50 (EC50)**	**Standard deviation**
CHLOROFORM	Organic	31	1.916623717	0.755106088	10	1.565486167	0.985151789
3-TRIFLUOROMETHYL-4-NITROPHENOL	Organic	86	1.021012329	0.520200875	17	0.756009202	0.36335451
BIS(2-ETHYLHEXYL)PHTHALATE	Organic	33	0.766538547	1.306871056	19	0.291368603	1.407701961
ACETONE	Organic	59	3.674907392	0.856811137	11	3.295184456	0.938440609
TOLUENE	Organic	52	1.748229685	0.655731182	4	1.672720382	0.308529824
1,1,2-TRICHLOROETHANE	Organic	36	1.940246229	0.334056333	22	1.75725096	0.515553499
TRICHLOROETHENE	Organic	45	1.737638857	0.455656598	5	2.110612348	0.546179668
1,2,3-TRICHLOROBENZENE	Organic	12	0.307752242	0.37036775	5	-0.476191894	0.418189806
1,2-DICHLOROBENZENE	Organic	23	0.853727615	0.649687071	4	0.860828486	0.845569792
3,4-DICHLOROANILINE	Organic	44	0.480691306	0.763860897	26	0.182601847	0.660910561
4-CHLOROPHENOL	Organic	36	0.982098841	0.822852068	11	0.768947016	0.631575017
CHLOROBENZENE	Organic	24	1.002404194	1.004866654	11	0.79648849	1.098028575
PHENOL	Organic	227	1.798010314	0.865502145	25	0.998324424	1.081441014
1,2,4-TRICHLOROBENZENE	Organic	46	0.373831129	0.544975818	7	0.464522944	0.521178366
PENTACHLOROBENZENE	Organic	14	-0.447622647	0.779060046	9	-0.789554054	0.882817917
METHYLENE BISTHIOCYANATE	Organic	11	-0.688197433	0.905555988	3	-1.349791377	0.173901006
1,4-DICHLOROBENZENE	Organic	20	0.81719202	0.505498935	7	0.410004931	0.84234249
1,1,1-TRICHLOROETHANE	Organic	23	2.075240223	0.860798285	4	2.095160381	0.958441471
LEAD	Inorganic	21	0.625780848	0.800536166	13	0.344719631	1.206676141
NICKEL	Inorganic	39	0.937373013	0.976159938	8	-0.09127676	1.318549551
SILVER	Inorganic	22	-1.246393695	1.018982225	3	-1.608356135	0.72695666
CADMIUM	Inorganic	93	0.153797525	1.207534573	28	-0.325667002	1.322407716
CHROMIUM	Inorganic	46	0.701615487	1.138355688	9	0.404813266	0.814056536
COPPER	Inorganic	96	-0.460882876	1.301304544	30	-0.756312174	0.770997247
ZINC	Inorganic	66	0.504176559	0.975335595	18	0.058162411	0.878705594
ZINC SULFATE HEPTAHYDRATE	Inorganic	18	-0.033630419	1.233314941	5	-0.454208645	1.312047488
Copper chloride (CuCl2)	Inorganic	115	-0.828098543	0.915656808	44	-1.083346842	1.257539781
Potassium chloride	Inorganic	38	3.026999619	0.51791944	6	2.634037614	0.463778826
Zinc chloride (ZnCl2)	Inorganic	146	0.388682547	1.035189903	27	-0.008935697	0.929100763
SODIUM CHLORIDE	Inorganic	49	3.849922239	0.484368039	8	3.625329732	0.200384985

		Acute			Chronic		
Chemical name	**Class**	**N species**	**Log HC50 (EC50)**	**Standard deviation**	**N species**	**Log HC50 (EC50)**	**Standard deviation**
AMMONIA	Inorganic	84	0.514919515	0.862586197	5	1.079078639	0.997461421
Nickel chloride (NiCl2)	Inorganic	71	1.06525542	0.948060439	18	-0.197466226	1.23192034
LEAD CHLORIDE	Inorganic	49	0.560487273	0.925885809	23	0.127326717	1.228767208
SILVER NITRATE	Inorganic	89	-1.361039389	0.954812268	21	-1.593102065	0.754511421
SELENIUM	Inorganic	15	0.63729415	0.884912912	7	0.228388962	0.989970666
LEAD NITRATE	Inorganic	86	0.907335477	1.1710546	15	-0.107458087	1.643226457
Cadmium dichloride	Inorganic	351	0.194644766	1.155125764	93	-0.661926549	1.381104823
AMMONIUM CHLORIDE	Inorganic	157	0.928487671	0.932157359	30	0.435288947	0.995182052
MERCURY CHLORIDE	Pesticide	232	-0.868079561	0.899496985	37	-1.385770421	0.729666126
COPPER SULFATE PENTAHYDRATE	Pesticide	34	-0.59076465	0.936707584	16	-1.171787204	0.858899969
AMITROLE	Pesticide	27	1.716516295	1.3091071	6	0.645461303	0.880055872
SIMAZINE	Pesticide	62	1.200789476	1.07669006	27	-0.570037112	1.179194188
DIURON	Pesticide	56	-0.118926586	1.213865508	21	-0.775738178	1.718930854
BROMOXYNIL	Pesticide	7	0.416077278	0.548369902	5	0.449678748	0.888405638
ATRAZINE	Pesticide	103	0.304116179	1.091457659	54	-0.50539187	0.858152679
		Acute			**Chronic**		
Chemical name	**Classe**	N species	Log HC50 (EC50)	Standard deviation	N species	Log HC50 (EC50)	Standard deviation
ETHOFUMESATE	Pesticide	12	1.059490827	0.542360187	5	0.769355091	0.477958687
METOLACHLOR	Pesticide	21	0.373024084	0.82047155	10	-0.408860533	0.808364739
HEXAZINONE	Pesticide	27	0.95795935	1.70670869	12	-1.017528241	1.088078109
P,P'-DDT	Pesticide	223	-1.477163592	1.232934298	23	-0.513244356	1.902131626
FENTHION	Pesticide	124	-1.011898125	1.384871498	7	-0.808309448	1.507328528
PARATHION	Pesticide	140	-1.127027177	1.425388806	14	-2.543242068	1.389882762
GAMMA-HEXACHLOROCYCLOHEXANE	Pesticide	175	-0.747146439	1.214250449	25	-1.172699519	1.473948548
DIMETHOATE	Pesticide	68	0.06141115	1.740447457	12	1.034057757	0.962291381
DIELDRIN	Pesticide	134	-1.493413875	1.018230255	30	-1.645302988	1.196454711
METHOXYCHLOR	Pesticide	96	-1.570811733	0.806222614	20	-1.321207662	1.845390785
METHYL AZINPHOS	Pesticide	76	-1.424916087	1.421855603	17	-1.047858748	1.901929133
ENDOSULFAN	Pesticide	158	-1.703114011	1.401233703	15	-1.107216474	1.392514764
MALATHION	Pesticide	111	-0.363693653	1.19907562	11	-0.99995399	1.345944451
FENITROTHION	Pesticide	140	-0.76655813	1.444279313	17	-0.21357597	1.486871785
KEPONE	Pesticide	29	-1.018377901	0.550092454	9	-1.585853336	0.951401283
PARATHION METHYL	Pesticide	125	-0.199432402	1.52583616	10	0.412928299	1.206803472
ALDRIN	Pesticide	79	-1.221059913	1.092843362	7	-1.714764503	1.024618792

		Acute			Chronic		
Chemical name	**Class**	**N species**	**Log HC50 (EC50)**	**Standard deviation**	**N species**	**Log HC50 (EC50)**	**Standard deviation**
DIAZINON	Pesticide	105	-0.631484374	1.317641067	6	-0.47661643	1.918979636
PROPARGITE	Pesticide	18	-0.31919822	1.340779482	7	-0.795006836	2.10852329
TOXAPHENE	Pesticide	77	-1.641620368	1.03634137	8	-2.063488671	0.99003569
DODECYL SULFATE, SODIUM SALT	Pesticide	103	0.9349557	0.819198405	10	1.358930157	0.929783177
NICLOSAMIDE ETHANOLAMINE SALT	Pesticide	90	-0.263013979	1.037427878	11	-0.723976006	0.538661235
PENTACHLOROPHENOL	Pesticide	165	-0.255249016	0.935174972	43	-0.407854918	0.772164136
HEXACHLOROBENZENE	Pesticide	22	-0.605122652	1.63842328	6	-1.512128728	0.703493122
CAPTAN	Pesticide	39	-0.159757816	1.019242647	7	0.171918726	0.957223018
CARBOXIN	Pesticide	10	0.696187072	1.03637595	4	-0.101982605	0.120286619
Cymoxanil	Pesticide	14	0.912806247	0.939532994	9	0.293429448	1.229609507
PROCHLORAZ	Pesticide	8	-0.140313678	0.729733409	4	-1.03409982	0.51745372
2,4,5-T triethylammonium salt	Pesticide	18	2.190797951	0.693842986	8	1.742405891	0.82929489
MECOPROP	Pesticide	6	1.667461606	0.734751579	4	1.355566358	0.753717743
2-METHYL-4-CHLOROPHENOXYACETIC ACID	Pesticide	26	1.425586894	1.142512964	9	0.688566126	1.268982842
2,4-DICHLOROPHENOXYACETIC ACID	Pesticide	67	1.542618345	1.252646173	15	1.12704954	1.133623576
MOLINATE	Herbicide (thiocarbamate)	47	0.804359529	0.716469121	15	0.323019393	0.767398544
THIOBENCARB	Herbicide (thiocarbamate)	54	0.137211129	0.594401221	12	-0.656257387	0.757310776
CARBOFURAN	Insecticide (carbamate)	63	-0.46312603	1.197201267	3	0.068813862	2.205237921
1-NAPHTHYL-N-METHYLCARBAMATE	Insecticide (carbamate)	169	-0.023477262	1.241560693	11	0.121904648	1.010066217
NABAM	Fungicide (dithiocarbamate)	12	0.22152827	0.654800842	4	-0.046228706	0.702772007
MANEB	Fungicide (dithiocarbamate)	25	0.275691506	1.061778381	5	-0.342710263	1.286857379
THIRAM	Fungicide (Dimethyldithiocarbamate)	31	-0.889067439	1.515989597	4	-1.446617799	1.177277582
ZIRAM	Fungicide (Dimethyldithiocarbamate)	20	-0.522427402	0.711272918	5	-1.267533877	1.127635978

		Acute			Chronic		
Chemical name	**Class**	**N species**	**Log HC50 (EC50)**	**Standard deviation**	**N species**	**Log HC50 (EC50)**	**Standard deviation**
FERBAM	Dimethyl thiocarbamate	17	-0.20384682	0.828950491	3	-1.374817624	1.579126745
DIMETHYLDITHIOCARBAMIC ACID, SODIUM SALT	Dimethyl thiocarbamate	13	0.065063671	1.076347663	3	-1.51589733	1.283810788
TRIPHENYLTIN HYDROXIDE	Organotin	29	-0.977785779	1.569313029	4	-4.009983936	2.246254834
Tributylstannane	Organotin	8	-2.243973708	1.300769776	5	-2.131574011	1.566727418
TRIBUTYLCHLOROSTANNANE	Organotin	35	-2.137465523	1.006658835	19	-3.251947229	1.341029684
TRI-N-BUTYLTIN FLUORIDE	Organotin	15	-2.344384611	0.932513527	3	-3.426613566	1.018076703
BIS(TRI-N-BUTYLTIN) OXIDE	Organotin	62	-1.744438525	1.31565359	29	-2.386519622	0.720610406

Appendix 2 : Distinction between acute and chronic ecotoxicity data

Determination of the exposure duration for acute, sub-chronic and chronic tests used in this analysis originates from guidelines from ISO, OECD, US-EPA. FIFRA, ASTM, UBA, and publications from Heger et Al, (Heger, Jung et al. 1995), ECETOC (ECETOC 1993), and the European Technical Guidance Document (EU-Commission 2002).

Table A1: Description of the time duration retained for the distinction between acute, sub-chronic and chronic aquatic toxicity tests

	Acute	Sub-chronic	Chronic (1) (2)
Vertebrates	Tests < 7 days	7 days Tests < 32 days	32 days Tests
Invertebrates	Tests < 7 days	7 days Tests < 21 days	21 days Tests
Plants	Tests < 7 days	-	7 days Tests
Algae	Tests < 3 days	-	3 days Tests

(1) For chronic tests, the endpoint addresses a whole Life Cycle or a sensitive life stage (ex: larvae, young, etc); the endpoint can be biochemical or histo-pathological effects, Growth (length and/or weight), hatch, reproduction, larval development or mortality, young development or mortality, emergence, behaviour, and daphnia immobilization or mortality.
(2) The time duration indicated for the selection between acute and sub-chronic is from Heger et al. (1995) for the distinction between acute and sub-chronic. Distinction between sub-chronic and chronic is much more complex since the relevant time duration for a chronic test depends on the species and life stage tested. For this reason, we have presented here the time duration of chronic tests that is mostly found in the available databases. Nevertheless, in some cases, chronic tests can be carried out with shorter time duration both for fishes and invertebrates. This is the case for example for fishes with the ASTM 7-day test on larvae, or for crustaceans with the 7-day test on *Ceridaphnia* dubia. Therefore, the above table presents only general indications but is not valid for all tests. It is recommended that the original guidelines be referred to.

Appendix 3: Ecotoxicity data used for calculation of the $HC50_{EC50}$ of the Chlorothalonil and Propiconazole.

Chronic aquatic toxicity data for Chlorothalonil (1897-45-6)

Species	Phylum	Measure	Tox. Level (mg/l)	Est. EC50	Ref. database
Mysidopsis bahia	Crustacea	LOEC	0.001	0.00252	Aquire (US-EPA 2001)
Pimephales promelas	Fish	LOEC	0.007	0.01365	Aquire (US-EPA 2001)
Daphnia magna	Crustacea	LOEC	0.079	0.1659	Aquire (US-EPA 2001)
Scenedesmus subspicatus	Algae	EC50	0.570	0.57	IUCLID (EU-Commission 2000)

Chronic aquatic toxicity data for Propiconazole (60207-90-1)

Species	Phylum	Measure	Tox. Level (mg/l)	Est. EC50	Ref. database
Anabaena flosaquae	Algae	EC50	8.63	8.63	Aquire (US-EPA 2001)
Anabaena flosaquae	Algae	EC50	13.58	13.58	Aquire (US-EPA 2001)
Cyclotella	Algae	EC50	3.30	3.3	Aquire (US-EPA 2001)
Cyprinodon variegatus	Fish	LOEC	0.29	0.609	Aquire (US-EPA 2001)
Daphnia magna	Crustacea	LOEC	0.69	1.449	Aquire (US-EPA 2001)
Lemna gibba	Aquatic plant	EC50	9.02	9.02	Aquire (US-EPA 2001)
Microcystis aeruginosa	Algae	EC50	1.00	1	Aquire (US-EPA 2001)
Mysidopsis bahia	Crustacea	LOEC	0.51	1.071	Aquire (US-EPA 2001)
Navicula seminulum	Algae	EC50	0.09	0.093	Aquire (US-EPA 2001)
Pimephales promelas	Fish	LOEC	0.18	0.378	Aquire (US-EPA 2001)
Pimephales promelas	Fish	LOEC	0.21	0.441	Aquire (US-EPA 2001)
Raphidocelis subcapitata	Algae	EC50	0.72	0.72	Aquire (US-EPA 2001)
Raphidocelis subcapitata	Algae	EC50	1.50	1.5	Aquire (US-EPA 2001)
Scenedesmus subspicatus	Algae	EC50	6.30	6.3	Aquire (US-EPA 2001)
Skeletonema costatum	Algae	EC50	0.02	0.021	Aquire (US-EPA 2001)
Synechococcus leopoliensis	Algae	EC50	4.50	4.5	Aquire (US-EPA 2001)

CHAPTER 3

Comparison of Existing Methods for Life Cycle Impact Assessment on Aquatic Ecosystems*

* To be submitted

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Abstract

Four methods are currently used for the development of Effect Factors for the Life Cycle Impact Assessment (LCIA) on Ecosystems: AMI (Assessment of the Mean Impact) based on $HC50_{EC50}$s; the Eco-Indicator based on $HC50_{NOEC}$s; USES-LCA based on both $HC5_{NOEC}$s and the most sensitive species, and the PNEC (Predicted No-Effect Concentration) based on the most sensitive species. After presentation of the LCIA framework and its main divergences from Environmental Risk Assessment for chemical regulation, the four methods are detailed and applied for the calculation of Effect Factors for 83 substances, covering inorganics, non-pesticide organics, and pesticide organics. Each method is therefore analysed concerning three key points: applicability in the LCA framework, environmental relevance, and statistical reliability.

Particular attention is paid to possible bias and the uncertainty, highlighting the following findings: (1) $HC5_{NOEC}$s are on average 50 times higher than the most sensitive species, and this difference in conservatism introduces a bias in the analyses for the method mixing $HC5_{NOEC}$s and most sensitive species. (2) Effect Factors based on the most sensitive species increase the relative weight of the most toxic chemicals by two orders of magnitude, depending on whether the study is based on US or European ecotoxicity databases. (3) the methods based on $HC50_{EC50}$s and $HC5_{NOEC}$s are the only ones able to provide a Confidence Interval on the Effect Factor, but the Confidence Interval on the $HC5_{NOEC}$s can be more than 4 orders of magnitude greater than that of the $HC50_{EC50}$s. (4) compared with the Confidence Interval on the $HC50_{EC50}$s, the most sensitive species cannot be distinguished from $HC50_{EC50}$s for chemicals characterised by fewer than five species, and the $HC5_{NOEC}$s cannot be distinguished from the $HC50_{EC50}$s for chemicals characterised by fewer than eight species.

Key words: LCIA, ecosystems, AMI, PNEC, HC50, HC5, Uncertainty

Introduction

The aim of Life Cycle Assessment (LCA) is to compare environmental impacts of products providing an equivalent service. This assessment is based on several steps. For a given product, the emissions of each substance in each environmental compartment (air, water and soil) are integrated over time and space, thus setting up the Life Cycle Inventory (LCI) of the product. Next, emission inventories are converted into impacts on the environment by the Life Cycle Impact Assessment (LCIA), considering several classes of impact such as global change, human health, etc. For impact assessment on ecosystems, a Fate Factor converts the amount of pollutant emitted by a product in all media into a change in concentration in a given medium (water for example). The change in concentration is converted into an impact on the ecosystem using an Effect Factor. This value of toxic impact on aquatic or terrestrial ecosystems is compared with other classes of impact related to ecosystems (such as eutrophication or acidification), and possibly added up expressing a final impact on the ecosystem due to several stressors.

When comparing methods, the evaluation criteria are closely related to the objective of the considered application. The assessment of impacts on ecosystems in LCIA is mainly based on knowledge developed in Environmental Risk Assessment (ERA) for chemical regulation. Nevertheless, LCIA presents considerable specificities regarding the following points.

- The principle of threshold is debated, but commonly used in risk assessment (Cairns 1992; Forbes and Forbes 1994). In LCIA, impact assessment is based on a functional unit (e.g: a unit of product or service) and can concern very small emissions of compounds, which may only constitute a minute fraction of total chemical emissions. *LCIA aims at assessing the impact of marginal increase in a chemical emission in a comparative way.*

- ERA for chemical regulation is based on a conservative approach; the goal is to protect ecosystems against unacceptable levels of risks and consequences (EU-Commission 2002). This choice leads to a bias with the overestimation of risks of some chemicals. Uncertainty in estimation can vary from one substance to another because of the data available and differences in extrapolation techniques. This approach does not fulfil LCA requirements. Indeed, *LCA aims at comparison and an unbiased method is required, associated with the estimation of uncertainty.*

- An LCIA approach must consider all the chemicals listed in the Life Cycle Inventory (usually several hundreds of chemicals varying between studies), which may include larger emissions of many low-toxic chemicals (with little data available). *LCIA methods should enable good and reliable discrimination for a large number of substances.*

-Several ecotoxicological measures can be used as a basis for the LCIA on ecosystems, the NOEC or LOEC (No -or Lowest observed Effect Concentration), or the ECx (Effect concentration affecting X% of the individuals of a population). The NOEC and LOEC are criticised for being strongly dependent on experimental design (Laskowski 1995; Chèvre 2000; Isnard, Flammarion et al. 2001), while EC5 or EC10 are typically below the minimum level of observed effect (Forbes and Forbes 1994; Kooijman 1996; Crane and Newman 2000; Isnard, Flammarion et al. 2001). In *LCIA, the ecotoxicological measure must be based on an actual effect level, independent of experimental design.*

-LCIA simultaneously considers several classes of impact on ecosystems such as eutrophication, acidification, etc. *It is therefore important that effect measures for toxic impact can be converted as far as possible into impact on biodiversity* (Snell and Serra 2000), and therefore combined with impacts on ecosystems due to other stressors.

Four main methods for impact assessment on ecosystems have been adapted from ERA to LCA (Schulze, Jödicke et al. 2001; Hauschild and Pennington 2003). Each method will be analysed regarding several questions in particular: (1) How far can the methods be applied to a large number of chemicals? (2) Are there any sources of bias in the Effect Factors? (3) How stable are the Effect Factors, regarding the selection of tested species, addition of new data, or change in databases? (4) To what extent can the methods rank the chemicals on the basis of toxicity, associating a Confidence Interval with the Effect Factor? (5) How relevant are the results regarding environmental conditions?

Addressing the above questions, the analysis starts with a description of the four methods and then identifies the strengths and weaknesses of each. On the basis of a dataset of 83 Effect Factors calculated using the PNEC, $HC5_{NOEC}$, Combi-PAF and AMI methods, a comparison is made regarding the uncertainty range of the different methods and considering their applicability to LCA, environmental relevance, and statistical reliability.

Description of Current Methods

The four methods for LCIA on ecosystems are presented below. The PNEC method (Predicted No-Effect Concentration) includes two versions, one based on the most sensitive species and the other on the $HC5_{NOEC}$ [Hazardous Concentration affecting 5% of the species above the NOEC] and is directly adapted from ERA (Guinée, Heijungs et al. 1996; Wenzel, Hauschild et al. 1998; Huijbregts 1999). The Combi-PAF method was developed for the effect assessment of mixtures in ERA (Hamers, Aldenberg et al. 1996) and adapted

to LCA (Goedkoop, Effting et al. 2000). The AMI method was developed especially for LCA (Payet and Jolliet 2004) and is based on the $HC50_{EC50}$.

PNEC Based on the most sensitive Species

The PNEC based on most sensitive species is currently used by the EDIP (Eco-Design for Industrial Products) method (Hauschild and Wenzel 1998; Wenzel, Hauschild et al. 1998). This method is directly based on the raw data, using as basis for calculation of the Effect Factor the lowest toxicological data among different tests results concerning different trophic levels. Depending on data quality, the PNEC is assessed applying a safety factor on the lowest EC50 or the lowest NOEC. Effect Factors based on PNEC are calculated as described below.

$$EF = \frac{1}{PNEC} \qquad (1)$$

where EF is the Effect Factor, or Aquatic Ecotoxicity Potential (unit : $m^3.g^{-1}$) and PNEC : Predicted No-Effect Concentration for the substance in the aquatic ecosystem (unit : mg/l).

As developed in EDIP, this method does not use a reference substance, and provides both acute and chronic Effect Factors. The Effect Factor is multiplied by a change in concentration in the water ecosystem for impact calculation. The use of the PNEC in the context of LCA requires the assumption of a linear extrapolation from PNEC down to 0.

The $HC5_{NOEC}$ Method

A possible alternative to the PNEC based on the most sensitive species is use of the $HC5_{NOEC}$ value instead. As presented in Figure 12, the $HC5_{NOEC}$ is calculated on the basis of an SSD (Species Sensitivity Distributions) (Kooijman 1987; Sloof 1992; Aldenberg and Slob 1993; Hauschild and Wenzel 1998; Wenzel, Hauschild et al. 1998; Stephan 2001), assessing the concentration affecting 5% of species above their NOEC level. This method has been adapted for LCIA (Huijbregts, Thissen et al. 2000) with the USES-LCA approach using the Effect Factor below.

Calculation of SSDs has been described in detail in several publications (Aldenberg and Jaworska 2000; DeZwart 2002). The SSD relates a concentration of toxic in a particular medium (water for example) to a Fraction

of Affected Species over a given ecotoxicological measure as illustrated in Figure 12.

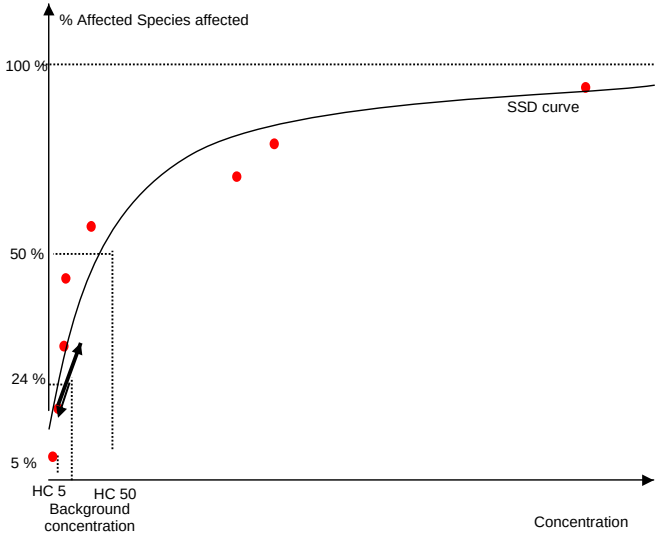

Figure 12: Using an SSD curve based on NOECs for 8 species for calculation of the $HC5_{NOEC}$ (USES-LCA); the slope of effect at the background concentration (Eco-Indicator), and the $HC50_{EC50}$ (AMI).

The equation is shown below.

$$F(C) = \frac{1}{1 + e^{\frac{\alpha - \log C}{\beta}}} \qquad (2)$$

where F(C) is the Fraction of Affected Species at a given concentration, α is the average of the Log10-transformed toxicity values and β is the scale parameter estimated from the standard deviation (S) of the log-transformed toxicity values (β=0.55 x S)

Based on this equation, the HCx for a given Fraction of Affected Species (F) can therefore be calculated using:

$$LogHCx = \alpha - \beta \times Ln\left(\frac{1}{F} - 1\right) \quad (3)$$

This calculation is required for assessment of the Effect Factors based on the $HC5_{NOEC}$ and Combi-PAF methods, as presented below.

$$EF = \frac{0.05}{HC5_{NOEC}} \quad (4)$$

where EF is the Effect Factor, or Aquatic Ecotoxicity Potential (unit : $m^3.g^{-1}$) and $HC5_{NOEC}$ is the Hazardous Concentration of substance affecting 5% of the species above their NOEC in the aquatic ecosystem (unit : mg/l).

Unlike the EDIP method, USES-LCA uses both the $HC5_{NOEC}$ and PNEC based on the most sensitive species. Nevertheless, with USES-LCA, impacts are always related to a reference substance and the Effect Factors address only chronic exposure.
The method has been further improved with an assessment of the potential influence of mixtures, comparing effect additive and concentration additive models (Huijbregts, VandeMeent et al. 2002). Furthermore, the need for a chronic NOEC-based SSD has been often considered a restriction, since many data are required. In order to solve this problem, an extrapolation method has been proposed enabling the calculation of a chronic SSD using acute EC50s (DeZwart 2002; Roelofs, Huijbregts et al. 2003). Two attempts have been made for considering mixtures and acute ecotoxicity data (Roelofs, Huijbregts et al. 2003) in USES-LCA but characterisation factors have not been provided yet.

The Combi-PAF Method

The Combi-PAF method presented in Eco-Indicator (Goedkoop, Effting et al. 2000) is based on SSD. This method aims at calculating the Fraction of Affected Species (PAF) due to a change in toxic concentration. This method has several specificities. Effect Factors are calculated using a theoretical SSD curve of mixture, Combi-PAF, characterised by a β=0.4. A reference value is chosen on the Combi-PAF curve representing the current ambient level of toxic stress (working point) and the marginal change in Fraction of Affected Species at the working point is used as basis for impact calculation (current level of toxic stress is estimated at 24% and converted into a slope of effect of 0.593 on the Combi-PAF). Toxics arriving in a given medium are converted into Hazard Units (Equation 5) and summed up, following the indications of a

concentration additive mixture model (Hamers, Aldenberg et al. 1996). Change in Fraction of Affected Species due to change in Hazard Units is calculated on the basis of the slope of effect.

The Effect Factor is based on the marginal variation of the Fraction of Affected Species due to a change in Hazard Unit in an environmental medium, and can be expressed as:

$$\Delta HU = \frac{\Delta C}{HC50_{NOEC}} \quad (5)$$

$$EF = \frac{\Delta PAF}{\Delta HU} = \frac{Se}{HC50_{NOEC}} \quad (6)$$

where ΔHU is the change in Hazard Units due to a change in the concentration ΔC of a substance in the medium; EF is the change in the *Potentially Affected Fraction* of species that experiences an *Increase* in toxic unit (TU) [$m^3.g^{-1}$], Se : Slope of effect at 24% of Fraction of Affected Species on the Combi-PAF curve (Se=0.593), and $HC50_{NOEC}$ is the Hazardous Concentration affecting 50% of species over their NOEC expressed in mg/l.

The AMI Method

The AMI (Assessment of the Mean Impact) method (Payet and Jolliet 2004), has been developed for LCA and recently implemented in the IMPACT 2002 model for LCIA (Jolliet, Margni et al. 2003). This method can use both acute and chronic toxicity data and does not require the calculation of an SSD curve.

The method is typically based on assessment of the average chronic toxicity for a chemical tested with several species from at least three groups of organisms (typically one vertebrate, one invertebrate, and one plant). The geometric mean of the chronic EC50s ($HC50_{EC50}$) has been retained as the best statistical estimator for calculation of Effect Factors. Furthermore, the uncertainty on the Effect Factor is calculated on the basis of the 95% limits of the Confidence Interval around the geometric mean based on Student, as shown below.

$$EF = \frac{\Delta PAF}{\Delta C} = \frac{0.5}{HC50_{EC50} \times 1000} \quad (7)$$

where EF is the Change in the *Potentially Affected Fraction* of species experiencing an increase in stress due to a change in contaminant

concentration [m^3 kg^{-1}]; ΔC is the change in exposure concentration [kg m^{-3}]; $HC50_{EC50}$ is the Hazardous concentration affecting 50% of the species tested over their chronic EC50 (in mg/l) and PAF is the Potentially Affected Fraction of species due to exposure to the chemical for which an EF is derived.

The above equation can be interpreted as a change in Fraction of Affected Species in the aquatic ecosystem beyond the concentration range. The Effect Factor will get lower when the average toxicity of the substance decreases (increasing HC50). The Confidence Interval on the $HC50_{EC50}$ corresponds to the standard error on the mean and has the property of decreasing when the number of EC50s available for calculation of the $HC50_{EC50}$ increases. This method is based on the underlying assumption of a log-normal distribution of EC50s. If this assumption cannot be fulfilled, an alternative calculation of the Effect Factor can be made using a non-parametric estimator based on the median with an uncertainty based on bootstrap [Payet & Jolliet, 2004]. Another limit identified was the low availability of chronic EC50s, making it impossible to obtain reliable Effect Factors for hundreds of chemicals. Best-estimate extrapolation factors were determined to calculate a chronic $HC50_{EC50}$ value and its Confidence Interval based on acute data.

Results

For comparison between methods, Effect Factors for 83 chemicals have been computed, covering 23 inorganic chemicals, 30 non-pesticide organics, 30 pesticide organics. All Effect Factors are calculated using EC50s and NOECs from several databases (Mayer and Ellersieck 1986; EU-Commission 2000; US-EPA 2001; ECETOC 2002; US-EPA 2002). From these databases, all the chronic EC50s for these 83 substances were selected and served as a basis for the calculation of Effect Factors. Therefore, Effect Factors differ only by the computation method and ecotoxicity measure (EC50 or NOEC).

As for Effect Factors based on an SSD, typically, a minimum number of data of 8 to 20 species tested with chronic NOECs is required for calculation of an SSD (Host, Regal et al. 1991; EU-Commission 2002). Aldenberg and Jaworska (2000) suggests that this extrapolation could be performed down to 2 species. In order to study a wider range of chemicals, here we will calculate the $HC5_{NOEC}$s and $HC50_{NOEC}$s down to three species (and three different animal phyla or plant taxa) and discuss the related uncertainty.

Comparing Statistical estimators

Statistical regressions between the Effect Factors from each method have been performed for each group of chemicals mentioned above. For the comparison, inorganics, non-pesticide organics, and pesticide organics are gathered into one group. Results are presented in Figure 13.

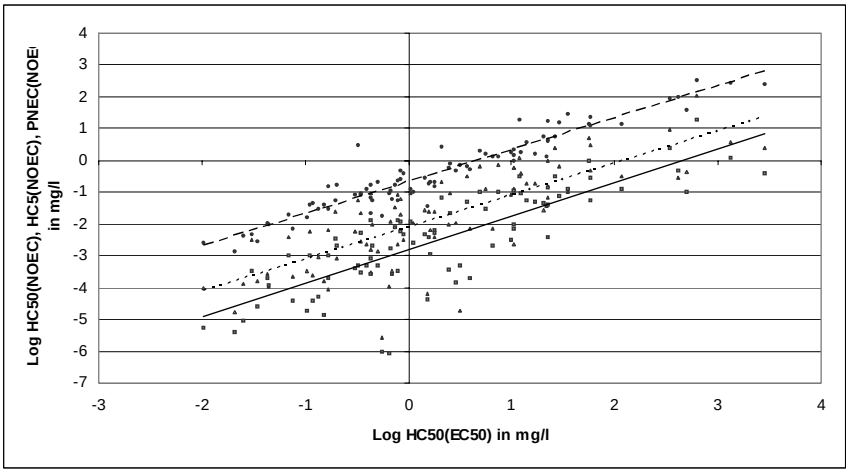

Figure 13 : Comparison between indicators: $HC50_{NOEC}$ (Eco-Indicator) •, $HC5_{NOEC}$ (USES-LCA) Δ , $PNEC_{NOEC}$ (EDIP) ■ versus the $HC50_{EC50}$ (AMI). Regressions: dotted line: $HC50_{NOEC}$ vs. $HC50_{EC50}$; dashed line: $HC5_{NOEC}$ vs. $HC50_{EC50}$; black line: $PNEC_{NOEC}$ vs. the $HC50_{EC50}$

The equations of the regression lines shown in Figure 13 are detailed in Table 8.

Table 8 : Relation between the $HC50_{NOEC}$ (Eco-Indicator), $HC5_{NOEC}$ (USES-LCA) , $PNEC_{NOEC}$ (EDIP) versus the $HC50_{EC50}$ (AMI). Each equation is represented with the confidence interval on the slope and on the intercept.

Equations	Slope CI	Intercept CI	R^2
$HC50_{NOEC}$= 1.00 $HC50_{EC50}$ -0.67	[0.93; 1.07]	[-0.75; -0.58]	0.91
$HC5_{NOEC}$ = 1.01 $HC50_{EC50}$ - 2.12	[0.83; 1.18]	[-2.33; -1.90]	0.61
$PNEC_{NOEC}$ =1.06 $HC50_{EC50}$ - 2.79	[0.89; 1.22]	[-2.99; -2.59]	0.67

Small Sample Size

Figure 13 presents good relations between the indicators, nevertheless the quality of the relation depends on the number of species available for the assessment. In order to better understand the influence of small samples on the different indicators, we made a regression between the $HC50_{EC50}$ (AMI) on the one hand and the $HC50_{NOEC}$ (Eco-Indicator) the $HC5_{NOEC}$ (USES-LCA), and the PNEC (EDIP) on the other hand for chemicals tested with 3 or 4 species (N=34 substances) or with 5 species and more (N=49 substances). The results indicate a constant R square independently of the sample size both for the $HC50_{NOEC}$ and the $PNEC_{NOEC}$ with respective R square of R^2=0.90 and R^2=0.63. However, for the $HC5_{NOEC}$, we observed a difference with a R^2=0.44 for small samples and a R^2=0.68 for large samples. This suggests that the $HC5_{NOEC}$ model tends to overestimate the relative influence of the lowest NOECs compared to the highest ones.

Uncertainty Calculation

The comparison between methods also addresses the question of uncertainty on the Effect Factors. Among the four methods, USES-LCA and AMI can provide a Confidence Interval on the Effect Factor. Calculation of the 95% Confidence Interval on the $HC50_{EC50}$ (AMI) is based on Student, and the 90% Confidence Interval on the $HC5_{NOEC}$ (USES-LCA) is based on Aldenberg's table (Aldenberg and Jaworska 2000). The level of confidence differs between the two indicators but the 95% CI on the $HC5_{NOEC}$ is not provided in Aldenberg's table and the comparison is based on existing tools.
A first comparison addresses the reliability of each Effect Factor. The most sensitive species (PNEC) and the upper limit of the 90% Confidence Interval on the $HC5_{NOEC}$ are compared to the 95% Confidence Interval on the $HC50_{EC50}$ (AMI). Furthermore, in order to ascertain whether the Confidence Interval on the $HC50_{EC50}$ (AMI) is able to cover all the phyla tested, the geometric mean of the EC50s of the most sensitive phyla (called MP in Table 9) is also compared to the Confidence Interval on the $HC50_{EC50}$. Results are presented in Table 9.

Table 9 Comparison between the geometric mean of the most sensitive phylum (MP), the most sensitive species (MS) versus the Confidence Interval on the $HC50_{EC50}$.

N species[1] (number of chemicals concerned)[2]	MP included[3]	MS included[4]
3 (3)	3	3
4 (8)	8	8
5 (6)	6	3
6 (8)	4	0
7 (3)	3	2
8 (8)	4	0
9 (4)	3	0
10 (6)	4	0
11-15 (11)	7	0
16-30 (17)	3	0
31-68 (9)	1	0

(1) Number of species considered
(2) in brackets, number of chemicals assessed with N species
(3) Number of cases where the geometric mean of the most sensitive phylum is included in the confidence interval on the $HC50_{EC50}$
(4) Number of cases where the most sensitive species is included in the confidence interval on the $HC50_{EC50}$

If fewer than 5 species are available for calculation of the Effect Factor, it appears that the most sensitive species (MS) is not statistically different from the geometric mean $HC50_{EC50}$. Comparing the $HC50_{EC50}$ and geometric mean of the most sensitive phylum (MP), there are no differences between values for fewer than 5 species, and the geometric mean of the most sensitive phylum is generally included in the Confidence Interval of the $HC50_{EC50}$ until 15 species have been considered. In order to better understand the influence of the HC50 and the HC5 model, we have compared the HC50 to the HC5 calculated using similar EC50s, the threshold of 8 species appears to be the limit below which the Confidence Interval of the HC50 and HC5 are overlapping.

Calculation of the uncertainty of the Effect Factor also helps in interpretation of results. A reliable interpretation of the LCIA results is only possible if the Confidence Interval is not too great compared to the spread of the Effect Factors, ensuring a good discriminating power between toxics. As presented in Figure 14, the maximum spread of the Confidence Interval on the $HC50_{EC50}$ or $HC5_{NOEC}$ can cover respectively more than 7 or 11 orders of magnitude. Furthermore, the Confidence Interval on the $HC50_{EC50}$ depends on the number of species tested and is less than 2 orders of magnitude for 91% of the chemicals considered in the study and less than 5 orders of magnitude for 97% of the toxics. At the same time, the Confidence Interval on the $HC5_{NOEC}$ is also

dependent on the number of species tested per chemical but is lower than 2 orders of magnitude for only 47% of the chemicals, and lower than 5 orders of magnitude for 84% of toxics.

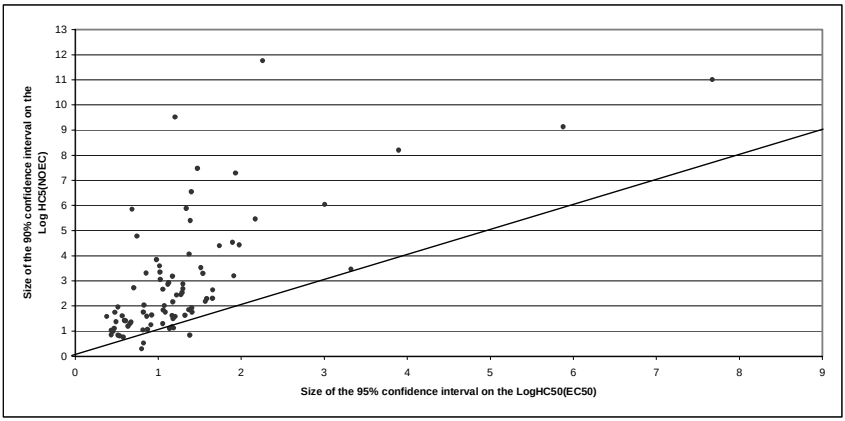

Figure 14: Comparison between the size of the 90% confidence interval of the $HC5_{NOEC}$ and those of the $HC50_{EC50}$ based on 83 substances (black line represent the 1to 1 line)

Figure 14 shows the comparison between sizes of the 90% Confidence Interval of the $HC5_{NOEC}$ versus the 95% Confidence Interval $HC50_{EC50}$ for 83 substances. The Confidence Interval of the $HC5_{NOEC}$ is on average 45 times higher than the CI of the $HC50_{EC50}$. Nevertheless, for the smaller range of Confidence Intervals, the CI on the $HC5_{NOEC}$ or $HC50_{EC50}$ is nearly the same size while there is nearly 4 orders of magnitude difference for the greater range.

Discussion

The comparison between AMI ($HC50_{EC50}$) and Eco-indicator ($HC50_{NOEC}$) because Effects Factors does not differ strongly, but the two methods are using different theoretical approaches, and different input data (AMI is based on EC50s while Eco-Indicator uses NOECs). Eco-indicator places the emphasis on the mixture model with an explicit concentration additive approach and the background concentration of chemicals. AMI highlights the importance of data availability, calculation of uncertainty, and transparency.

Comparing AMI ($HC50_{EC50}$) with EDIP (PNEC), USES-LCA ($HC5_{NOEC}$) and Eco-Indicator ($HC50_{NOEC}$) it appears that methods give the same relative weight to the chemicals. Figure 13 also shows an average difference of a factor of 50 between EDIP (PNEC) and USES-LCA ($HC5_{NOEC}$).
The PNEC based on the most sensitive species is on average 5 times more sensitive than the $HC5_{NOEC}$. The difference in the intercept of the regressions between EDIP (PNEC) and USES-LCA ($HC5_{NOEC}$) indicates that it would not be appropriate to mix Effect Factors based on the $HC5_{NOEC}$ and Effect Factors based on the most sensitive species in the same LCA study. This is likely to introduce a bias in the study, confirming the assumptions of formers studies (Heijungs, Guinée et al. 1992; Guinée and Heijungs 1993).

The analysis of the methods will be carried out according to the method's applicability for LCA, its environmental relevance and the statistical reliability of the Effect Factor.

Applicability in LCA Framework

Applicability in LCA depends on data availability for calculating Effect Factors for a large number of chemicals and compatibility with fate modelling assumptions.

Data availability: the LCI typically covers several hundred chemicals, and the LCIA method applied for impact assessment must provide factors for most of these chemicals. Currently, Effect Factors in most LCIA methods do not cover enough chemicals, with 76 factors provided for EDIP methods, 46 substances for Eco-Indicator, 181 for USES-LCA, and 522 for AMI. The restriction is usually data accessibility. Eco-Indicator and USES-LCA are based on chronic NOECs, while AMI is based on chronic EC50s and provides a set of best-estimate extrapolation factors if only acute EC50s are available.

Compatibility with fate modelling assumptions: Effect Factors must be compatible with fate. Indeed, fate models in LCIA work under steady-state assumption (Guinée and Heijungs, 1996), and with integration of the concentration of toxic over time and space. The unit and time and space scale of the effect model must thus be compatible. This is the case for the four methods presented here since they are already applied to LCA studies.

Environmental Relevance Criteria

Environmental relevance has often been addressed in LCA: ISO addresses the question of environmental relevance mainly at a general level, considering the ability of the category indicator to reflect the consequences of the Life Cycle Inventory (LCI) on the category endpoint (ISO 2000). Furthermore, ISO 14 042 requires a clear description of the spatial and temporal aspects, and the uncertainty of the links between the characterisation model and changes in category endpoints. This definition of the environmental relevance is general and needs to be clarified focusing on questions related to ecosystems. Indeed, the four methods satisfy the definition of environmental relevance as described above, except concerning uncertainty, which cannot be addressed by the PNEC method. As to the other points, three of the methods translate the midpoint and endpoint (USES-LCA does not), and there is always a clear description of spatial and temporal aspects. In order to go further on this point, we address the question of environmental relevance for the impact assessment on ecosystems in the following questions.

Taking account of mixtures: The four methods are based on the assumption of a concentration additive model of the toxic impacts, this being well detailed in the Eco-Indicator with reference to Hamers work (Hamers, Aldenberg et al. 1996). This assumption is validated by research mentioning that complex mixtures of toxics tend to fit the concentration additive model for mixtures (Pedersen, Kristensen et al. 1994).

Chronic or acute exposure: Another aspect of environmental relevance for ecotoxicity indicators is exposure duration. LCIA works with time-integrated models and must strive for long-term exposure (Hauschild and Pennington 2003). At the same time, new tendencies in LCA are to include accidental situations and eventually background concentration of chemicals. This will lead to situations where high concentration exposure of chemicals will have to be considered, corresponding to acute exposure testing in ecotoxicology. Currently only chronic Effect Factors are applied in most LCA studies, and all four methods provide chronic factors. Nevertheless, if acute factors are required, only EDIP and AMI provide both acute and chronic factors. Furthermore, to enable the use of most of the data available, extrapolation from acute to chronic exposure are required. Such an extrapolation is possible with the EDIP and AMI method.

Multiple stressors and compatibility with endpoint modelling: The main aspect of the environmental relevance of the indicator is interpretability of results in terms of quantitative weighting of the ecotoxicity impact score against the

scores of other impact categories (ISO 2000; Hauschild and Pennington 2003). LCIA deals with multiple environmental stressors, such as ecotoxicity, eutrophication, acidification, etc. and the model selected for ecotoxicity assessment must be interpretable in the same unit as other impact categories. This unit could be a change in biodiversity for example. Using EC50 data as basis for calculation of Effect Factors offers the possibility of making a link with the endpoint indicator, such as biodiversity losses. AMI in IMPACT 2002 and Combi-PAF in Eco-Indicator make a link with other stressors affecting ecosystems, expressing results in terms of Fraction of Affected Species. EDIP makes the link on the basis of the volume of medium altered at the No- Effect level for each class of impact.

Background of effect or background of exposure to chemicals: The question of the working point (which is the actual level of damage on the ecosystems) is often discussed in LCA (Goedkoop, Effting et al. 2000; Pennington, Payet et al. 2004). On the one hand, monitoring of chemicals in the environment generally identifies very small concentrations of individual chemicals in the environmental medium, and this exposure is thus considered environmentally relevant. This corresponds to concentrations ranging typically in the same order of magnitude as the $HC5_{NOEC}$ or PNEC. It would better make sense to take into account that 10 to 50% of species are already affected (Kleeper, Bakker et al. 1998) and consider the toxic impact on the present species. For this second situation the Eco-Indicator method, calculating impact at a background level of 24% of species affected, and the AMI method, taking a linear model from the $HC50_{EC50}$ down to 0 (which is very close to the average of the slope between 10 and 50%) are more relevant. Species are exposed to several stressors simultaneously and therefore stressor effects are assumed to be additive (possibly synergistic) (Payet, Margand et al. 2004). For example, a species already exposed to a lack of dissolved oxygen in water can be more sensitive to toxic stress (Stuijfzand, Helms et al. 2000). Furthermore, in terms of biodiversity, the most sensitive species to the reduction in oxygen are also likely to be sensitive to toxic stress. On this basis, the Eco-indicator and AMI methods present a better ecological realism.

Structure and function of the ecosystem: this question is recurrent in Environmental Risk Assessment, and must also be addressed in LCIA. The structure typically corresponds to the biodiversity of the ecosystem while the energy and material flows characterise the function. Some keystone species may represent only a very small part of the biodiversity but can at the same time play a major role in energy transfer. In ERA for chemical regulation, the underlying assumption is that by protecting all species, the functions of the system are also protected. LCIA models do not consider thresholds but work with linear dose-effect relations. Therefore, LCIA aims to compare levels of

stress to ecosystems, but does not aim at ensuring the protection of ecosystem species. The underlying assumption is therefore that all species in ecosystems have the same likelyhood of being keystone species, and the potential reduction of the number of species affected by toxic stress is likely to have a repercussion on both the structure and function of the ecosystem.

Statistical Reliability

The reliability of the Effect Factor can also be analysed at the statistical level. This is discussed below, confronting each method with several questions.

The Stability of the effect indicator is strongly influenced by the considered method. AMI and the Combi-PAF are both based on the geometric mean of species' responses while the PNEC uses the most sensitive species. As shown in Figure 15, the EC50 of the most sensitive species can vary greatly from one database to another.

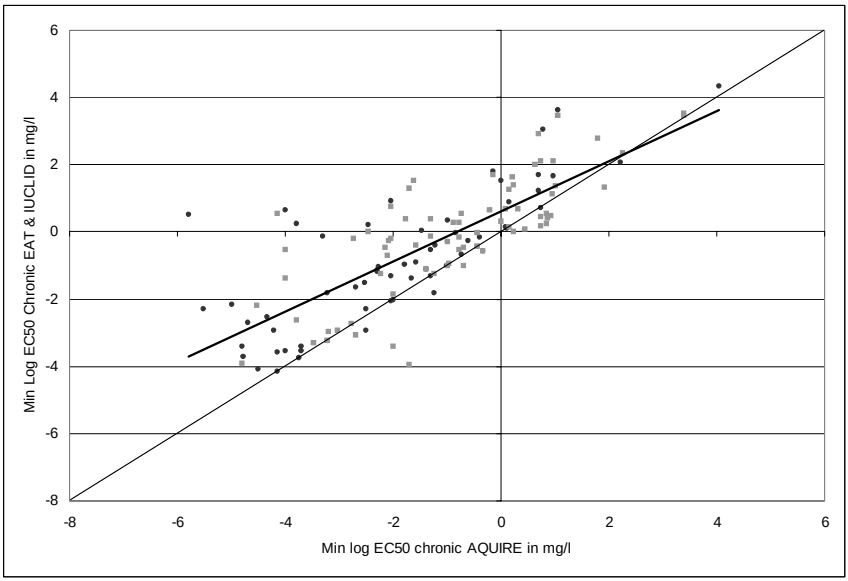

Figure 15: Comparison between most sensitive species based on AQUIRE versus EAT (●) or IUCLID (■) database for 115 substances (Regression: y= -1.01+0.83x; Intercept [-1.22; 1.80]; Slope [0.72; 0.94])

Based on the lowest EC50 for 115 substances, correlation between the Aquire versus the EAT and IUCLID databases is not good, with an R^2 of 0.62 and 0.60 respectively. For Diuron, for example, depending whether the most sensitive species is taken from the Aquire or EAT database, the lowest EC50 will vary by more than 6 orders of magnitude. Similarly with the Parathion, whether the lowest EC50 comes from Aquire or IUCLID, it varies by nearly 5 orders of magnitude. A complementary indication is presented in Figure 15 with calculation of the regression line between the lowest chronic EC50 of the US database Aquire and that of the European databases EAT and IUCLID. At the same time, the 1:1 line is presented. In terms of comparison between substances, the difference in slope between the two lines indicates that the most toxic substances appear 100 times more toxic based on the US database than on the European databases. This can lead to a considerable bias between studies based on the US or European databases. This problem results from a difference in the number of data in Aquire versus the EAT or IUCLID databases. Aquire has far more chronic EC50s than EAT or IUCLID and the "most sensitive" species can only get lower when new chronic data are included in the database (Forbes and Forbes 1993). Since more data are available for the most toxic and all well-known chemicals, the lowest EC50 for these substances will be biased compared to other substances. This explains the bias observed between the US and European databases.

Robustness against deviation due to statistical assumptions: an indicator based on the tail part of a distribution like the $HC5_{NOEC}$ will be much more sensitive to deviation due to statistical assumptions compared to an indicator based on the middle of the distribution like the $HC50_{EC50}$(AMI) or $HC50_{NOEC}$ (Combi PAF).

Uncertainty calculation: interpretability of the LCA results is facilitated if users can assess the level of certainty of the result. The method should therefore be able to take into account the uncertainty of the results. For the Effects Factors in a comparative assessment, uncertainty depends on the number of data available. As soon as the number of data increases, uncertainty decreases. AMI provides the 95% Confidence Interval based on Student for all Effect Factors, and USES-LCA can also provide 90% Confidence Interval on the $HC5_{NOEC}$. On average, the $HC5_{NOEC}$ presents a lower discriminating power than the $HC50_{EC50}$. Observing the discrimination between the $HC5_{NOEC}$ and $HC50_{EC50}$, it is interesting to note that both values are so uncertain that it is not feasible to ensure that the $HC5_{NOEC}$ is different from the $HC50_{EC50}$ for chemicals tested with fewer than 8 species. Similarly, the most sensitive species is always included in the Confidence Interval of the $HC50_{EC50}$ for chemicals tested with 3 or 4 species (Table 9). At the ecological level, the

exclusion of one whole phylum from the assessment can pose a problem. The Confidence Interval of the $HC50_{EC50}$ includes the geometric mean of the most sensitive phylum in most cases if fewer than 15 species are used for the assessment. Nevertheless, if this is not so, the AMI method provides the geometric mean of the most sensitive phylum as complementary data, enabling this parameter to be used in the assessment.

Avoiding bias for comparison: an example of bias due to databases has been highlighted above with the comparison of the most sensitive species from US or European databases. Another example has been given with the mix of data from the $HC5_{NOEC}$ and the most sensitive species in calculation of a PNEC. Since the level of conservatism is not the same for both methods, results obtained cannot be compared (Heijungs, Guinée et al. 1992; Emans, VandePlassche et al. 1993; Guinée and Heijungs 1993). Another source of bias is due to the use of conservative extrapolation factors. Indeed, the lack of ecotoxicological data leads to the use of extrapolation factors for extrapolation from acute to chronic data for example. At this time, the EDIP and USES-LCA methods are based on conservative factors while a set of best-estimate extrapolation factors has been developed for the AMI method.

Small samples: for most chemicals, only a small number of data is available. One of the strengths of the PNEC method is that it provides Effect Factors even when only one data is available. The AMI method (parametric version) requires at least 3 EC50s (acute or chronic) from 3 different phyla. The Combi-PAF does not mention the minimum data required for calculation of each Effect Factor. Nevertheless, like AMI, Combi-PAF uses the HC50 as basis for assessment of the effect indicator and therefore the minimum data requirement is also assumed to be 3 species. For the $HC5_{NOEC}$, it is necessary to build a reliable SSD. Aldenberg's table provides data from 2 available species, but this appears to be an underestimation since other publications focussing on this point mention a minimum 4 (Sloof 1992), 8 (Host, Regal et al. 1991) or 10 species (EU-Commission 2002) in order to build a reliable curve. For small samples, comparison between the $HC50_{EC50}$ on the one hand and the most sensitive species and the $HC50_{NOEC}$ on the other hand presents a better relation (R^2=0.63 and R^2=0.9) than the $HC50_{EC50}$ versus the $HC5_{NOEC}$ (R^2=0.44). This confirms the weakness of the $HC5_{NOEC}$ for small samples.

Conclusions

The analysis of methods for the LCIA on ecosystems has revealed some important points. The four methods can be divided into two groups: the AMI and Eco-Indicator methods, using the $HC50_{EC50}$ and $HC50_{NOEC}$ respectively as

basis for calculation of the Effect Factor; USES-LCA and the EDIP methods, based on the $HC5_{NOEC}$ and most sensitive species PNEC respectively. On the basis of the results presented in the discussion, the question presented at the beginning of this chapter can therefore be answered for each method:

1- In terms of ability to cover numerous chemicals, the AMI methods based on $HC50_{EC50}$s, taking into account both acute and chronic EC50s, currently provides the most important database (522 chemicals) which is to be extended to more than 1500 substances in the coming months. The Eco-indicator method is more restricted, and does not provide extrapolation factors from acute to chronic, nor the minimum number of NOECs necessary for calculation of a reliable $HC50_{NOEC}$. Only a small number of Effect Factors are available with this method. The $HC5_{NOEC}$ method is based on the SSD and therefore requires calculation of a reliable chronic SSD for assessment of an Effect Factor. This necessitates many chronic data and explains the limited number of Effect Factors based on the $HC5_{NOEC}$. The latest research published by Huijbregts (2000), providing a method for extrapolation of a chronic SSD based on acute data, provides a possible solution. The EDIP method, based on the most sensitive species, does not provide a large database of Effect Factors, but the method is well-known in ERA and offers LCA practitioners the possibility of calculating Effect Factors based on their own ecotoxicological databases.

2- Regarding bias, three sources have been identified. For extrapolation factors between acute and chronic data, EDIP is based on conservative extrapolation factors, which is a source of bias. A second source of bias in the USES-LCA method is the mix between the PNEC based on the most sensitive species and the HC5, due to the differences in level of conservatism between these approaches. The last source of bias has been observed both for the $HC5_{NOEC}$ and the most sensitive species; for the most toxic substances, Effect Factors based on the US database appear to be two orders of magnitude more toxic than those based on European database.

3- In terms of effect indicator stability, EDIP is very sensitive to the addition of new species in the database, since an effect indicator can vary by several orders of magnitude depending on whether a very sensitive species is included in the dataset or not. Methods based on the $HC50_{EC50}$ or $HC50_{NOEC}$ (AMI, Combi-PAF) are much more robust in this respect. The $HC5_{NOEC}$ will be very sensitive on this point for small datasets but not if many species are used for calculation of the $HC5_{NOEC}$.

4- Discrimination between chemicals: all methods have almost the same range of variability of Effect Factors, but only AMI currently provides 95%

Confidence Intervals for the Effect Factors. USES-LCA can possibly provide a 90% Confidence Interval on the $HC5_{NOEC}$ but it is can be up to 10 orders of magnitude larger than the 95% Confidence Interval on the $HC50_{EC50}$.

5- In terms of environmental relevance, the discussion points out that LCA addresses several stressors and the toxic stress can not be separated from the other stressors. Therefore it makes sense to base Effect Factors close to the actual level of affected species in ecosystems, taking all stressors into account. The Combi-PAF and AMI methods are regarded as more relevant, since both are based on the actual level of effect in the field (based on the marginal slope at 24% of affected species for the Combi-PAF and the average slope under the $HC50_{EC50}$ level for AMI). USES-LCA and EDIP do not appear to be environmentally relevant since both methods focus on toxic stress, ignoring other stressors.

A recent meeting of the UNEP-SETAC Life Cycle Initiative (UNEP-SETAC 2003) and a publication relating to impact assessment on ecosystems (Pennington, Payet et al. 2004) have pointed out that a method based on the Hazardous Concentration affecting 50 percent of species over their chronic EC50 level ($HC50_{EC50}$) is a well-adapted indicator for Life Cycle Impact Assessment on ecosystems. Analysing several methods with regard to the LCIA framework on the basis of actual Effect Factors, this chapter confirms these suggestions. At the same time, the path explored by Eco-indicator, with the possibility of working on the marginal slope of effect at a given working point, is promising but requires further research in order to develop a reliable curve linking the effect and the exposure to several substances and several stressors.

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Appendix: List of substances used for the comparison of methods and corresponding $HC50_{EC50}$, $HC50_{NOEC}$, $HC5_{NOEC}$, and $PNEC_{NOEC}$ expressed in log scale in mg/l (MP: geometric mean of the most sensitive phylium; MS: most sensitive species)

Chem-name	Chem. Group	CAS	N. Species EC50	$HC50_{EC50}$ 2.5% CI	Log $HC50_{EC50}$	$HC50_{EC50}$ 97.5% CI	MP EC50	MS EC50	N species NOEC	$HC50_{NOEC}$	$HC5_{NOEC}$ 5% CI	$HC5_{NOEC}$ 50%	$HC5_{NOEC}$ 95% CI	PNEC NOEC
2,4-Dinitrophenol	Non-pesticide organic	51-28-5	7	0.63	**1.09**	1.54	0.85	0.67	5	0.25	-1.30	-0.41	-0.05	-1.02
(1alpha,2alpha,3beta,4alpha,5alpha,6beta)-1,2,3,4,5,6-Hexachlorocyclohexane	Pesticide organic	58-89-9	32	-1.64	**-1.11**	-0.59	-2.20	-5.00	17	-2.14	-4.43	-3.64	-3.14	-4.40
4-Chloro-3-methylphenol	Non-pesticide organic	59-50-7	4	0.64	**0.87**	1.10	0.70	0.70	3	0.13	-0.95	-0.14	0.04	-1.00
dimethoate	Pesticide organic	60-51-5	12	0.42	**1.02**	1.63	-0.58	-0.58	3	-0.02	-10.40	-2.65	-0.89	-2.15
chloroform	Non-pesticide organic	67-66-3	12	1.11	**1.76**	2.41	1.42	0.20	6	1.35	-2.17	-0.31	0.52	-1.25
1,1,1-trichloroethane	Non-pesticide organic	71-55-6	6	1.11	**2.06**	3.02	0.79	0.79	5	1.17	-2.80	-0.51	0.40	-0.89
1,1,2-trichloroethane	Non-pesticide organic	79-00-5	23	1.55	**1.77**	1.99	1.39	0.46	6	1.10	-0.25	0.46	0.78	-0.52
chloroacetic acid	Non-pesticide organic	79-11-8	4	-3.33	**0.51**	4.34	-1.57	-1.60	4	-0.13	-13.00	-4.71	-1.99	-3.30
1,1,2,2-tetrachloroethane	Non-pesticide organic	79-34-5	6	0.89	**1.35**	1.81	1.10	0.81	4	0.68	-1.23	0.00	0.40	-0.85
1,2-Benzenedicarboxylic acid, Dibutyl ester	Non-pesticide organic	84-74-2	6	-0.78	**-0.07**	0.63	-0.38	-1.27	5	-0.30	-2.49	-1.23	-0.73	-1.91
1,2-Benzenedicarboxylic acid, Butyl phenylmethyl ester	Non-pesticide organic	85-68-7	8	-0.42	**0.16**	0.75	0.14	-0.58	6	-0.56	-2.67	-1.56	-1.06	-2.31
PENTACHLOROPHENOL	Pesticide organic	87-86-5	52	-0.56	**-0.30**	-0.04	-0.82	-2.00	14	-0.69	-4.07	-2.87	-2.11	-3.30
1-Methyl-2-nitrobenzene	Non-pesticide organic	88-72-2	4	0.71	**1.22**	1.73	0.92	0.92	3	0.21	-3.44	-0.72	-0.10	-1.30
(2,4-Dichlorophenoxy)acetic acid	Pesticide organic	94-75-7	20	0.79	**1.36**	1.92	1.30	-1.40	7	1.24	-2.76	-0.80	0.16	-2.40
1,2-dichlorobenzene	Non-pesticide organic	95-50-1	8	0.65	**1.34**	2.04	0.25	0.25	3	0.12	-5.76	-1.37	-0.37	-1.43
3,4-dichloroaniline	Non-pesticide organic	95-76-1	32	-0.42	**-0.11**	0.21	-0.69	-2.64	16	-1.24	-3.35	-2.63	-2.16	-3.49

Chem-name	Chem. Group	CAS	N. Species EC50	$HC50_{EC50}$ 2.5% CI	**$HC50_{EC50}$**	$HC50_{EC50}$ 97.5% CI	MP EC50	MS EC50	N species NOEC	$HC50_{NOEC}$	$HC5_{NOEC}$ 5% CI	$HC5_{NOEC}$ 50%	$HC5_{NOEC}$ 95% CI	PNEC NOEC
1-chloro-2,4-dinitrobenzene	Non-pesticide organic	97-00-7	5	-0.87	**-0.45**	-0.04	-0.73	-0.85	3	-1.07	-3.29	-1.64	-1.26	-2.30
NITROBENZENE	Non-pesticide organic	98-95-3	6	1.16	**1.42**	1.68	1.29	1.01	4	0.73	-0.24	0.39	0.59	-0.49
1,3,5-Trinitrobenzene	Non-pesticide organic	99-35-4	4	-0.68	**-0.14**	0.41	-0.42	-0.45	4	-0.76	-2.81	-1.49	-1.05	-2.10
1,3-dinitrobenzene	Non-pesticide organic	99-65-0	4	-0.02	**0.57**	1.15	0.44	0.09	3	-0.19	-1.47	-0.52	-0.30	-1.32
4-nitrophenol	Non-pesticide organic	100-02-7	10	0.22	**0.75**	1.28	0.40	-0.16	4	0.23	-2.89	-0.88	-0.22	-1.52
1,4-Dichlorobenzene	Non-pesticide organic	106-46-7	9	-0.19	**0.40**	1.00	-0.35	-0.83	6	-0.10	-2.06	-1.03	-0.57	-1.64
4-chlorophenol	Non-pesticide organic	106-48-9	13	-0.01	**0.60**	1.21	-0.82	-2.06	8	-0.27	-3.76	-2.15	-1.32	-3.71
2-Propenal	Non-pesticide organic	107-02-8	10	-1.40	**-1.16**	-0.91	-1.19	-1.55	5	-1.70	-3.40	-2.42	-2.03	-3.00
Methylbenzene	Non-pesticide organic	108-88-3	6	0.88	**1.55**	2.22	1.09	0.80	4	1.45	-5.43	-1.00	0.46	-0.90
CHLOROBENZENE	Non-pesticide organic	108-90-7	12	0.28	**1.08**	1.87	0.42	-1.05	5	1.30	-1.55	0.09	0.74	-0.51
PHENOL	Non-pesticide organic	108-95-2	26	0.56	**0.99**	1.42	0.44	-1.40	9	0.25	-2.11	-1.08	-0.52	-2.50
6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	Pesticide organic	115-29-7	20	-2.52	**-1.69**	-0.86	-3.61	-6.00	9	-2.85	-6.27	-4.78	-3.96	-5.40
HEXACHLOROBENZENE	Pesticide organic	118-74-1	9	-1.92	**-1.52**	-1.12	-1.76	-2.15	5	-2.34	-2.70	-2.49	-2.41	-3.49
1,2,4-trichlorobenzene	Non-pesticide organic	120-82-1	9	-0.26	**0.39**	1.03	0.05	-1.62	7	-0.25	-3.72	-2.02	-1.19	-3.42
2,4-dichlorophenol	Non-pesticide organic	120-83-2	13	-1.22	**-0.26**	0.71	-0.67	-4.30	5	-1.75	-10.80	-5.58	-3.51	-6.00
2,4-dinitrotoluene	Non-pesticide organic	121-14-2	14	-0.09	**0.25**	0.59	0.10	-0.82	3	-0.82	-7.20	-2.43	-1.35	-2.30
[(Dimethoxyphosphinothioyl)thio]butanedioic acid, Diethyl ester	Pesticide organic	121-75-5	17	-1.47	**-0.82**	-0.17	-2.44	-3.32	9	-1.38	-5.66	-3.80	-2.78	-4.86
FENITROTHION	Pesticide organic	122-14-5	18	-0.94	**-0.19**	0.57	-2.91	-4.27	9	-1.01	-6.24	-3.96	-2.72	-6.05

Chem-name	**Chem. Group**	**CAS**	N. Species EC50	$HC50_{EC50}$ 2.5% CI	**$HC50_{EC50}$**	$HC50_{EC50}$ 97.5% CI	MP EC50	MS EC50	N species NOEC	$HC50_{NOEC}$	$HC5_{NOEC}$ 5% CI	$HC5_{NOEC}$ 50%	$HC5_{NOEC}$ 95% CI	PNEC NOEC
Tetrachloroethene	Non-pesticide organic	127-18-4	8	0.44	**1.31**	2.18	0.37	0.15	5	0.76	-4.69	-1.55	-0.30	-1.33
1,2-Benzenedicarboxylic acid, Dimethyl ester	Non-pesticide organic	131-11-3	5	1.46	**1.75**	2.03	1.68	1.42	3	1.14	-0.61	0.70	1.00	-0.02
SODIUM DODECYL SULPHATE	Pesticide organic	151-21-3	28	0.74	**1.03**	1.32	0.15	-0.30	23	0.15	-1.35	-0.90	-0.58	-2.00
FLUORANTHENE	Non-pesticide organic	206-44-0	10	-1.69	**-0.70**	0.29	-1.44	-2.15	5	-0.76	-6.26	-3.09	-1.83	-2.68
METHYL PARATHION	Pesticide organic	298-00-0	13	-0.51	**0.19**	0.89	-2.75	-2.75	4	-1.45	-9.10	-4.17	-2.56	-4.35
DIURON	Pesticide organic	330-54-1	27	-1.17	**-0.49**	0.20	-1.89	-4.89	11	0.50	-2.43	-1.26	-0.58	-3.30
LINURON	Pesticide organic	330-55-2	27	-1.20	**-0.87**	-0.54	-1.56	-2.60	18	-1.52	-3.81	-3.02	-2.51	-4.30
DIAZINON	Pesticide organic	333-41-5	17	-1.69	**-0.99**	-0.29	-1.72	-3.32	10	-1.77	-4.73	-3.50	-2.81	-4.73
1,3-dichlorobenzene	Non-pesticide organic	541-73-1	6	0.50	**1.14**	1.78	0.49	0.49	5	0.55	-2.49	-0.74	-0.04	-1.27
(2-Chloroethyl)trimethyl ammonium, Chloride	Pesticide organic	999-81-5	3	-0.32	**2.62**	5.56	1.40	1.40	3	2.00	-7.96	-0.52	1.17	-0.30
glyphosate	Pesticide organic	1071-83-6	11	0.93	**1.47**	2.00	1.26	0.20	7	1.22	-1.54	-0.19	0.47	-1.11
Aluminosilicic acid, Sodium salt	Inorganic	1344-00-9	5	2.32	**2.69**	3.06	2.50	2.26	4	1.61	-3.99	-0.38	0.80	-1.00
2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine	Pesticide organic	1582-09-8	10	-1.88	**-1.36**	-0.85	-2.03	-2.36	5	-1.95	-5.74	-3.56	-2.69	-3.70
3,5-Dibromo-4-hydroxybenzonitrile	Pesticide organic	1689-84-5	6	-0.26	**0.82**	1.91	-1.06	-1.06	4	0.14	-6.24	-2.13	-0.79	-2.70
5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone	Pesticide organic	1698-60-8	8	-0.36	**0.32**	1.01	-0.05	-0.77	3	0.45	-3.99	-0.67	0.08	-1.15
6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine	Pesticide organic	1912-24-9	65	-0.73	**-0.51**	-0.30	-0.80	-3.00	22	-1.10	-2.75	-2.25	-1.91	-3.40
CHLORPYRIFOS	Pesticide organic	2921-88-2	29	-2.39	**-1.99**	-1.58	-2.34	-4.10	23	-2.59	-4.66	-4.03	-3.61	-5.25
5,6-Dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide	Pesticide organic	5234-68-4	4	-0.29	**-0.10**	0.09	-0.20	-0.23	3	-0.63	-2.35	-1.06	-0.77	-1.89
cadmium	Inorganic	7440-43-9	29	-0.87	**-0.37**	0.14	-0.89	-2.91	5	-1.65	-6.11	-3.54	-2.52	-3.59
Copper	Inorganic	7440-50-8	33	-1.04	**-0.77**	-0.51	-1.40	-2.51	9	-1.52	-2.72	-2.19	-1.91	-3.00
Zinc	Inorganic	7440-66-6	19	-0.39	**0.02**	0.43	-1.12	-2.08	3	-0.88	-1.46	-1.03	-0.93	-1.93
Sulfuric acid, Dithallium (1+) salt	Inorganic	7446-18-6	4	-1.17	**-0.35**	0.48	-1.05	-1.05	3	-1.27	-4.15	-2.00	-1.51	-2.70
Cupric chloride	Inorganic	7447-39-4	47	-1.28	**-0.92**	-0.57	-2.66	-4.11	9	-1.33	-5.37	-3.61	-2.65	-4.40
Potassium chloride	Inorganic	7447-40-7	8	2.39	**2.80**	3.21	2.32	1.89	3	2.53	0.62	2.05	2.37	1.28
Mercuric chloride	Inorganic	7487-94-7	40	-1.70	**-1.46**	-1.22	-2.81	-2.81	7	-2.56	-4.95	-3.78	-3.21	-4.60

Chem-name	**Chem. Group**	**CAS**	N. Species EC50	$HC50_{EC50}$ 2.5% CI	**$HC50_{EC50}$**	$HC50_{EC50}$ 97.5% CI	MP EC50	MS EC50	N species NOEC	$HC50_{NOEC}$	$HC5_{NOEC}$ 5% CI	$HC5_{NOEC}$ 50%	$HC5_{NOEC}$ 95% CI	PNEC NOEC
Zinc chloride	Inorganic	7646-85-7	28	-0.29	**0.04**	0.38	-0.35	-1.59	7	-1.00	-2.87	-1.95	-1.50	-2.60
SODIUM CHLORIDE	Inorganic	7647-14-5	12	2.96	**3.45**	3.94	2.36	1.16	5	2.39	-2.38	0.37	1.46	-0.40
BROMIDE	Inorganic	7647-15-6	6	2.18	**3.13**	4.08	1.56	1.56	4	2.44	-2.86	0.56	1.67	0.08
Hypochlorous acid, Sodium salt	Inorganic	7681-52-9	8	-1.40	**-0.71**	-0.01	-0.86	-2.38	4	-1.27	-2.25	-1.62	-1.42	-2.44
NICKEL	Inorganic	7718-54-9	21	-0.60	**-0.07**	0.46	-0.96	-2.74	6	-0.58	-2.99	-1.72	-1.15	-2.21
Sulfuric acid, Zinc salt (1:1)	Inorganic	7733-02-0	68	-0.03	**0.21**	0.45	-0.30	-2.00	26	-0.70	-2.85	-2.20	-1.75	-2.95
Nitric acid, silver (1+) salt	Inorganic	7761-88-8	25	-1.91	**-1.60**	-1.30	-2.10	-2.92	13	-2.38	-4.74	-3.87	-3.34	-5.05
Selenium	Inorganic	7782-49-2	9	-0.46	**0.20**	0.86	-0.45	-1.15	5	-0.74	-2.76	-1.59	-1.13	-2.40
Hydrogen sulfide	Inorganic	7783-06-4	10	-1.94	**-1.36**	-0.77	-1.61	-1.98	6	-2.01	-6.18	-3.98	-3.00	-3.94
manganese sulphate	Inorganic	7785-87-7	5	0.44	**1.03**	1.62	0.68	0.42	3	0.37	-1.99	-0.23	0.17	-0.96
BORIC ACID	Inorganic	10043-35-3	10	0.79	**1.35**	1.91	0.66	0.25	6	0.61	-3.13	-1.15	-0.27	-1.40
LEAD NITRATE	Inorganic	10099-74-8	23	-0.75	**-0.18**	0.39	-0.74	-2.28	10	-0.93	-2.62	-1.92	-1.52	-2.77
Selenious acid, Disodium salt	Inorganic	10102-18-8	8	-0.79	**-0.05**	0.68	-1.24	-1.24	3	-0.42	-8.57	-2.48	-1.10	-2.31
COBALTOUS SULFATE	Inorganic	10124-43-3	4	-2.11	**-0.16**	1.79	-1.72	-1.72	3	-1.21	-10.16	-3.48	-1.96	-3.55
Ammonium chloride	Inorganic	12125-02-9	39	0.16	**0.45**	0.75	0.27	-0.96	16	-0.32	-2.84	-1.97	-1.41	-3.82
(3-Methylphenyl)carbamic acid 3-[(methoxycarbonyl)amino]phenyl ester	Pesticide organic	13684-63-4	3	-1.86	**-0.36**	1.14	-0.89	-0.89	3	-1.16	-7.76	-2.83	-1.72	-3.10
2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	Pesticide organic	15972-60-8	7	-1.24	**-0.45**	0.33	-0.93	-2.02	7	-1.05	-4.03	-2.57	-1.86	-3.52
2-Ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranol methane sulfonate	Pesticide organic	26225-79-6	5	0.10	**0.69**	1.29	0.35	0.26	4	0.30	-1.01	-0.17	0.11	-1.00
3-(4-isopropylphenyl)-1,1-dimethylurea	Pesticide organic	34123-59-6	7	-1.91	**-0.78**	0.35	-1.67	-1.85	3	-0.80	-13.64	-4.05	-1.87	-3.70
3-(3,5-dichlorophenyl)-2,4-dioxo-N-isopropylimidazolidine-1-carboxamide	Pesticide organic	36734-19-7	8	-0.81	**-0.37**	0.07	-0.68	-1.52	3	-0.75	-1.91	-1.04	-0.84	-1.89
3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide, sodium salt	Pesticide organic	50723-80-3	3	0.87	**2.53**	4.19	1.79	1.79	3	1.94	-1.85	0.98	1.62	0.41

Chem-name	**Chem. Group**	**CAS**	N. Species EC50	$HC50_{EC50}$ 2.5% CI	**$HC50_{EC50}$**	$HC50_{EC50}$ 97.5% CI	MP EC50	MS EC50	N species NOEC	$HC50_{NOEC}$	*$HC5_{NOEC}$ 5% CI*	*$HC5_{NOEC}$ 50%*	*$HC5_{NOEC}$ 95% CI*	*PNEC NOEC*
2-chloro-2'-ethyl-N-(2-methoxy-1-methylethyl)-6'-methylacetanilide	Pesticide organic	51218-45-2	12	-0.83	**-0.40**	0.03	-0.58	-1.29	5	-0.88	-4.99	-2.62	-1.68	-3.29
2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	Pesticide organic	57966-95-7	11	-0.52	**0.25**	1.02	-0.27	-0.69	3	-0.69	-4.28	-1.60	-0.99	-2.17
N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]-1H-imidazole-1-carboxamide	Pesticide organic	67747-09-5	5	-1.55	**-0.95**	-0.35	-1.33	-1.40	5	-1.40	-3.36	-2.23	-1.78	-3.00

CHAPTER 4

Statistical Estimator for Assessing Impact on Aquatic Ecosystems in LCA*

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Abstract

The chapter compares two statistical estimators aiming at calculating the average toxicity of substances on biological species. The two methods provide an estimation of the $HC50_{EC50}$ and the associated confidence interval. On the one hand, a parametric method using the geometric mean and a calculation of the confidence interval with Student is proposed. On the other hand, a distribution-free method calculates the $HC50_{EC50}$ based on the median response of species and the confidence interval based on bootstrap. In order to facilitate the use of the non-parametric method, a table linking the number of species tested and the size of the confidence interval is provided for samples from 5 to 500 species. The comparison is based on actual data concerning 191 substances covering inorganics, non-Pesticide organics, and Pesticide organics. The mean and width of the chronic EC50s samples for all the substances are presented. The Shapiro-Wilk test is performed for the 191 EC50s samples and the assumption of log-normality of the distribution failed in more than 20% of the cases. Two causes of this non Log-normality are identified; (1) the skewness is shown to be an important issue for the assessment of the average toxicity of chemicals while (2) the multi-modal distributions are not likely to influence considerably the final result. A detailed application of the two methods is done with the comparison of two herbicides, the Sulfosulfuron and the Prosulfuron, and the distribution-free method appears to be more powerful than the parametric for a substance-to-substance comparison. Nevertheless, the distribution-free method requires a minimum of 5 chronic EC50s that cannot be satisfied in most cases. We therefore suggest to take the geometric mean with the Student confidence interval as a baseline, and to calculate the non parametric median and the bootstrap confidence interval as a sensitivity study, to test the influence of the substance with non-lognormal distributions.

Key words: HC50, EC50, confidence interval, LCIA, Ecosystems, non-parametric.

Introduction

Life Cycle Assessment quantifies and compares the environmental impact of substances emitted during a product life cycle. Thus, the potential toxicity of hundreds of substances emitted in the aquatic ecosystems needs to be quantified and compared. Some proposals have been made for using the $HC50_{EC50}$ (Concentration of toxic affecting 50% of the species over their EC50) for the calculation of Effect Factors quantifying the potential effects of chemicals on the ecosystems (Payet, Larsen et al. 2003; Pennington, Payet et al. 2004). Nevertheless, the $HC50_{EC50}$ can be based on a parametric (i.e. the geometric mean) or a non-parametric (i.e the median) statistical estimator. It is the purpose of this chapter to compare the two estimators.

Until now, two methods of Life Cycle Impact Assessment (LCIA) for ecosystems have been developed on the basis of existing Environmental Risk Assessment (ERA) methods. The EDIP method (Wenzel, Hauschild et al. 1998) has been developed on the basis of the European method for the ERA for chemical regulation (EU-Commission 1994). This method is using the Predicted No Effect Concentration (PNEC) as an indicator of the toxicity of substances. The PNEC is assumed to be the threshold level under which a given substance has no adverse effect on the ecosystems. It is calculated on the basis of the lowest acute or chronic ecotoxicity measure (EC50, Lowest Observed Effect Concentration (LOEC) or No Observed Effect Concentration (NOEC)) applying extrapolation factors for calculating the threshold level. The assumption of a linear relation from the PNEC down to 0 (under the threshold level) has to be made for applying the method in LCA. Nevertheless, a limitation remains since the threshold is strongly dependent on the set of species tested and can vary by several orders of magnitude from one database to another. The second method adapted to LCIA is based on Species Sensitivity Distributions (SSD). The $HC5_{NOEC}$ (Hazardous Concentration affecting 5% of the species above their NOEC level) has been developed in ERA in order to ensure the protection of 95% of the species in the ecosystems (Kooijman 1987; Aldenberg and Slob 1993; EU-Commission 1994; Aldenberg and Jaworska 2000). Assuming a linear relation from the HC5 down to 0, Huijbregts (Huijbregts, Thissen et al. 2000) has adapted the method for LCA. The main limitation for LCA concerns the data requirement. The new European guidance document for ERA (EU-Commission 2002; Huijbregts, VandeMeent et al. 2002) requires a minimum of

10 chronic data for the calculation of an $HC5_{NOEC}$, and this is possible only for a very limited group of chemicals.

More recently, several publications have highlighted the strength of an Effect Factor based on the $HC50_{EC50}$ for LCA (UNEP-SETAC 2003; Pennington, Payet et al. 2004). Indeed, such an indicator appears to provide reliable results for comparative assessment with a better ecological realism, and to enable the calculation of a confidence interval on the effect factors. Nevertheless this method raises several questions. First, the calculation of the Effect Factors is based on ecotoxicity data that do not necessarily fit a log Normal Distribution (Newman, Ownby et al. 2000), and the question is therefore *to know if better Effect factors are obtained with the underlying assumption of a Normal Distribution or if non-parametric methods would be more relevant.* Second, ecotoxicity data are scarced considering the large number of substances daily used in industrial processes. Therefore, the question of the *applicability of a parametric or non parametric estimator to small samples* (three or four EC50s) is of central importance. Third still related to the lack of chronic data, the question also concern the *compatibility of the estimator with an extrapolation from an acute to a chronic* $HC50_{EC50}$.

The present paper aims to address these three questions by comparing the $HC50_{EC50}$ and the related confidence intervals by non-parametric and parametric estimates.

Specifically, the paper presents a distribution-free method developed for the assessment of the median toxicity of substances with the calculation of the corresponding confidence interval. Using this method, the average toxicity of 191 substances is compared to similar estimate based on the geometric mean and the confidence interval based on Student. For presenting in more details the influence of the method on the average toxicity, a comparison focusing on two substitutable herbicides is provided.

The paper is structured as follows: with the purpose of improving the assessment of the Effect Factors, the method that provides a parametric estimate of the $HC50_{EC50}$ is presented. Its alternative based on a non-parametric assessment of the $HC50_{EC50}$ is presented. In order to base the discussion on actual results, the $HC50_{EC50}$ is then calculated with both methods for 191 substances. The substances, and the ecotoxicity data retained for the calculation

of their effect factors are therefore presented together with the results of the calculation of the $HC50_{EC50}$, a discussion is conducted, aiming at answering the questions stated above.

Presentation of the parametric and non-parametric methods

The statistical analyses of multiple species ecotoxicity data are typically based on the assumption of a Log-normal (sometimes log-logistic or log-triangular) distribution of ecotoxicity data (Host, Regal et al. 1991; Aldenberg, Jaworska et al. 2002). Nevertheless, after analysing a set of ecotoxicity data for 23 substances for freshwater and/or salt water, Newman (Newman, Ownby et al. 2000) mentioned that the normality assumption was rejected for 50% of the dataset using the Shapiro-Wilk test. We therefore present shortly in this section the assessment of the $HC50_{EC50}$ based on the geometric mean with a confidence interval calculated with Student. After this, the distribution-free method that we have developed for the calculation of the median based $HC50_{EC50}$ is presented in detail.

a) Parametric method

As an alternative, we compared the results of the non-parametric $HC50_{EC50}$ with a parametric method using the geometric mean to estimate the average toxicity of a chemical and the Student distribution for calculating the confidence interval on the mean as presented in the equation below.

$$Log(HC50_{EC50}) \pm \frac{1}{\sqrt{N}} \times t_{N-1}^{0.05} \times SDev(LogEC50) \qquad (1)$$

where N is the size of the sample, $t_{n-1}^{0.05}$ is the t value from the Student table for a 95% confidence interval with N-1 degree of freedom, and Sdev is the Standard deviation of the LogEC50s.

Using the example of the bis(2-ethylhexyl) phthalate presented below, the average of the logarithmic values is 0.30 with a standard deviation of 1.26. For N=20, t=2.093 for the calculation of the 95% confidence interval on the mean. The results of the parametric $HC50_{EC50}$ and its confidence limits is GeoM=2 [0.52; 7.77] mg/l.

b) Non-parametric method

In order to provide a possible solution to the problem of non Log-normal EC50s distributions, we have developed a distribution-free method for the calculation of the $HC50_{EC50}$. This method is based on the median of the EC50s and enables to calculate the confidence interval on the $HC50_{EC50}$ by bootstrapping the median.
The median was selected as the most suitable statistical estimator when no assumption can be made concerning the distribution.
The calculation of the confidence interval on the median can be done with different distribution-free methods. The main two ones are the jackknife method (Miller 1974) and the bootstrap method (Efron 1981). Both methods have been compared and the results outlined the accuracy of the bootstrap method compared to the jackknife one (Gosh, Parr et al. 1984; Nagao 1991). Nevertheless, resampling procedure is not necessary for the calculation of the confidence interval for the median, the observation fits a binomial distribution (Vessereau 1987) and this is illustrated with an exercise of calculation of the exact resampling density given in Davison and Hinkley's book (Davison and Hinkley 1997). Based on this property, and considering a symmetric binomial cumulative distribution, Owen (Owen 1962) is providing a formula for the calculation of the confidence interval on the median.

$$B(n, A, \frac{1}{2}) = \sum_{j=0}^{A} \frac{n!}{j!(n-j)!2^n} \leq \gamma \qquad (2)$$

where n is the number of binomial trial, A is given such as the binomial sum with p=1/2 is small, and γ is the limit of the confidence interval (0.005, 0.01, 0.025, 0.05 and 0.1 in the table).

The results of the calculation provided in Owens book are presented in a statistical table providing distribution-free confidence limits for the median for a sample from 4 to 1,000 observations. MacKinnon (MacKinnon 1964) is going further with this idea providing a table for binomial critical values for distribution-free confidence interval on the median. This table presents the two-tailed probability of the confidence interval of 0.001, 0.01, 0.02, 0.05, 0.1 and 0.5 for a sample size from 2 to 1,000 observations. After this table, the author also presents an example (example 4, p. 951) illustrating the use of the table for the calculation of the confidence interval on the median.

The method mentioned above is very useful for a first estimate of the confidence interval on the $HC50_{EC50}$ based on the median, but a limitation remains: the distribution is not smoothed, and therefore, the confidence interval on a median can be unstable when consecutive points of the distribution present several orders of magnitude of differences; this is an important point for the ecotoxicity data since most of the time, data are spread irregularly on 4 to 8 orders of magnitude. In order to make feasible the calculation of distribution-free confidence limits on the median with ecotoxicity data, we have adapted the method presented above, with the assumption of a linear relation between the consecutive EC50s.

For that purpose, we did a resampling of different data set from N=5 to N=100 observations using an Excel macro performing 50,000 resampling. The confidence interval (CI) obtained for each dataset was divided by the total width of the dataset, and this ratio was then plotted with the number of observations N of the dataset. After the Log-logistic transformation of these data, a linear relation was obtained between the relative size of the confidence interval and the size N of the sample. The relation between the sample size and the percentile is presented in the equation below.

$$Log(CI) = -0.532 Log(N) + 0.372 \quad (3)$$

where CI is the fraction of the width of the distribution that is covered by the confidence interval on the median, and N is the number of observations in the sample. Therefore, the lower and upper percentile (P_{min} and P_{max}) of the confidence limit on the median θ can be calculated using the equations (3) and (4).

$$P_{max} = 0.50 + 10^{\frac{-0.532 Log(N) + 0.372}{2}} \quad (4)$$

$$P_{min} = 0.50 - 10^{\frac{-0.532 Log(N) + 0.372}{2}} \quad (5)$$

where P_{max} and P_{min} are the percentiles corresponding to the upper and lower limits of the 95% confidence interval on the median of a sample of N observations.

Extrapolating from N=5 to N=500, the equation (3), (4) and (5) have been used for calculating the values of the 95% confidence limit on the median. Results are presented in Table 10.

Table 10: Distribution-free 95% Confidence interval on the median of a population and the centile corresponding to the lower (Pmin) and the upper (Pmax) limits of the confidence interval.

Sample size	***CI/width sample***	**Pmin**	**Pmax**
5	*1.00*	0.00	1.00
6	*0.91*	0.05	0.95
7	*0.84*	0.08	0.92
8	*0.78*	0.11	0.89
9	*0.73*	0.13	0.87
10	*0.69*	0.15	0.85
11	*0.66*	0.17	0.83
12	*0.63*	0.19	0.81
13	*0.60*	0.20	0.80
14	*0.58*	0.21	0.79
15	*0.56*	0.22	0.78
16	*0.54*	0.23	0.77
17	*0.52*	0.24	0.76
18	*0.51*	0.25	0.75
19	*0.49*	0.25	0.75
20	*0.48*	0.26	0.74
21	*0.47*	0.27	0.73
22	*0.45*	0.27	0.73
23	*0.44*	0.28	0.72
24	*0.43*	0.28	0.72
25	*0.42*	0.29	0.71
26	*0.42*	0.29	0.71
27	*0.41*	0.30	0.70
28	*0.40*	0.30	0.70
29	*0.39*	0.30	0.70
30	*0.39*	0.31	0.69
31	*0.38*	0.31	0.69
32	*0.37*	0.31	0.69
33	*0.37*	0.32	0.68
34	*0.36*	0.32	0.68
35	*0.36*	0.32	0.68
36	*0.35*	0.33	0.67
37	*0.34*	0.33	0.67
38	*0.34*	0.33	0.67
39	*0.34*	0.33	0.67
40	*0.33*	0.33	0.67
41	*0.33*	0.34	0.66
42	*0.32*	0.34	0.66
43	*0.32*	0.34	0.66
44	*0.31*	0.34	0.66
45	*0.31*	0.34	0.66
46	*0.31*	0.35	0.65
47	*0.30*	0.35	0.65
48	*0.30*	0.35	0.65
49	*0.30*	0.35	0.65
50	*0.29*	0.35	0.65
51	*0.29*	0.35	0.65
52	*0.29*	0.36	0.64
53	*0.28*	0.36	0.64
54	*0.28*	0.36	0.64
55	*0.28*	0.36	0.64
56	*0.28*	0.36	0.64
57	*0.27*	0.36	0.64
58	*0.27*	0.36	0.64
59	*0.27*	0.37	0.63
60	*0.27*	0.37	0.63
61	*0.26*	0.37	0.63
62	*0.26*	0.37	0.63
63	*0.26*	0.37	0.63
64	*0.26*	0.37	0.63
65	*0.26*	0.37	0.63
66	*0.25*	0.37	0.63
67	*0.25*	0.37	0.63
68	*0.25*	0.38	0.62
69	*0.25*	0.38	0.62
70	*0.25*	0.38	0.62
71	*0.24*	0.38	0.62
72	*0.24*	0.38	0.62
73	*0.24*	0.38	0.62
74	*0.24*	0.38	0.62
75	*0.24*	0.38	0.62
76	*0.24*	0.38	0.62
77	*0.23*	0.38	0.62
78	*0.23*	0.38	0.62
79	*0.23*	0.38	0.62
80	*0.23*	0.39	0.61
81	*0.23*	0.39	0.61
82	*0.23*	0.39	0.61
83	*0.22*	0.39	0.61
84	*0.22*	0.39	0.61
85	*0.22*	0.39	0.61
86	*0.22*	0.39	0.61
87	*0.22*	0.39	0.61
88	*0.22*	0.39	0.61
89	*0.22*	0.39	0.61
90	*0.21*	0.39	0.61
91	*0.21*	0.39	0.61
92	*0.21*	0.39	0.61
93	*0.21*	0.39	0.61
94	*0.21*	0.39	0.61
95	*0.21*	0.40	0.60
96	*0.21*	0.40	0.60
97	*0.21*	0.40	0.60
98	*0.21*	0.40	0.60
99	*0.20*	0.40	0.60
100 to124	*0.20*	0.40	0.60
125 to 149	*0.18*	0.41	0.59
150 to 199	*0.16*	0.42	0.58
200 to 249	*0.14*	0.43	0.57
250 to 299	*0.12*	0.44	0.56
300 to 349	*0.11*	0.44	0.56
350 to 500	*0.10*	0.45	0.55

For illustrating the use of the table for the calculation of the $HC50_{EC50}$ and the associated confidence interval with the non-parametric method, we propose to estimate the toxicity of the organic chemical: bis(2-ethylhexyl) phthalate (CAS: 117-81-7). Chronic toxicity data collected in the Aquire database (US-EPA 2001) are presented in Table 11.

Table 11: Chronic EC50 data for the bis(2-ethylhexyl) phthalate (CAS: 117-81-7)

Species name	Phyla (or taxa)	EC50 in mg/l	Log EC50
Scenedesmus subspicatus	Algae	0.10	-1.000000
Pimephales promelas	Chordata	0.31	-0.508638
Anacystis aeruginosa	Algae	0.32	-0.494850
Chlorella pyrenoidosa	Algae	0.32	-0.494850
Euglena gracilis	Plant	0.32	-0.494850
Stephanodiscus hantzschii	Plant	0.32	-0.494850
Pseudo-kirchneriella subcapitata	Algae	0.41	-0.382577
Poecilia reticulata	Chordata	0.48	-0.320108
Brachydanio rerio	Chordata	0.72	-0.145365
Oryzias latipes	Chordata	0.78	-0.105638
Jordanella floridae	Chordata	0.94	-0.028870
Gammarus pulex	Arthropod	1.00	0.000000
Gasterosteus aculeatus	Chordata	1.01	0.004414
Daphnia magna	Arthropod	1.76	0.245750
Salmo gairdneri	Chordata	3.54	0.549245
Bufo woodhousei fowleri	Chordata	3.88	0.588832
Rana pipiens	Chordata	4.44	0.647383
Micropterus salmoides	Chordata	50.34	1.701933
Carassius auratus	Chordata	188.48	2.275273
Karenia brevis	Algae	31000.00	4.491362

For N=20, the median is the average of the 10^{th} and 11^{th} values. As indicated in Table 10, the confidence interval on the median represents 48% of the width of the sample and corresponds to the 0.24 and the 0.76 percentile. The results for calculation of the non-parametric estimate of the $HC50_{EC50}$ and the confidence interval of the bis(2-ethylhexyl) phthalate are $Log\theta = 0.86$ [0.32; 3,56].mg/l

Aquatic toxicity data for the comparison of statistical estimators

In order to compare the two statistical estimators on the basis of actual data, we have checked in the database Aquire (US-EPA, 2001) 191 chemicals represented with at least 5 chronic aquatic EC50s covering three different phyla or taxa as required by AMI (Payet and Jolliet 2004). After controlling the reliability of the results, we have selected 7,697 tests providing EC50s for 378 biological species. The chemicals used for the analysis are gathered within 3 groups: inorganics (48 substances), non-Pesticide organics (38 substances) and Pesticide organics (105 substances). The calculation of the average response of species is based on a two-step procedure, first EC50s for one species for a chemical are aggregated together using the geometric mean, in order to obtain one EC50 per species per chemical. The next step is the calculation of the average of these EC50s on the basis of the median or the geometric mean (depending whether we work under non-parametric or parametric assumptions).

Analysis of the Aquatic toxicity data

In this section, the comparison of the average EC50s estimate per substance and the associated confidence interval is presented. Then, the main results of the non-parametric and parametric assessment of the $HC50_{EC50}$ for the 191 substances are summarized regarding three key aspects: the assumption of Log-normal distribution of the data; the differences between the median and the geometric mean for the calculation of the average toxicity of chemicals; and the comparison of the size of the confidence interval on the median or the geometric mean depending on the sample size. More detailed results are presented in Appendix.

Comparison of the average response of species

Using the selected aquatic toxicity data presented in the previous section, the mean of the logEC50s per species for the 3 groups of substances is calculated (Figure 16). It presents a clear distinction of the toxicity between the 3 groups. The geometric mean of the 191 substances ranks from $2.3.10^{-4}$ mg/l for the most toxic substance to $2,3.10^{3}$ mg/l for the less toxic one.

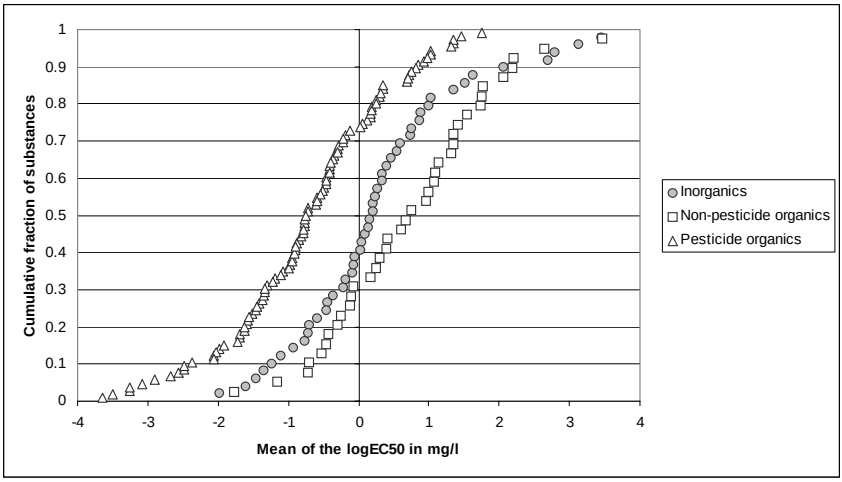

Figure 16: Mean toxicity of the 191 studied substances calculated with the Log EC50s.

Comparison of the EC50s spread

Regarding the spread of the EC50s per chemical, the results presented in Figure 17 indicates the width of the EC50s sample per chemical for the 3 groups of substances covered.

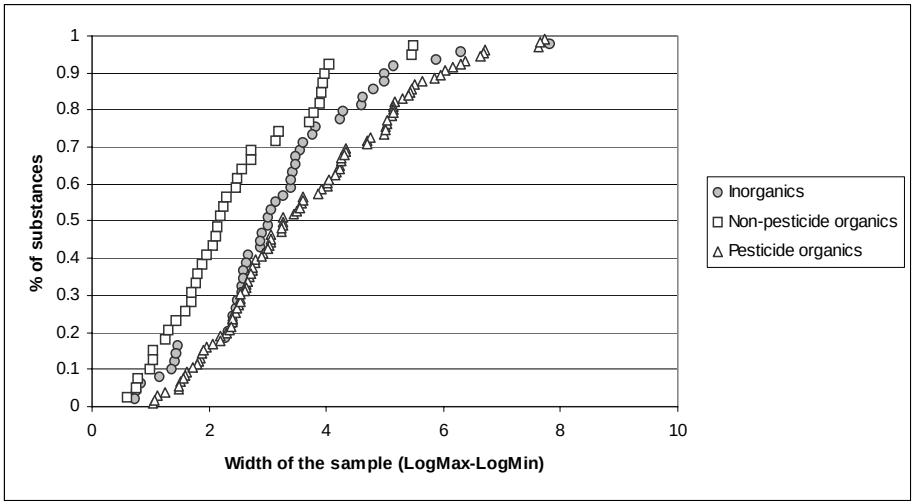

Figure 17: Comparison of the width of the EC50s per chemical for the 191 substances.

The 3 groups present some substances for which EC50s are spread on less that 1 order of magnitude. But for the largest width, the EC50s cover about 5 orders of magnitude for non-Pesticide organics while it covers nearly 8 orders of magnitude for Pesticide organics and inorganics. This can be due to different reasons whether the substance is a Pesticide organic or an inorganic. Some pesticide organics are quite specific regarding the biological target, and do not considerably affect the organisms that are physiologically far from this target species. The width of the EC50s is therefore considerable. For inorganics, the toxicity depends much more on the media condition than on the species sensitivity (Simonnin, Payet et al. 2004), and the available EC50s cover a wide range of media conditions presenting large differences in bioavailability.

Log-normal assumption

Following the publication of Newman's article related to the questionable assumption of log-normality of NOECs data for building Species Sensitivity Distributions [Newman, 2000], the question remains open concerning the relative importance of the non Log-normal distribution of ecotoxicity data. In

this chapter, we have checked the validity of the assumption of the Log-normality of EC50s for the 191 substances considered. The test is based on the Shapiro-Wilk test of normality after the log transformation of the EC50s, and it has been performed using "Statistica 6" software, considering the limit of the 95% chance to accept the assumption of log-normality. The results are presented in Table 12.

Table 12: Validation of the assumption of Log-normal EC50s distribution for 191 substances using Shapiro Wilk test at 95% confidence level.

	Inorganics	Non-Pesticide organics	Pesticide organics	**Total**
Number of non Log-normal	10	13	22	**45**
Total substances	49	38	105	**192**
% of non log-normal	20.4	34.2	20.9	**23.4**

The assumption of Log-normality is rejected in nearly 1 case on 4, and the non-pesticide organics appears to have a higher fraction of non Log-normal distribution than the other two groups. With the purpose of verifying the assumption that the normality is improved with an increase of the sample size, we have analysed the validation of the log-normality assumption regarding the number of species tested (Figure 18).

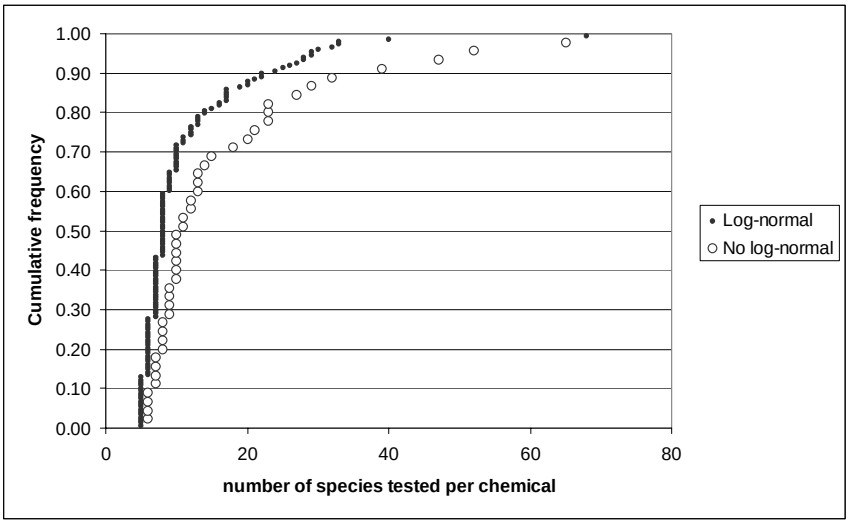

Figure 18: Relation between the number of species tested and the assumption of Log-normality

The results presented in Figure 18 indicate a tendency to observe larger samples associated with the non Log-normal distributions.
The consequences of non log-normal distribution are analysed in the next section

Median versus Geometric mean

The comparison between the median and the geometric mean has been done after a log transformation of the EC50s in order to improve the linearity of the relation (Figure 19).

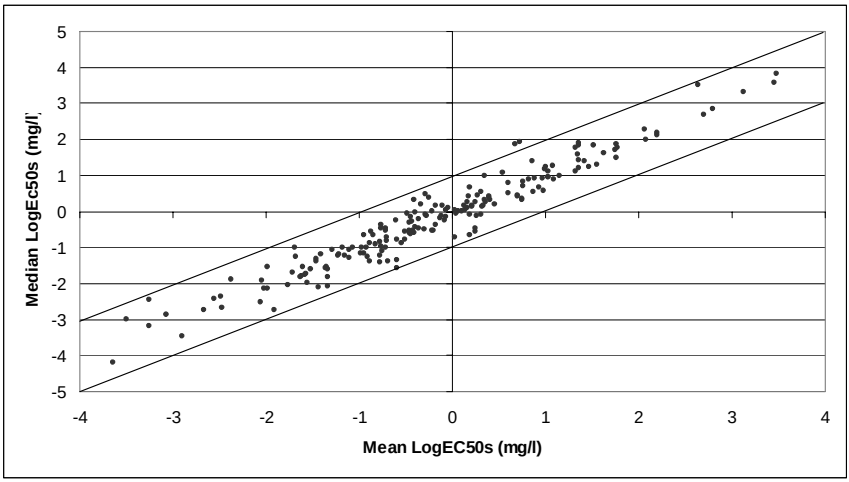

Figure 19 : Comparison between $HC50_{EC50}$ based on the median and based on the geometric mean after log transformation.

The median and the geometric mean present some differences. The two lines in Figure 19 indicate that nearly all the median values are included within an interval of plus or minus one order of magnitude around the geometric mean (99% of the median included). If we consider an interval covering only one order of magnitude around the mean (±0.5), 85% of the median will be included.

Non-parametric and parametric confidence interval

The Student confidence interval on the geometric mean and the distribution-free confidence interval on the median are compared in Figure 20. The relative size of the confidence interval are plotted from N=5 to N=200 assuming in both cases a size of 1 for N=5 (relying on the assumption of an equivalent width between the sample and the confidence interval for that sample size). The CI based on Student decreases faster and corresponds to 30% of the width of the sample at N=30 while this level is reached at N=40 for the Distribution-free CI.

As a consequence, the distribution-free CI is on average 20% larger than the Student one.

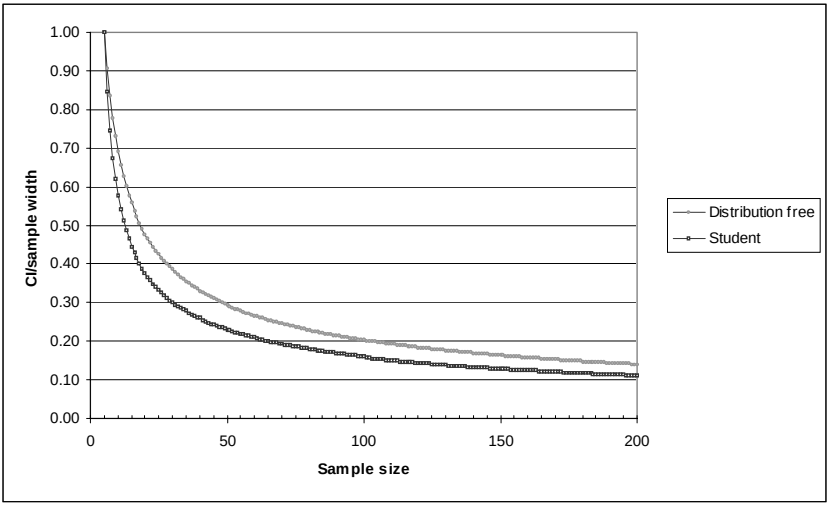

Figure 20 : Comparison of the 95% confidence interval (CI) based on Distribution-free method and the 95% confidence interval based on student.

Beyond the differences in the relative size of the distribution-free and the Student confidence interval, the skewness of the distribution will considerably influence the limits of the confidence interval. Figure 21 compares the confidence interval on the geometric mean and on the median for the 191 substances. For the comparison, the substances have been ranked from the one presenting the biggest asymmetry in favour of the most sensitive species to the one presenting the asymmetry in favour of the most robust species.

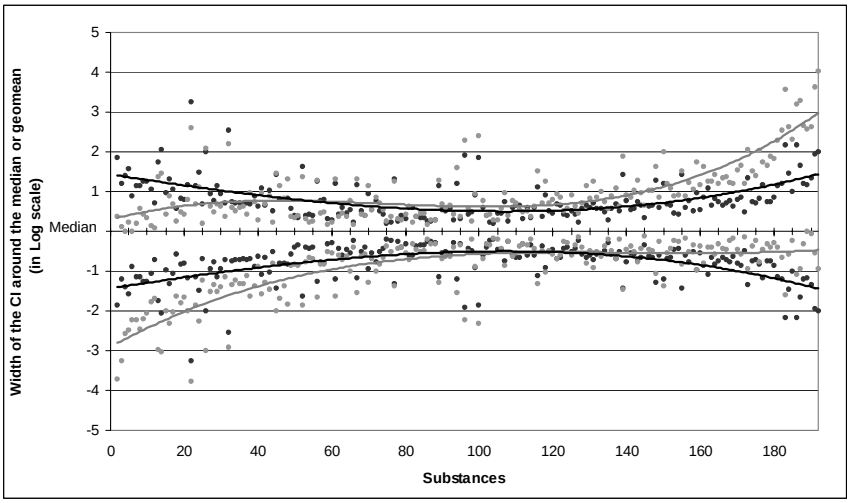

Figure 21 : Comparison of the confidence interval based on the distribution-free (grey) or Student (black) for 191 substances. The curves represent the third order polynomial curve of the confidence limits (Substances are ranking from the larger skewness in favour of the lowest sensitivity to the larger one in favour of the highest sensitivity).

The Student confidence interval is always symmetric in spite of the EC50s spread and is based on an average influence of the two tails of the distribution. On the contrary, the distribution-free confidence interval follows the spread of the EC50s sample. As indicated by the polynomial curve, for the first part of the curve the distribution-free confidence interval is shift on average from one order of magnitude in favour of the most sensitive species, while the discrepancy is similar but in favour of the most robust species in the last part of the curve.

Discussion

On the basis of the results above, we discussed in this section the strength and weaknesses of each method regarding some key aspects: the distribution assumption, the validity of the method for small samples, and the feasibility of an acute to chronic extrapolation.

The question of the *Distribution assumption* has often been discussed about the SSDs. To be modelled, the density probability of the NOECs or EC50s must fit a distribution, which usually means uni-modal and symmetric distributions after log transformation. Three sorts of regression models have been explored: Log-logistic, Log-normal, and Log-triangular. The comparison of the results obtained with the 3 models present very small differences in the potentially observable range (Pennington et al. 2004). The most common is the log-normal regression model. On the other hand, the problem is more complicated concerning the validity of the assumption of Log-normal distribution. Indeed, ecotoxicity data are often asymmetric after log transformation, and cases of multi-modal distributions have been described (Newman, Ownby et al. 2000; DeZwart 2002). The asymmetry can influence strongly the slope at the 5^{th} percentile and therefore, several authors have proposed non-parametric methods for the calculation of the $HC5_{NOEC}$s (Newman, Ownby et al. 2000; Verdonck, Jaworska et al. 2001; Grist, Leung et al. 2002). Nevertheless, the problem of the asymmetry of the distribution has still not been addressed concerning the $HC50_{EC50}$.

The following example aims at illustrating this problem with the comparison of the $HC50_{EC50}$s of the toxicity to aquatic species of two herbicides: the Sulfosulfuron and the Prosulfuron. For the two herbicides, chronic EC50s for aquatic species have been collected. The results are presented in Table 13.

Table 13: Chronic EC50s for the Sulfosulfuron and the Prosulfuron (US-EPA 2001)

		EC50s in mg/l	
Species	**Phyla (or taxa)**	**Sulfosulfuron**	**Prosulfuron**
Daphnia magna	Arthropod	204	296
Pimephales promelas	Chordata	-	292
Salmo gairdneri	Chordata	200	-
Anabaena flosaquae	Algae	0.68	0.027
Lemna gibba	Plant	0.001	0.0012
Navicula pelliculosa	Algae	87	0.084
Pseudo-kirchneriella subcapitata	Algae	0.402	0.01
Skeletonema costatum	Algae	103	0.028

The two pesticide organics present similar levels of toxicity for the most sensitive species, and have the same EC50s spread, covering more than 5 orders of magnitude. Furthermore, in both case, the same phyla (or taxa for plants) and the same species are represented, with
an exception for the chordata, represented by two different fishes.

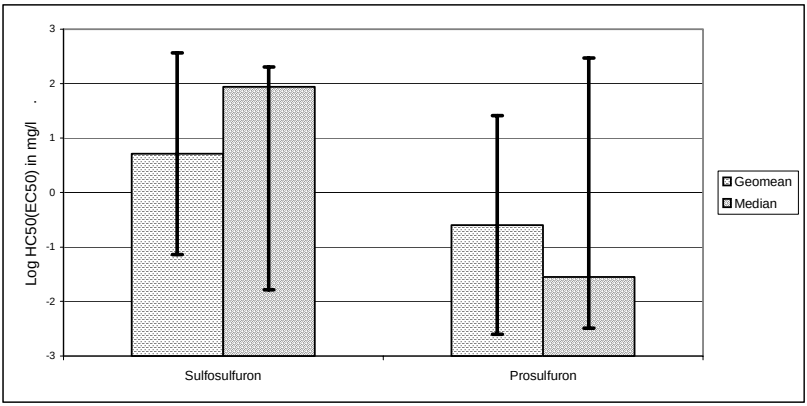

Figure 22: Comparison of the Chronics $HC50_{EC50}$s and their confidence interval for two herbicides.

As a result, we can see similar patterns for the $HC50_{EC50}$ based on the geometric mean, with only one order of magnitude of difference and a symmetric confidence interval (in logarithmic scale) covering in both cases between 3 and 4 orders of magnitude. On the opposite the $HC50_{EC50}$s calculated with the median presents more than 3 orders of magnitude of differences between Sulfosulfuron and Prosulfuron, associated with an asymmetric confidence interval skewed in favour of the sensitive species for Sulfosulfuron and in favour of the robust species for Prosulfuron. Looking back to the EC50s, the difference in sensitivity of algae can explain it. The Sulfosulfuron is very toxic for plants but not so toxic for algae, while the Prosulfuron presents a high toxicity both for algae and plants. A comparison using parametric statistics hides this information while a comparison based on distribution-free method highlights it. At the ecological level, assuming that the two pesticide organics are used at similar doses, we can expect a higher impact on aquatic communities from the Prosulfuron that strongly affects both algae and plants compared to the Sulfosulfuron that strongly affects the plants but presents a lower toxicity for algae. This difference is better illustrated with the distribution-free method (3 order of magnitude of difference between the two medians) than with the parametric one (1 order of magnitude of difference between the two geometric mean).

The other weakness of the ecotoxicity data regarding the assumption of log-normality is the multi-modal distribution. De Zwart (DeZwart 2002) has presented different Species Sensitivity Distributions (SSD) for Pesticide organics that are characterized by this particularity. In that case, it is possible to observe some gaps between EC50s that can reach one order of magnitude in the middle of the distribution. If the median is close to such a gap, the $HC50_{EC50}$ can vary by a factor 4 or 5 whether a data is removed or added to the sample. The geometric mean is less sensitive to this problem. Nevertheless, this is not expected to be a major issue in LCA, firstly because the factor 4 of variability will only have a small influence on the final result, and secondly, because pesticide organics were expected to be concerned at first by multi-modal distributions but after checking the validity of the log-normal assumption, the pesticide organics are not the group that presents the largest part of the non Log-normal distributions. Nevertheless, in order to resolve the problem of multi-modal distributions, the possibility of using a multi-PAF approach based on separate phyla has been explored both for the $HC5_{NOEC}$s calculation (DeZwart,

Posthuma et al. 2000) and for the $HC50_{EC50}$ (Larsen and Payet 2003). The main limitation for assessing separate $HC50_{EC50}$ per phyla is the lack of EC50s.

The *applicability to small samples* (3, 4 or 5 EC50s) is also a key aspect in the choice of the statistical estimator. Indeed, the distribution-free method requires at least 5 EC50s for calculating the $HC50_{EC50}$ of a chemical. On the contrary, the student method can be applied with a minimum of 3 EC50s. The constraints of 5 EC50s per substance would reduce drastically the number of substances considered.

Still considering the data availability, it must be noticed that most of the *data available concern acute EC50s, while chronic $HC50_{EC50}$s are required in LCA.* The statistical estimator must therefore enable the assessment of chronic $HC50_{EC50}$s on the basis of acute EC50s. The strength of the distribution-free method is to conserve the information on the asymmetry of the distribution. Nevertheless, different species, and different types of effect are tested for acute EC50s compared to chronic ones, and therefore, no relations are to be expected between the asymmetry observed for chronic data and those eventually observed with acute EC50s. Extrapolation from acute to chronic $HC50_{EC50}$s using a distribution-free method for the acute $HC50_{EC50}$s calculation would not be relevant.

Conclusion

The method presented in this chapter for the calculation of $HC50_{EC50}$s and their associated confidence interval provides the possibility to apply a distribution-free approach for comparative assessment. Since a large part of the substances does not present a Log-normal distribution, the possibility to use a non parametric approach is promising and should be developed further. Nevertheless, for LCIA purpose, the key issue is the number of substances covered, and typically, only a small number of acute data are available for the calculation of the average toxicity and the confidence interval. It is therefore relevant to use in priority the parametric method, based on the geometric mean and Student for two reasons: (1) a minimum requirement of 3 acute EC50s available can be satisfying, while the distribution-free method requires at least 5 chronic EC50s; (2) an extrapolation from acute to chronic $HC50_{EC50}$ is feasible

with the geometric mean and the confidence interval based on Student while it is disputable for the median and the distribution-free confidence interval.

Nevertheless, based on the comparison between the distribution-free method and the parametric one, several conclusions have to be highlighted.
1- Concerning the rejection of the assumption of lognormal distribution, we have shown that it is not a consequence of the lack of data, and it is more likely a property of the substance. Furthermore the non-lognormal distributed substances are not randomly distributed among the type of substances. This indicates that in spite of the relatively small number of the non-lognormal distribution (23% on 191 substances), it can sometimes influence the results of an LCA study.
2- For skewed distributions, the use of the distribution-free method provides a better estimate of the $HC50_{EC50}$, and the confidence interval fits better with the data spread. The distribution-free method would be more relevant where at least 5 chronic EC50s are available and if the distribution of the EC50s does not fit with a Log-normal distribution.
3- Concerning the comparison between the geometric mean and the median, we have observed a good consistency between the median and the geometric mean. This suggest that a LCA study considering hundreds of substances would be quite robust regarding this assumption. Nevertheless, as presented with the comparison between the Prosulfuron and the Sulfosulfuron, this discrepancy can affect an LCA study if the concerned substance has a major influence on the LCA results.

Therefore, the relative influence of the non-lognormal substances on the LCA results must not be under estimated, and we would therefore suggest the strategy below.
As a first approach, we suggest to take the geometric mean with the Student confidence interval as a baseline, and calculate the non parametric median and the bootstrap confidence interval as a sensitivity study, to test skewness and its consequences when non-parametric $HC50_{EC50}$s are available. If the result significantly differs and this influence the decision, special care in the analysis should be taken.
An alternative is possible in mixing the parametric and the distribution-free method (i.e, some values calculated by one method, others by the other), but further researches are needed to confirm that this does not introduce any bias in the results.

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Appendix: Results of the analysis of the 191 substances regarding Log-normality testing, Geometric mean and median calculation.

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
Sulfosulfuron (Herbicide)	141776-32-1	Pesticide organic	7	0.81704	0.06013	**0.714**	-1.138	2.566	**1.940**	-1.788	2.306
1,4-Naphthalenedione	130-15-4	Non pest.Organic	10	0.71484	0.00133	**0.668**	-0.541	1.877	**1.889**	-1.367	2.000
Silver chloride	7783-90-6	Inorganic	6	0.49609	0.00002	**0.534**	-0.869	1.936	**1.079**	-1.480	1.079
4-Chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazinone	27314-13-2	Pesticide organic	5	0.80985	0.09726	**-0.295**	-1.862	1.273	**0.477**	-2.013	0.716
Benomyl (fungicide)	17804-35-2	Pesticide organic	8	0.70047	0.00228	**-0.412**	-1.302	0.478	**0.337**	-1.899	0.342
Acetic acid ethyl ester	141-78-6	Non pest.Organic	7	0.71208	0.00502	**2.638**	1.486	3.790	**3.519**	1.298	3.725
2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	34256-82-1	Pesticide organic	8	0.83719	0.07047	**-0.405**	-1.562	0.752	**-0.008**	-2.469	0.559
2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid	81334-34-1	Pesticide organic	7	0.71444	0.00532	**1.350**	0.080	2.619	**1.851**	-0.360	2.277
BENSULIDE	741-58-2	Pesticide organic	5	0.87236	0.27611	**-0.341**	-1.586	0.903	**0.198**	-1.860	0.554
5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile	120068-37-3	Pesticide organic	9	0.69845	0.00136	**-1.695**	-2.761	-0.629	**-1.000**	-2.765	-0.855
4-Chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinene	96489-71-3	Pesticide organic	9	0.91705	0.3684	**-2.375**	-3.098	-1.652	**-1.873**	-3.572	-1.785
2-Chloro-6-[4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid sodium salt	123343-16-8	Pesticide organic	8	0.91482	0.38925	**0.850**	-0.888	2.589	**1.406**	-1.558	2.766
Hydroxytriphenylstannane	76-87-9	Pesticide organic	6	0.89179	0.32768	**-3.505**	-5.560	-1.451	**-2.996**	-6.037	-1.530
2,4-dichlorophenol	120-83-2	Non pest.Organic	13	0.79263	0.00558	**-0.258**	-1.224	0.708	**0.403**	-1.588	0.833
Strontium chloride	10476-85-4	Inorganic	7	0.8411	0.10173	**1.516**	0.214	2.819	**1.856**	-0.467	2.734
Dibutyldichlorostannane	683-18-1	Pesticide organic	16	0.97036	0.84435	**-0.773**	-1.819	0.274	**-0.361**	-2.402	0.327

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
(2,4-Dichlorophenoxy)acetic acid	94-75-7	Pesticide organic	20	0.91337	0.07392	**1.356**	0.793	1.920	**1.923**	0.305	2.193
6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	115-29-7	Pesticide organic	20	0.9596	0.53587	**-1.687**	-2.515	-0.859	**-1.246**	-3.048	-0.752
(2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine (1:1)	2008-39-1	Pesticide organic	8	0.86327	0.12939	**1.322**	0.528	2.115	**1.781**	0.137	2.199
2-Chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide	64902-72-3	Pesticide organic	10	0.89674	0.20171	**0.342**	-0.819	1.502	**1.000**	-1.014	1.798
(Acetyloxy)triphenylstannane	900-95-8	Pesticide organic	5	0.92536	0.5651	**-3.253**	-6.509	0.004	**-2.436**	-6.215	0.155
1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone	59756-60-4	Pesticide organic	6	0.85242	0.16464	**0.306**	-0.845	1.457	**0.540**	-1.328	1.228
6,7-Dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium, Dibromide	85-00-7	Pesticide organic	6	0.91919	0.49952	**-0.948**	-2.435	0.539	**-0.652**	-2.900	0.452
METHYL PARATHION	298-00-0	Pesticide organic	13	0.84087	0.02174	**0.188**	-0.512	0.888	**0.663**	-0.576	0.864
3-Chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1H-pyrazole-4-carboxylic acid, Mether ester	100784-20-1	Pesticide organic	7	0.97565	0.93591	**-0.772**	-2.773	1.229	**-0.456**	-3.451	1.637
BROMIDE	7647-15-6	Inorganic	6	0.89791	0.3617	**3.127**	2.180	4.075	**3.330**	1.823	3.954
2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	15972-60-8	Pesticide organic	7	0.90928	0.39093	**-0.455**	-1.239	0.329	**-0.138**	-1.489	0.336
2,3,4,6-Tetrachlorophenol	58-90-2	Non pest.Organic	7	0.71797	0.0058	**-0.296**	-1.427	0.835	**-0.093**	-1.617	0.590
1,1,1-trichloroethane	71-55-6	Non pest.Organic	6	0.87451	0.2448	**2.062**	1.107	3.016	**2.293**	0.888	2.911
3-[2,4-Dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2(3H)-one	19666-30-9	Pesticide organic	6	0.86535	0.2083	**-1.419**	-2.133	-0.706	**-1.173**	-2.326	-0.802
Flumetsulam	98967-40-9	Pesticide organic	6	0.83436	0.11699	**0.170**	-2.363	2.702	**0.412**	-2.506	2.601
Selenious acid, Disodium salt	10102-18-8	Inorganic	8	0.93118	0.52691	**-0.053**	-0.790	0.683	**0.106**	-1.227	0.717
lead	7439-92-1	Inorganic	14	0.97763	0.95873	**0.273**	-0.434	0.981	**0.464**	-0.762	0.972
Chromium oxide	1333-82-0	Inorganic	9	0.94565	0.64249	**-0.443**	-1.168	0.282	**-0.280**	-1.582	0.327
1,2-dichlorobenzene	95-50-1	Non pest.Organic	8	0.88364	0.20396	**1.344**	0.650	2.038	**1.590**	0.261	2.227
1,2-Benzenedicarboxylic acid, Dibutyl ester	84-74-2	Non pest.Organic	6	0.85991	0.18886	**-0.075**	-0.779	0.630	**0.063**	-1.051	0.499

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
DIURON	330-54-1	Pesticide organic	27	0.92415	0.04975	**-0.489**	-1.174	0.197	**-0.056**	-1.685	0.902
3-Methoxy-2-methylbenzoic acid, 2-(3,5-Dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide	161050-58-4	Pesticide organic	7	0.91459	0.42855	**-0.280**	-1.176	0.616	**-0.108**	-1.488	0.630
2-Propanone	67-64-1	Non pest.Organic	12	0.75579	0.00307	**3.473**	2.843	4.103	**3.847**	2.957	4.135
3,5-Dibromo-4-hydroxybenzonitrile	1689-84-5	Pesticide organic	6	0.88491	0.29236	**0.821**	-0.264	1.906	**0.905**	-0.669	1.877
chloroform	67-66-3	Non pest.Organic	12	0.954	0.69591	**1.761**	1.112	2.411	**1.886**	0.602	2.574
2-Chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide	87674-68-8	Pesticide organic	7	0.87238	0.19473	**-0.723**	-1.745	0.298	**-0.444**	-1.905	0.440
Bis(8-quinolinolato-N1,08)copper	10380-28-6	Pesticide organic	5	0.88619	0.33832	**-2.054**	-2.561	-1.547	**-1.918**	-2.721	-1.682
2-[[[[[4,6-Bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid, Methyl ester	86209-51-0	Pesticide organic	7	0.95625	0.78595	**-0.856**	-2.280	0.569	**-0.653**	-2.661	0.799
1,1'-(2,2,2-Trichloroethylidene)bis(4-chlorobenzene)	50-29-3	Pesticide organic	24	0.94127	0.17409	**-0.604**	-1.412	0.204	**-0.251**	-1.936	0.890
CHLOROBENZENE	108-90-7	Non pest.Organic	12	0.92752	0.35454	**1.076**	0.283	1.869	**1.292**	-0.067	2.125
1,1'-(2,2,2-Trichloroethylidene)bis[4-methoxybenzene]	72-43-5	Pesticide organic	21	0.89633	0.02973	**-1.295**	-2.116	-0.475	**-1.071**	-2.886	0.237
Arsenenous acid, Sodium salt	7784-46-5	Inorganic	13	0.91518	0.21578	**0.995**	0.434	1.555	**1.255**	0.399	1.615
Diethylcarbamothioic acid, S-[(4-Chlorophenyl)methyl]ester	28249-77-6	Pesticide organic	16	0.96574	0.76571	**-0.724**	-1.132	-0.316	**-0.524**	-1.345	-0.183
LINURON	330-55-2	Pesticide organic	27	0.92633	0.05622	**-0.874**	-1.203	-0.544	**-0.562**	-1.431	-0.167
4"-Deoxy-4"-(methylamino)-avermectin B1 benzote (salt)	137512-74-4	Pesticide organic	5	0.96363	0.83303	**-2.560**	-4.202	-0.919	**-2.409**	-4.252	-1.027
CHLORPYRIFOS	2921-88-2	Pesticide organic	29	0.90502	0.01295	**-1.986**	-2.394	-1.579	**-1.533**	-2.298	-1.207
3-(3,5-dichlorophenyl)-2,4-dioxo-N-isopropylimidazolidine-1-carboxamide	36734-19-7	Pesticide organic	8	0.80788	0.03474	**-0.370**	-0.807	0.066	**-0.215**	-0.879	0.021
N-(3,4-Dichlorophenyl)propanamide	709-98-8	Pesticide organic	7	0.92485	0.50799	**-1.189**	-1.595	-0.782	**-1.002**	-1.676	-0.738

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
Chlorotriphenylstannane	639-58-7	Pesticide organic	8	0.90335	0.3096	**-3.075**	-4.341	-1.808	**-2.869**	-4.523	-1.594
FENITROTHION	122-14-5	Pesticide organic	18	0.88064	0.02676	**-0.185**	-0.943	0.573	**0.174**	-0.658	0.640
COBALT	7646-79-9	Inorganic	15	0.94358	0.42958	**-0.461**	-1.255	0.333	**-0.523**	-1.360	-0.035
3,4-dichloroaniline	95-76-1	Non pest.Organic	32	0.90913	0.01066	**-0.106**	-0.424	0.213	**0.180**	-0.348	0.364
bis(tributyltin) oxide	56-35-9	Pesticide organic	33	0.97479	0.6224	**-2.485**	-2.766	-2.203	**-2.350**	-2.894	-2.119
fenthion	55-38-9	Pesticide organic	10	0.97361	0.92211	**-1.073**	-2.276	0.130	**-0.983**	-2.599	0.337
6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine	122-34-9	Pesticide organic	29	0.94236	0.11558	**-0.463**	-0.904	-0.022	**-0.296**	-0.908	0.065
manganese sulphate	7785-87-7	Inorganic	5	0.95719	0.78826	**1.026**	0.438	1.615	**1.118**	0.417	1.570
1,1,2,2-tetrachloroethane	79-34-5	Non pest.Organic	6	0.90279	0.39068	**1.349**	0.889	1.810	**1.446**	0.817	1.827
4-nitrophenol	100-02-7	Non pest.Organic	10	0.8826	0.13976	**0.751**	0.222	1.280	**0.849**	-0.158	1.609
6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine	1912-24-9	Pesticide organic	65	0.94615	0.00685	**-0.514**	-0.731	-0.296	**-0.554**	-0.960	-0.383
N-[2,4-Dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide	122836-35-5	Pesticide organic	7	0.87683	0.21275	**-0.224**	-1.401	0.952	**0.009**	-1.526	1.310
CADMIUM	10124-36-4	Inorganic	11	0.92702	0.38146	**-1.231**	-1.757	-0.706	**-1.222**	-1.849	-0.803
POTASSIUM	7447-40-7	Inorganic	8	0.88933	0.23071	**2.796**	2.385	3.206	**2.868**	2.309	3.262
1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-(1a,4a,4ab,5a,8a,8ab)-1,4:5,8-dimethanonaphthalene	309-00-2	Pesticide organic	7	0.96224	0.8377	**-1.715**	-2.662	-0.767	**-1.699**	-2.996	-0.562
MANGANESE	7773-01-5	Inorganic	6	0.97168	0.90353	**0.880**	0.341	1.419	**0.927**	0.239	1.478
1,2-Dichloroethane	107-06-2	Non pest.Organic	6	0.94972	0.73798	**2.191**	1.422	2.960	**2.184**	1.193	3.040
cadmium	7440-43-9	Inorganic	29	0.97588	0.72589	**-0.366**	-0.875	0.144	**-0.467**	-1.309	0.242
2-chloro-2'-ethyl-N-(2-methoxy-1-methylethyl)-6'-methylacetanilide	51218-45-2	Pesticide organic	12	0.9534	0.68715	**-0.400**	-0.828	0.027	**-0.430**	-1.093	0.101
barium chloride	10361-37-2	Inorganic	9	0.87674	0.14507	**1.623**	1.410	1.835	**1.630**	1.259	1.875
Mercuric chloride	7487-94-7	Inorganic	40	0.96199	0.19578	**-1.460**	-1.699	-1.221	**-1.362**	-1.866	-0.984
Zinc dimethyldithiocarbamate	137-30-4	Pesticide organic	5	0.97649	0.91501	**-1.225**	-2.540	0.090	**-1.174**	-2.550	0.079

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
copper	7440-50-8	Inorganic	33	0.96741	0.41228	**-0.775**	-1.042	-0.508	**-0.824**	-1.254	-0.507
HEXACHLOROBENZENE	118-74-1	Pesticide organic	9	0.81185	0.02786	**-1.518**	-1.919	-1.117	**-1.602**	-1.992	-1.323
Nitric acid, silver (1+) salt	7761-88-8	Inorganic	25	0.94795	0.2253	**-1.604**	-1.907	-1.300	**-1.528**	-2.070	-1.090
Tin chloride	7772-99-8	Inorganic	6	0.96735	0.87421	**0.388**	-0.204	0.980	**0.405**	-0.321	1.040
Hydrogen sulfide	7783-06-4	Inorganic	10	0.61508	0.00008	**-1.357**	-1.944	-0.770	**-1.540**	-1.841	-1.326
Aluminosilicic acid, Sodium salt	1344-00-9	Inorganic	5	0.95123	0.74596	**2.690**	2.317	3.063	**2.713**	2.255	3.097
2,4-dinitrotoluene	121-14-2	Non pest.Organic	14	0.91303	0.17437	**0.249**	-0.093	0.591	**0.276**	-0.227	0.729
ZINC	7646-85-7	Inorganic	28	0.98251	0.90563	**0.042**	-0.295	0.378	**-0.033**	-0.520	0.418
2-Propenal	107-02-8	Non pest.Organic	10	0.82369	0.02808	**-1.155**	-1.402	-0.909	**-1.213**	-1.407	-1.053
NITROBENZENE	98-95-3	Non pest.Organic	6	0.88533	0.29441	**1.416**	1.157	1.676	**1.419**	1.097	1.718
1,2-Benzenedicarboxylic acid, Dimethyl ester	131-11-3	Non pest.Organic	5	0.97799	0.92357	**1.745**	1.462	2.029	**1.726**	1.417	2.020
Arsenic	7440-38-2	Inorganic	5	0.96539	0.84492	**0.596**	-0.560	1.752	**0.512**	-0.770	1.785
Trichloroethene	79-01-6	Non pest.Organic	8	0.97096	0.90546	**2.200**	1.646	2.754	**2.133**	1.435	2.825
DIFLUBENZURON	35367-38-5	Pesticide organic	13	0.8858	0.08548	**-2.477**	-2.987	-1.967	**-2.655**	-3.270	-2.044
1,2-Benzenedicarboxylic acid, Butyl phenylmethyl ester	85-68-7	Non pest.Organic	8	0.8729	0.16086	**0.163**	-0.422	0.748	**0.107**	-0.535	0.754
SODIUM DODECYL SULPHATE	151-21-3	Pesticide organic	28	0.96022	0.35293	**1.027**	0.738	1.316	**0.972**	0.563	1.398
TIN	688-73-3	Pesticide organic	8	0.94171	0.62795	**-2.671**	-3.874	-1.469	**-2.739**	-4.279	-1.152
VANADIUM	1314-62-1	Inorganic	5	0.90855	0.45891	**0.339**	0.023	0.655	**0.323**	-0.012	0.707
2-[[[[(4,6-Dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid, Methylester	74222-97-2	Pesticide organic	6	0.98908	0.98682	**-0.896**	-2.813	1.021	**-0.882**	-3.105	1.394
PHENOL	108-95-2	Non pest.Organic	26	0.94026	0.13627	**0.992**	0.560	1.424	**1.174**	0.667	1.735
SODIUM CHLORIDE	7647-14-5	Inorganic	12	0.67434	0.00048	**3.447**	2.956	3.938	**3.587**	3.382	3.854
4-Chlorobenzoic acid, 2-Benzoyl-2-(1,1-dimethylethyl)hydrazide	112226-61-6	Pesticide organic	5	0.97695	0.91767	**-0.126**	-1.027	0.775	**-0.108**	-0.983	0.833

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid, (3-Phenoxyphenyl)methyl ester	52645-53-1	Pesticide organic	7	0.93963	0.63539	**-1.465**	-3.335	0.404	**-1.301**	-3.621	1.091
dimethoate	60-51-5	Pesticide organic	12	0.96638	0.86939	**1.024**	0.423	1.626	**0.965**	0.270	1.742
Copper sulfate, pentahydrate	7758-99-8	Inorganic	17	0.96592	0.74384	**-1.109**	-1.517	-0.700	**-1.068**	-1.387	-0.667
Sulfuric acid, Zinc salt (1:1)	7733-02-0	Inorganic	68	0.99056	0.89073	**0.210**	-0.025	0.445	**0.137**	-0.214	0.572
4-Nitro-3-(trifluoromethyl)phenol	88-30-2	Pesticide organic	17	0.98123	0.96748	**0.756**	0.569	0.943	**0.716**	0.532	0.993
1-Naphthalenol, Methylcarbamate	63-25-2	Pesticide organic	15	0.78052	0.00209	**0.117**	-0.351	0.586	**0.184**	-0.010	0.478
1-Methyl-3-nitrobenzene	99-08-1	Non pest.Organic	5	0.99532	0.99461	**0.964**	0.498	1.429	**0.919**	0.477	1.477
1,2,3-Trichlorobenzene	87-61-6	Non pest.Organic	7	0.96922	0.89274	**-0.431**	-1.186	0.324	**-0.509**	-1.353	0.472
PENTACHLOROPHENOL	87-86-5	Pesticide organic	52	0.94021	0.01142	**-0.299**	-0.557	-0.042	**-0.490**	-0.756	-0.081
Tributylchlorostannane	1461-22-9	Pesticide organic	22	0.93986	0.19663	**-3.252**	-3.859	-2.646	**-3.162**	-3.781	-2.393
Selenium	7782-49-2	Inorganic	9	0.964	0.83913	**0.203**	-0.459	0.864	**0.180**	-0.366	0.892
1,2,4-trichlorobenzene	120-82-1	Non pest.Organic	9	0.76896	0.00898	**0.389**	-0.255	1.034	**0.439**	0.060	0.988
ACENAPHTHENE	83-32-9	Non pest.Organic	7	0.94687	0.70112	**-0.128**	-0.450	0.194	**-0.169**	-0.479	0.325
4-chlorophenol	106-48-9	Non pest.Organic	13	0.82201	0.0126	**0.601**	-0.010	1.212	**0.807**	0.467	1.333
COPPER	7447-39-4	Inorganic	47	0.90746	0.00126	**-0.923**	-1.277	-0.569	**-1.004**	-1.198	-0.621
1,2-Benzenedicarboxylic acid, bis(2-Ethylhexyl)ester	117-81-7	Non pest.Organic	20	0.73799	0.00012	**0.302**	-0.287	0.890	**-0.067**	-0.495	0.559
Nickel	7440-02-0	Inorganic	8	0.95234	0.73477	**-0.091**	-1.194	1.011	**-0.227**	-1.542	1.292
Hexahydro-1H-azepine-1-carbothioic acid, S-Ethyl ester	2212-67-1	Pesticide organic	17	0.95577	0.55377	**0.355**	-0.018	0.728	**0.255**	-0.238	0.964
1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one	143-50-0	Pesticide organic	13	0.94858	0.57687	**-1.585**	-2.502	-0.667	**-1.737**	-2.762	-0.490
(1alpha,2alpha,3beta,4alpha,5alpha,6beta)-1,2,3,4,5,6-Hexachlorocyclohexane	58-89-9	Pesticide organic	32	0.97456	0.63348	**-1.114**	-1.642	-0.586	**-1.288**	-1.768	-0.578

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
(1a alpha, 2 beta, 2a alpha, 3 beta, 6 beta, 6a alpha, 7 beta, 7a alpha)-3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene	60-57-1	Pesticide organic	30	0.94474	0.12206	**-1.632**	-2.078	-1.186	**-1.809**	-2.383	-1.000
CHLORINE	7681-52-9	Inorganic	8	0.89582	0.2648	**-0.705**	-1.396	-0.015	**-0.723**	-1.418	0.214
Propazine	139-40-2	Pesticide organic	7	0.85072	0.12478	**-0.981**	-1.634	-0.328	**-1.000**	-1.602	-0.153
1-chloro-2,4-dinitrobenzene	97-00-7	Non pest.Organic	5	0.86105	0.23204	**-0.455**	-0.870	-0.039	**-0.602**	-0.849	-0.097
zinc	7440-66-6	Inorganic	19	0.95251	0.4356	**0.023**	-0.390	0.435	**0.051**	-0.246	0.621
PENTACHLOROBENZENE	608-93-5	Non pest.Organic	10	0.94617	0.62344	**-0.712**	-1.236	-0.189	**-0.802**	-1.409	0.106
FLUORIDE	7681-49-4	Inorganic	7	0.88675	0.25815	**2.068**	1.651	2.484	**1.989**	1.652	2.630
1,1,2-trichloroethane	79-00-5	Non pest.Organic	23	0.88307	0.0115	**1.769**	1.551	1.987	**1.778**	1.632	2.230
LEAD	10099-74-8	Inorganic	23	0.90882	0.03858	**-0.178**	-0.749	0.392	**-0.377**	-0.781	0.346
[(Dimethoxyphosphinothioyl)thio]butanedioic acid, Diethyl ester	121-75-5	Pesticide organic	17	0.95554	0.54977	**-0.823**	-1.473	-0.174	**-0.892**	-1.702	0.240
1,4-Dichlorobenzene	106-46-7	Non pest.Organic	9	0.98353	0.97994	**0.404**	-0.186	0.995	**0.342**	-0.132	1.160
DIAZINON	333-41-5	Pesticide organic	17	0.94289	0.35413	**-0.987**	-1.689	-0.285	**-1.155**	-2.073	0.114
BORIC ACID	10043-35-3	Inorganic	10	0.9652	0.8431	**1.349**	0.791	1.907	**1.200**	0.699	2.053
chromium	7440-47-3	Inorganic	10	0.93251	0.47307	**0.326**	-0.251	0.903	**0.176**	-0.458	1.166
1-[[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole	60207-90-1	Pesticide organic	13	0.9295	0.33571	**0.059**	-0.415	0.534	**0.017**	-0.361	0.758
AMMONIA	12125-02-9	Inorganic	39	0.92444	0.01197	**0.454**	0.155	0.752	**0.190**	-0.002	0.761
[[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo [3,4-a]pyridazin-1-ylidine)amino]phenyl]thioacetic acid methyl ester	117337-19-6	Pesticide organic	7	0.90666	0.37321	**-2.028**	-2.599	-1.458	**-2.141**	-2.631	-1.264
Copper chloride	1344-67-8	Inorganic	7	0.97955	0.95732	**-1.986**	-2.686	-1.287	**-2.131**	-2.813	-1.036
LEAD	7758-95-4	Inorganic	23	0.86003	0.00416	**0.154**	-0.370	0.679	**0.269**	0.061	0.900
Cadmium nitrate	10325-94-7	Inorganic	6	0.86335	0.20095	**0.092**	-1.324	1.507	**0.001**	-1.463	1.894
2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	57966-95-7	Pesticide organic	11	0.75452	0.00238	**0.251**	-0.519	1.022	**-0.101**	-0.547	0.805
NICKEL	7718-54-9	Inorganic	21	0.96212	0.55981	**-0.072**	-0.602	0.459	**-0.148**	-0.507	0.698

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nickel sulphate	7786-81-4	Inorganic	10	0.95941	0.77914	**0.132**	-0.448	0.712	**0.040**	-0.473	1.067
2-[[6-(2-Cyanophenoxy)-4-pyrimidinyl]oxy-alpha-(methoxymethylene)benzeneacetic acid, E-Methyl ester	131860-33-8	Pesticide organic	8	0.93238	0.53803	**-0.458**	-1.208	0.292	**-0.594**	-1.359	0.686
alpha-[2-(4-Chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	107534-96-3	Pesticide organic	6	0.89913	0.36881	**-0.419**	-1.063	0.226	**-0.578**	-1.017	0.382
5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydrozybenzamide, compd. with 2-aminoethanol (1:1)	1420-04-8	Pesticide organic	11	0.83675	0.02863	**-0.763**	-1.106	-0.420	**-0.970**	-1.081	-0.324
1,3-dichlorobenzene	541-73-1	Non pest.Organic	6	0.93632	0.62969	**1.141**	0.503	1.780	**1.005**	0.538	2.020
1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene	76-44-8	Pesticide organic	6	0.93026	0.58213	**-1.576**	-2.245	-0.907	**-1.722**	-2.201	-0.680
2,3-Dihydro-2,2-dimethyl-7-benzofuranol, Methylcarbamate	1563-66-2	Pesticide organic	8	0.90671	0.33144	**-0.759**	-2.043	0.525	**-1.088**	-2.135	0.555
N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]-1H-imidazole-1-carboxamide	67747-09-5	Pesticide organic	5	0.89197	0.3671	**-0.950**	-1.553	-0.347	**-1.137**	-1.398	-0.276
DISULFOTON	298-04-4	Pesticide organic	9	0.90005	0.25228	**-1.626**	-2.839	-0.412	**-1.782**	-3.151	0.220
5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone	1698-60-8	Pesticide organic	8	0.95832	0.79393	**0.322**	-0.365	1.010	**0.146**	-0.395	1.332
Methylbenzene	108-88-3	Non pest.Organic	6	0.91328	0.45832	**1.548**	0.878	2.217	**1.324**	0.888	2.420
2,4-Dinitrophenol	51-28-5	Non pest.Organic	7	0.8307	0.08125	**1.085**	0.629	1.541	**0.900**	0.683	1.781
1-[[4-Bromo-2-(2,4-dichlorophenyl)tetrahydro-2-furaanyl]methyl]-1H-1,2,4-triazole	116255-48-2	Pesticide organic	8	0.87077	0.15336	**-0.596**	-1.036	-0.157	**-0.770**	-1.027	0.156
S-(2,3,3-Trichloro-2-propenyl)ester bis(1-methylethyl), carbamothioic acid	2303-17-5	Pesticide organic	5	0.82437	0.12612	**-0.219**	-1.656	1.217	**-0.522**	-1.356	1.000
2-Ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranol methane sulfonate	26225-79-6	Pesticide organic	5	0.86033	0.22944	**0.693**	0.100	1.285	**0.441**	0.255	1.334
4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid	1918-02-1	Pesticide organic	7	0.83047	0.08085	**0.926**	0.152	1.699	**0.667**	0.139	1.909
2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine	1582-09-8	Pesticide organic	10	0.91408	0.31019	**-1.363**	-1.877	-0.849	**-1.569**	-1.904	-0.503
(4-chloro-2-methylphenoxy)acetic acid	94-74-6	Pesticide organic	9	0.88545	0.17905	**0.697**	-0.285	1.680	**0.447**	-0.521	2.200

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glyphosate	1071-83-6	Pesticide organic	11	0.94592	0.59233	**1.466**	0.929	2.004	**1.230**	0.851	2.404
Tetrachloroethene	127-18-4	Non pest.Organic	8	0.90292	0.30688	**1.311**	0.443	2.180	**1.125**	0.312	2.751
3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-[2,7:3,6-dimethanonaphth[2,3-b]oxirene,[1a alpha,2 beta,2a beta,3 alpha,6 alpha,6a beta,7 beta,7a alpha]	72-20-8	Pesticide organic	10	0.73045	0.00206	**-2.906**	-3.989	-1.822	**-3.448**	-3.859	-2.198
3,5-Dinitro-N4, N4-dipropyl-sulfanilamide	19044-88-3	Pesticide organic	8	0.83704	0.07022	**-0.919**	-1.574	-0.265	**-1.260**	-1.671	-0.004
Ammonia	7664-41-7	Inorganic	7	0.96301	0.84408	**0.871**	0.048	1.694	**0.546**	-0.038	1.989
3-Cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione	51235-04-2	Pesticide organic	14	0.85292	0.02434	**-0.781**	-1.533	-0.029	**-1.222**	-1.544	-0.019
((3,5,6-Trichloro-2-pyridinyl)oxy)-acetic acid, Cmpd. with N,N-Diethylethanamine (1:1)	57213-69-1	Pesticide organic	8	0.89407	0.25522	**1.751**	1.092	2.411	**1.490**	1.060	2.820
3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3-(2H)-dione	133-06-2	Pesticide organic	8	0.89832	0.27902	**0.177**	-0.546	0.900	**-0.091**	-0.561	1.284
anthracene	120-12-7	Non pest.Organic	5	0.85615	0.21477	**-1.765**	-2.523	-1.006	**-2.022**	-2.252	-0.804
PHENANTHRENE	85-01-8	Non pest.Organic	6	0.77977	0.03835	**-0.518**	-1.153	0.118	**-0.773**	-0.952	0.419
2,4-Bis(isopropylamino)-6-methylthio-S-triazine	7287-19-6	Pesticide organic	10	0.90452	0.24544	**-1.346**	-2.142	-0.550	**-1.598**	-2.058	-0.063
aluminium chloride	7446-70-0	Inorganic	10	0.9293	0.44103	**0.740**	-0.115	1.594	**0.356**	-0.218	2.006
Selenic acid, Disodium salt	13410-01-0	Inorganic	11	0.9193	0.31284	**-0.211**	-0.706	0.285	**-0.532**	-0.665	0.679
[[1,2-Ethanediylbis[carbamodithioato]](2-)]manganese	12427-38-2	Pesticide organic	6	0.92458	0.53892	**-0.547**	-1.877	0.783	**-0.856**	-1.803	1.203
1H,1,2,4-Triazol-3-amine	61-82-5	Pesticide organic	8	0.88078	0.1916	**0.981**	0.231	1.732	**0.589**	0.205	2.108
2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile	1897-45-6	Pesticide organic	8	0.87428	0.16591	**-1.566**	-2.278	-0.855	**-1.986**	-2.228	-0.595
AZINPHOSMETHYL	86-50-0	Pesticide organic	22	0.91767	0.06804	**-1.442**	-2.258	-0.626	**-2.111**	-2.882	-0.086
N-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine	40487-42-1	Pesticide organic	8	0.72097	0.00388	**-1.338**	-2.499	-0.177	**-1.805**	-2.272	-0.064
Chromium chloride	10025-73-7	Inorganic	8	0.87884	0.18357	**0.746**	-0.002	1.494	**0.337**	0.023	1.995
TOXAPHENE	8001-35-2	Pesticide organic	9	0.82498	0.03921	**-2.062**	-2.816	-1.307	**-2.518**	-2.924	-0.620

Name	CAS	Chem group	Number species	S-W test	S-W proba.95%	Log GeoMean	Log Geomin	Log Geomax	Log Median	Log Medmin	Log Medmax
ALUMINIUM	10043-01-3	Inorganic	10	0.81342	0.02109	**0.242**	-0.627	1.112	**-0.447**	-0.722	1.394
3-(4-isopropylphenyl)-1,1-dimethylurea	34123-59-6	Pesticide organic	7	0.84769	0.11705	**-0.777**	-1.906	0.353	**-1.402**	-1.812	0.870
2-(tert-Butylamino)-4-(cyclopropylamino)-6-(methylthio)-s-triazine	28159-98-0	Pesticide organic	9	0.87807	0.14985	**-1.912**	-3.103	-0.721	**-2.721**	-3.367	-0.183
O,O-Diethyl S-[(ethylthio)methyl])ester, Phosphorodithioic acid	298-02-2	Pesticide organic	7	0.90024	0.33242	**-1.337**	-3.499	0.824	**-2.076**	-3.675	1.506
4-(Difluoromethoxy)-alpha-(1-methylethyl)benzeneacetic acid, (3-Phenoxyphenyl)methyl ester	70124-77-5	Pesticide organic	6	0.84343	0.13914	**-3.644**	-5.105	-2.182	**-4.183**	-4.750	-1.560
FLUORANTHENE	206-44-0	Non pest.Organic	10	0.79479	0.01253	**-0.696**	-1.685	0.293	**-1.361**	-1.597	0.947
silver	7440-22-4	Inorganic	5	0.81194	0.10103	**-0.717**	-2.878	1.443	**-0.979**	-2.056	2.217
2-[4-(1,1-Dimethylethyl)phenoxy]cyclohexyl-2-propynyl ester, Sulfurous acid	2312-35-8	Pesticide organic	8	0.87505	0.16877	**-0.890**	-2.537	0.758	**-1.366**	-2.309	1.909
TEBUTHIURON (herbicide)	34014-18-1	Pesticide organic	8	0.76833	0.01299	**0.187**	-1.002	1.377	**-0.644**	-0.963	2.010
Mepiquat Chloride (Plant growth regulator)	24307-26-4	Pesticide organic	7	0.64885	0.001	**0.023**	-1.145	1.190	**-0.699**	-0.699	1.859
Sethoxydim (herbicide)	74051-80-2	Pesticide organic	6	0.71798	0.00951	**0.244**	-1.097	1.585	**-0.536**	-0.595	2.101
Cupric nitrate	3251-23-8	Inorganic	6	0.73807	0.01519	**-0.596**	-2.539	1.348	**-1.334**	-1.881	2.286
Prosulfuron (Herbicide)	94125-34-5	Pesticide organic	7	0.80971	0.05103	**-0.597**	-2.604	1.409	**-1.553**	-2.492	2.469

CHAPTER 5

Application of the AMI Method for Comparative Assessment of Metals*

* In Payet, J. and O. Jolliet (2004). Comparative Assessment of the Toxic Impact of metals on aquatic ecosystems: the AMI method. In Life Cycle Assessment of Metals: Issues and research directions. A. Dubreuil Editor, SETAC Press, Pensacola (FL) USA (in press); Pages 172-175. (with slight modifications)

Abstract

The AMI (Assessment of Mean Impact) method enables the comparative assessment of the impacts of toxic substances on aquatic ecosystems. It is based on three key principles: (1) For ecotoxicological endpoints, the method is based mainly on single-species laboratory EC50s (Effect Concentration for 50% of the individuals of a species), which is the endpoint with the lowest uncertainty and NOEC (No Observed Effect Concentration), a commonly used endpoint in long-term studies. (2) Instead of assuming a specific distribution, the median of the test results is applied for calculation of the ecotoxicity indicator. (3) The uncertainty of the ecotoxicity indicator is calculated using a distribution-free method.

This chapter briefly describes the method and focuses on its application for the assessment of impact of metals on aquatic species. For that purpose, 9 metals are considered in the analysis, sometimes tested wit different salt and speciation. Two interesting results can be highlighted: the toxicity of metals covers the whole range of toxicity of chemicals; the spread of EC50s for test results on metals is on average twice as great for metals compared with other chemicals. This increase in the variability of ecotoxicological responses from species is likely to be due to the change in bioavailability of metals associated with a change of test conditions (pH, or Organic Matter).

Keywords: aquatic ecosystem, LCIA, LCA, metals, speciation

Nota Bene: This chapter has been submitted in the early development of the researches, it does not reflect all the details and improvement of the AMI methods applying to inorganics.

Introduction and presentation of AMI

AMI is the ecotoxicological effect component of IMPACT 2002 (Impact assessment of chemical toxicants), a new method developed at the EPFL to determine the Life Cycle Impacts of Toxics.

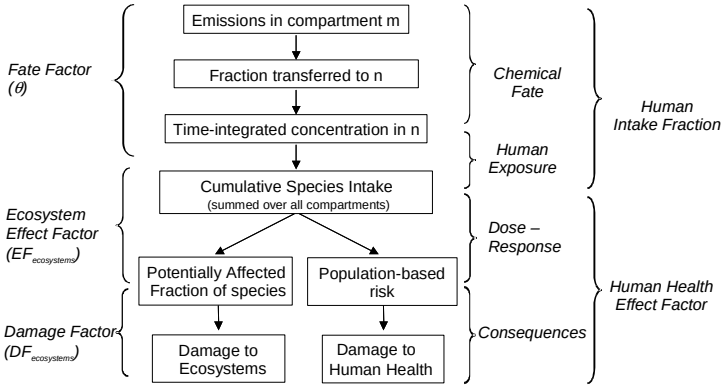

Figure 23: Impact 2002: general diagram

IMPACT 2002 provides a characterisation factor based on a generic default Effect Factor for chronic effects on aquatic (water column) ecosystems. Termed Ecotoxicological Damage Factor (EDF), it is calculated as the combination of two terms (Figure 23, lefthand side):

$$EDF = \theta \cdot EF_{ecosystem} \,.\, DF_{ecosystem} \qquad (1)$$

The fate factor θ consists of the equivalent residence time (the time- and space-integrated concentration in the aquatic freshwater per mass input of chemical released into the environment). The same fate model is applied as for human toxicity, but the interface between fate and effect is at the level of concentration for ecotoxicity. Exposure is implicitly taken into account in the Effect Factor.

The Effect Factor – EF – is the change in the Potentially Affected Fraction of species that experiences an increase in stress for a change in contaminant concentration.

Payet et al. (2002) proposed the AMI [Assessment of the Mean Impact] method to calculate ecotoxicological effect factors, along with estimates of the associated parameter (data) uncertainty. Effect Factors already exist for 300 chemicals. In the absence of field or mesocosm data, the use of median acute (LC50 or EC50) or median chronic (EC50) results of ecotoxicity tests for at least five species provides the preferable basis to estimate the median effect on multiple species systems (the median estimate of the $HC50_{EC50}$ which is the hazardous concentration of toxic affecting 50% of the species above their EC50). NOECs data does not provide a consistent basis for use in relative comparisons, hence is not retained except to estimate chronic EC50s via extrapolation. Both the median EC50 and the data uncertainty are estimated using a non-parametric (bootstrap) method to avoid unnecessary assumptions of the shape of a multiple species distribution (Species Sensitivity Distribution, SSD).

In short, the AMI method is based on three key principles: (1) For ecotoxicological endpoints, the method is based on single-species laboratory EC50s , which is the endpoint with the lowest uncertainty and NOECs (No Observed Effect Concentration), commonly used for endpoint in chronic studies. (2) Instead of assuming a specific distribution, the median of the test results is applied for calculation of the ecotoxicity indicator. (3) The uncertainty of the ecotoxicity indicator is calculated using a bootstrap method.

Application to metals

Metals are always present in the results of Life Cycle Inventory in Life Cycle Assessment and are often determinant in study results. Nevertheless, LCIA (Life Cycle Impact Assessment) methods for ecosystems so far do not enable reliable assessment of the toxicological impact on aquatic and terrestrial ecosystems. The toxicity is directly based on an NOEC [EDIP, 1997; USES-LCA 2000; ECO-INDICATOR 1999] or EC50 [AMI, 2002], without taking into account media conditions and the speciation of metals.

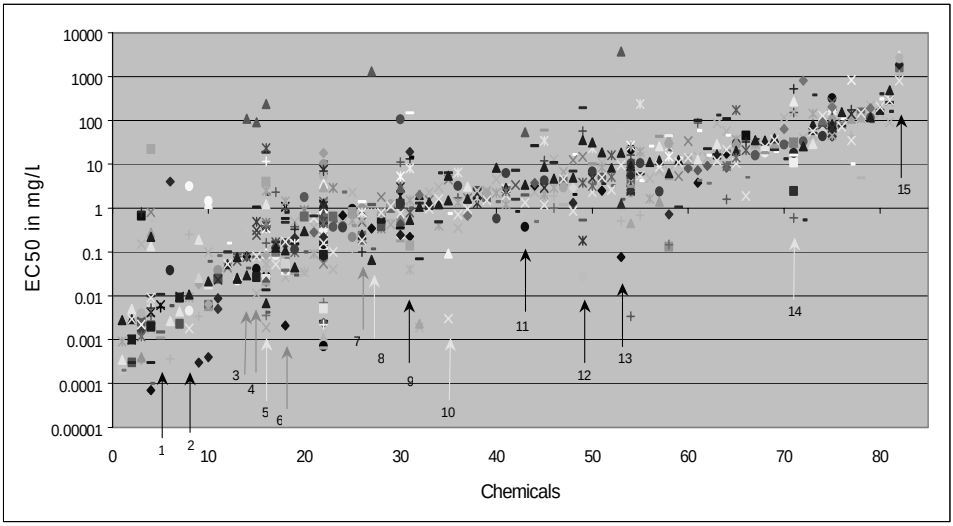

Figure 24: Comparison of the median toxicity of 82 chemicals based on 217 species. Arrows indicate the position of the following substances: Tributyltin (1); Silver (2); Copper (3) (4) (6) (7); Cadmium (5) (8) (10) (14); Zinc (9); Lead (11); Chromium (12); Nickel (13); Molybdenum (15).

The comparison between the toxicity of metals and non-metal substances, presented in Figure 24, illustrates three interesting points.

a) The metals presented here cover the whole range of chemical toxicity. From silver which have median toxicity (Median EC50 for at least 5 species) of 0.005 mg/L, to molybdenum (median EC50 = 1740 mg/L). The accuracy of metal toxicity data is therefore as important as for other chemicals.

b) The same metal can be tested with different salts, and these formulations may influence the toxicity. The examples of copper and cadmium are presented in Table 14. For cadmium, the most toxic formulation is 370 times more toxic than the least toxic.

c) The last point is the importance of the spread of the EC50 for test results on metals. The spread is on average twice as great for metals compared with other chemicals. As presented in Table 14, the ratio between the maximum and minimum EC50 can attain more than 5 orders of magnitude for cadmium, and approximately 4 orders of magnitude for chromium. An important factor of EC50 variability is explained by the differences in sensitivity between species and life stages of single species. Furthermore, concerning metal toxicity, an increase in the variability of ecotoxicological responses from species can be due to the different salt tested but also to the change in bioavailability of metals associated with a change of test conditions (pH, or Organic Matter).

Table 14: Toxicological results for 9 metals (results expressed in mg/L).

CASNO	Salt	Speciation	Min EC50	Max EC50	Max/Min ratio	Median EC50
10108-64-2	Cadmium chloride (CdCl2)	CADMIUM II	2.000E-03	2.358E+02	1.238E+05	8.400E-02
7718-54-9	Nickel chloride (Cl2Ni)	NICKEL II	7.700E-02	3.722E+03	4.866E+04	5.535E+00
10022-68-1	Cadmium nitrate Tetrahydrate (CdH8N2O10	CADMIUM II	6.600E-02	1.307E+03	1.980E+04	5.610E-01
7447-39-4	Copper Chloride (Cl2Cu)	COPPER II	1.100E-02	9.023E+01	7.915E+03	8.000E-02
7778-50-9	Potassium Dichromate (Cr2K2O7)	CHROMIUM VI	2.800E-02	1.950E+02	7.040E+03	4.015E+00
7446-20-0	Sulfuric acid, zinc salt (1:1), heptahydrate (H14O11SZn)	ZINC II	4.000E-02	1.479E+02	3.698E+03	1.001E+00
10031-43-3	cupric nitrate trihydrate (CuH6N2O9)	COPPER II	3.000E-02	1.090E+02	3.695E+03	7.800E-02
7761-88-8	Silver nitrate (AgNO3)	SILVER I	2.000E-03	3.160E+00	1.756E+03	1.000E-02
7758-99-8	Copper Sulfate (pentahydrate)	COPPER II	2.000E-03	1.430E+00	6.842E+02	1.190E-01
10099-74-8	Lead nitrate	LEAD II	3.700E-01	5.390E+01	1.457E+02	2.565E+00
1461-22-9	Tributyltin Chloride	TRIBUTYLTIN	1.000E-03	1.080E-02	9.818E+00	5.000E-03
7631-95-0	Sodium molybdate	MOLYBDENUM VI	8.000E+02	3.057E+03	3.821E+00	1.740E+03

As presented in Figure 25, accuracy in the assessment of the toxicity of metals is crucial since the uncertainty is associated with the toxic value.

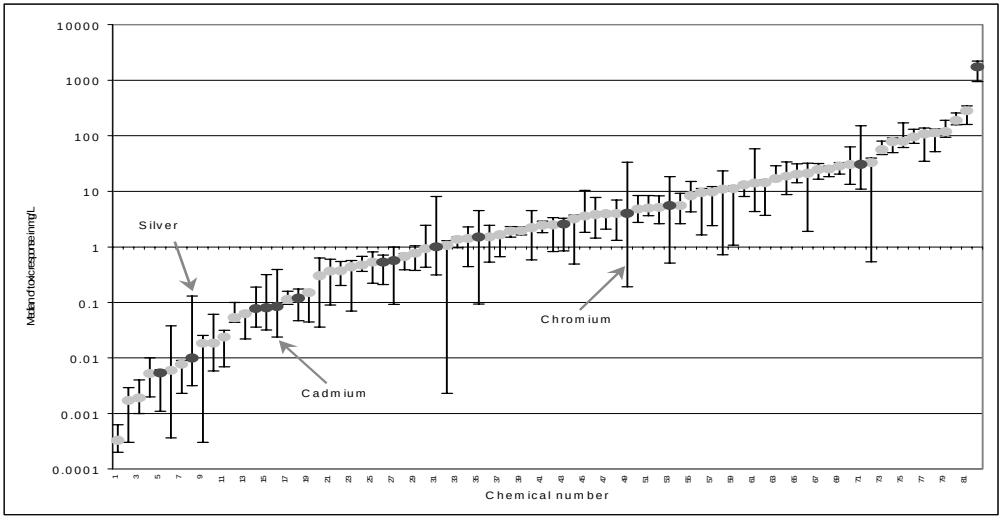

Figure 25: Median toxicity of 82 chemicals including 67 organics (grey dots) and 15 metals (black dots) ranked from most to least toxic. The associated uncertainty is calculated using the bootstrap technique as described in the AMI method [Payet et al, 2002].

Indeed, discrimination between the levels of toxicity of substances is particularly important in a comparative approach like LCA. A high degree of uncertainty in the assessment of toxicological impacts tends to reduce the interpretability of the final study results. It seems therefore important to better identify the effect of metal speciation, in order to improve the accuracy of the ecotoxicity indicator, which is used in the LCIA method.

Conclusions

The non parametric version of the AMI method and its integration in the IMPACT 2002 assessment framework offers interesting new insights for the comparative assessment of chemicals, for either LCIA or comparative risk assessment. Indeed, being close to the mode, the median is a good representative of the responses of the greater number of species. Furthermore, this estimator is not influenced by outliers and is a stable statistical estimator if sufficient data are considered. This is not right if the median is based on less than 5 EC50s. For three or four data for example, the gap between consecutive EC50s can be very large and the median, as a breakdown point indicator, would became unstable. Considering the confidence interval of the median, the bootstrap is a distribution-free method that fit the data spread. This is visible in Figure 25, where the asymmetry of the confidence interval follows the asymmetry of the EC50s spread. When a very sensitive species is tested while all other species present in average a good resistance to the substance, the confidence interval is skewed in favour of the lowest concentration. On the opposite, if the substance is in average very toxic for most of the species while only a small number are resistant, the confidence interval is skewed in favour of the high concentrations. The skewness of the distribution is therefore important in the description of the substances toxicity, and the only way to express this information is the use of a distribution-free method for the assessment of confidence intervals. Nevertheless, this is also likely to be a problem for small dataset or when a biological group of species is over-represented in the EC50s dataset for a substance. For the first point, the bootstrap based confidence interval requires at least 5 data and the calculation is not feasible for 3 or 4 species. For the second point, the over-representation of one phyla or taxa could lead to a biased estimate of the confidence interval excluding one whole phylum from the confidence interval of the median. In order to avoid it, three rules can be applied: (1) To require a minimum number of EC50s for the calculation of the Effect Factor (e.g.: a minimum of 5 EC50s or NOECs); (2) To fix a minimum diversity representation (e.g.: data covering at least three species from three different phyla or taxa); (3) To propose a flexible application of the confidence interval with some alternatives to the bootstrap (e.g. if the Geometric mean of one phyla is out of the range of the confidence interval, this geometric mean can be substituted to the confidence limit).

The application of the AMI method in its non-parametric version has provided interesting findings related to the comparative assessment of metals. Depending on the metal tested, the $HC50_{EC50}$ cover a broad range of toxicity (about 6 orders

of magnitude between the highest and the lowest $HC50_{EC50}$), as variable as the organic toxics. Depending on the formulation tested, which is linked to the metal speciation, the $HC50_{EC50}$ can vary by more than two order of magnitude. The variability of EC50s for metals is in average twice greater than the variability of EC50s for organic substances, and this can also be due to the speciation of metals. Indeed, the metals toxicity is conditioned by the speciation, and the speciation depends on the media condition. Therefore, the variability in pH, organic matter, hardness, etc. is likely to influence considerably the toxicity of the substance. This is highlighted by the AMI method since the indicator is based on the average response of species. A method based on the most sensitive species like the PNEC would not allow such an observation since only the lowest EC50 or NOEC (the one based on the most toxic speciation) would be considered in the assessment, and therefore, metals would simply appear as very toxic substances.

In terms of perspectives, these results are highlighting the strength of a method based on the mean response of species for comparative purpose. It is therefore possible to have a better perception of the toxicity of metals compared to other substances. Furthermore, it allows to make a distinction regarding the media quality for the calculation of Effect Factors for metals. This would allow the development of a spatially differentiated Effect Factors database, relating the intensity of the impact to the quality of the ecosystems biotope.

Acknowledgements

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CHAPTER 6

Analysis of Data Availability and Reliability for Calculation of LCIA Effect Factors for Aquatic Ecosystems*

* To be submitted

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Abstract

The purpose of the chapter is to analyse the reliability of existing aquatic toxicity databases and to quantify the number of Effect Factors for Life Cycle Impact Assessment (LCIA), that can be calculated on the basis of these databases. For that purpose, the main LCIA methods are presented focusing on their data requirement. It concerns: EDIP (based on the PNEC); AMI in its parametric version (based on the $HC50_{EC50}$); Eco-Indicator (based on the $HC50_{NOEC}$); USES-LCA (based on the $HC5_{NOEC}$). These requirements are compared to the aquatic toxicity data availability. Six ecotoxicity databases available in an electronic format are analysed: Aquire; Pesticide Ecotoxicity Database (PED); IUCLID; Acute Toxicity Database (ATD); Fathead Minnow database (FMD); and ECETOC Aquatic Toxicity Database (EAT). The analysis especially focuses on the identification of the substances and organisms the definition of the tests conditions, and the control procedure of the database. A selection of tests is done, retaining 128,864 tests results, acute, sub-chronic and chronic. A description of the data availability on the basis of the selected test is performed, considering the available EC50s (Effect Concentration affecting 50% of the individuals tested), LOECs and NOECs (Lowest or No Observed Effect Concentration). The number of covered substances is also analysed regarding the number of species or phyla considered. On that basis, an estimation of the maximum number of calculable Effect Factors is performed. The results highlight the discrepancy between the large number of test results available (128,864), and the relatively restricted number of Effect Factors (betweeen 34 and 4959 depending on the method)) that can be calculated for a comparative purpose like LCIA.

Key words*:* LCA, LCIA, ecotoxicity database, aquatic toxicity

Introduction:

Life Cycle Assessment is a comparative tool that aims at quantifying the environmental impacts of all the substances emitted during the Life-Cycle of a product. This chapter focuses on the impact of toxic substances on aquatic ecosystems. The main purpose is to analyze the availability of reliable data for the quantification of the potential hazard of chemicals on the aquatic ecosystems as a function of method requirements. Indeed, the LCI typically covers several hundreds of chemicals that have a potential impact on ecosystems, and finally only 15 to 50% are included in the impact assessment due to the lack of Effect Factors available.

During the last decade, several new methods for the Life Cycle Impact Assessment (LCIA) on ecosystems have been proposed (Wenzel, Hauschild et al. 1998; Huijbregts 1999; Goedkoop, Effting et al. 2000; Payet and Jolliet 2004). Most of the time, the number of Effect Factors available is an important weakness of a LCIA method. For the LCIA on aquatic ecosystems, EDIP bases Effect Factors on PNECs (Predicted No Effect Concentration). The PNEC can use both EC50s (Effect Concentration affecting 50% of the individuals tested) and NOECs (No observed Effect Concentration), and conservative extrapolation factors can be used for the calculation of chronic Effect Factors based on acute data. In Wenzel and Hauschild book (Wenzel, Hauschild et al. 1998), Effect factors for 76 substances are provided. The Eco-Indicator report (Goedkoop, Effting et al. 2000) provides indications for the calculation of Effects Factors based on chronic $HC50_{NOEC}$s only. The report also provides Effect Factors for 46 substances. USES-LCA proposes to use the chronic $HC5_{NOEC}$ as the indicator for the calculation of Effect Factors. Nevertheless, due to the lack of data, only part of the 181 factors provided (Huijbregts, Thissen et al. 2000) is based on the $HC5_{NOEC}$ while the other is based on a PNEC using the most sensitive species. More recently, the AMI method based on the $HC50_{EC50}$ has been proposed (Payet and Jolliet 2004), where acute and chronic EC50s, NOECs and LOECs (Lowest Observed Effects Concentration) can be used. Effect Factors for 522 substances are provided with this method.

In most of the new developments for the LCIA on ecosystems, the Effect Factors availability is the stumbling block of the methods. Addressing this question, the present chapter aims at analyzing how Effect Factors can be

calculated on the basis of existing databases in a consistent and reliable way with each method.

A review of the main existing database has identified 6 databases (Table 15).

Table 15: Presentation of the main available databases

Name	Origin	Author and version	Year (version)	Format	References
AQUIRE	US	US-EPA	2001	Table	(US-EPA 2001)
IUCLID	EC	ECB	2000	Table	(EU-Commission 2000)
ATD	US	Department of the interior	1986	Table	(Mayer and Ellersieck 1986)
PED	US	OPP	2001	Table	(US-EPA 2002)
FMD	US	Russom et al	1997	Table	(Russom, Bradbury et al. 1997)
EAT3	Priv.	ECETOC	1993	Table	(ECETOC 2002)

Four databases are from different US research centres, one is from the European Commission, and one database is from an European private association.

Each database is analysed throughout the framework of LCIA, especially addressing the following questions: (1) Which databases can be used for the development of LCIA effect factors on ecosystems? (2) What is the content and the respective reliability of these databases? (3) How many factors (Acute and chronic) can be potentially calculated for each method?
In order to answer the questions, the databases are first described. Then, they are analysed regarding their content and their level of reliability. After this step, the relevant data of all databases are extracted and combined. On the basis of the created dataset, we estimate the number of Effect Factors that can be potentially calculated with each method for acute and chronic aquatic toxicity.

Presentation of the databases

The databases selected for the analysis are described below.

ECOTOX database (US-EPA 2001)*:* created in 1999, the ECOTOX database has been developed by the MED-Duluth institute (US-EPA), and includes three components: AQUIRE, concerning the aquatic ecosystems; PHYTOTOX, concerning terrestrial plants; and TERRETOX for the terrestrial animal organisms. ECOTOX is based on the collection of ecotoxicological data from many scientific documents (articles, books, reports). After a detailed review, the results are reported in the database. This electronic database covers different types of chemicals (i.e.: pesticides, organic non pesticides, and inorganic substances). This database is the biggest available, covering nearly 8,000 substances, and 5,000 biological species, with 365,419 test results in November 2001. Data related to a chemical, or a set of chemicals can be obtained via the EPA website, entering the chemical name or its Chemical Abstract Service number (CAS). It is therefore possible to edit the ecotoxicological data as a table of data convertible in an Excel MS format for example. For the present study, we have focused on the AQUIRE database (AQUatic toxicity Information REtrivial). It is the main component of ECOTOX. This database was initiated in 1981 by the US-EPA, and the main part of the data covers tests performed in the last 30 years.
The way to provide data has been designed at first for Environmental Risk Assessment and access is provided for individual substances (or for a small group of substances). It is therefore very useful when a limited number of chemicals is under focus, but is time consuming when a large number of substances are considered.

IUCLID (EU-Commission 2000)*:* This database has been developed by the European Chemical Bureau, at the Joint Research Center. The IUCLID CD-Rom was first published in 1996. The 2000 version contains data for 2,604 substances, and provides information on the chemicals properties as well as human toxicity and ecotoxicity. The data of the CD-Rom have been submitted by the industries to the European Commission in the frame of the council regulation (EEC) N° 793/93 on the “Evaluation and Control of the Risk of Existing Substances”.
This database is the biggest one for Europe and does not provide as many data as AQUIRE. For aquatic ecotoxicity, acute toxicity data concern 1,100 to 1,700 substances, while chronic toxicity data are available only for about 400 chemicals (Allanou, Hansen et al. 1999). The IUCLID database is also available in a table format instead of a text format. Nevertheless, some errors have been

identified with the comparison between data in the two formats. It is therefore preferable to control the data for the calculation of effect factors.

Acute Toxicity Database (ATD) (Mayer and Ellersieck 1986): this database was published in 1986 by the Columbia Environmental Research Center. ATD is based on acute toxicity data developed by the Columbia National Fisheries Research Laboratory since 1965. The database is provided as a book, but can also be downloaded from the US-Geological Survey website as a text file convertible in MS excel format. The database describes 4,901 ecotoxicity tests concerning 410 chemicals and considers 66 freshwater species, mainly fishes. The electronic database presents nevertheless some errors for the identification of substances, in the assignment of the CAS numbers.

Pesticide Ecotoxicity Database (PED) (US-EPA 2002): the PED database was initiated in 1991, and is now developed by the Environmental Fate and Effect division of the Office of Pesticide Programs (US-EPA). It concerns only pesticides, and the data comes from three sources: the results of toxicological studies provided by pesticide companies in support to their products; the studies conducted by the US-EPA, and other US administrations over the last 25 years; some published data selected by the OPP. The database provides results for 600 pesticides (more than 14,000 tests results) for aquatic and terrestrial organisms. This database covers numerous pesticides, with a very small number of errors. In spite of some wrong CAS numbers, the database appears to have a good level of reliability. This is partly due to the 3 level quality insurance procedure that has been followed for the integration of data.

ECETOC Aquatic Toxicity database(EAT)(ECETOC 2002): The first version of the EAT1 database was published in 1993 (ECETOC, 1993). In the new version EAT3, the database proposed by the "European Center for Ecotoxicology and Toxycology of Chemicals", is based on a selection of 178 publications selected regarding their quality. The database concerns nearly 600 substances, tested through 5,460 tests, using 259 freshwater and marine species. Both acute and chronic exposures are addressed in the database. Nevertheless, the data reliability is affected by the lack of quality control, and numerous input errors would have been avoided with a rigorous control procedure.

Fathead Minnow Database (FMD) (Russom, Bradbury et al. 1997): The Mid-Continent Ecology Division (US-EPA) published the Fathead Minnow database

in 1997. The database has supported the development of a QSAR (Quantitative Structure Activity Relationship) estimating the toxicity of industrial organic compounds on Fathead Minnow on the basis of their mode of action. For this study, Russom and collaborators have analysed 753 acute toxicity tests results concerning 617 substances. Data are mainly from the Center for lake superior environmental studies, of the Wisconsin University. A high level of reliability of the database is observed, mainly due to the quality control procedure of the ecotoxicity data.

In addition, databases built on similar original data but provided in text format (pdf or html) like the Pesticide Manual (Tomlin 2001) are used for the control of ecotoxicity data.

Analysis of the databases

The calculation of Effect Factors in LCIA is most of the time based on existing data. The selection of data is therefore crucial in order to obtain reliable effect factors. The key points of the data selection concern especially four aspects and are described below.

Identification of the substances

The *identification of the substance* requires at least the CAS number, the name and the fraction of active ingredient used for the test, but it can also include the SMILES formula of the substance, its use, the molecular weight, the Kow, etc.
The main criteria (i.e.: CAS number, name and percent of active ingredient) are most of the time correctly described in all the databases. Nevertheless, there are some CAS errors in PED, EAT3, and ATD databases. Concerning the other criteria, it is important to mention the effort made for the identification of the use of the substances in ATD, PED and EAT3, and in their toxic mode of action (TMoA) in PED and FMD. We also have to point out the strength of the FMD mentioning the water solubility limit of the substance for all tests. In terms of details, the AQUIRE database in indisputably the one that gives the most technical information related to the substance, and also the one that covers the largest number of substances. Nevertheless, the commercial name of the substance is lacking, making a control of CAS numbers more complex.

Furthermore, it would have been useful to integrate the chemical use and the TMoA (Toxic Mode of Action) in the database for the data interpretation.
It is also important to mention that concerning partitioning chemicals and inorganics, the informations related to the speciation of the substances in the media tested is generally not specified in the database.

Identification of the organisms

The *identification of the organism* used in the test, requires at least the species latin name, and the phyla for animals or taxa for plants (algae or vascular plant). Some more details can also be mentioned like the development stage, the weight, the size, etc.
Among the 5 databases that cover several species (FMD excluded), only PED mentions both the Latin name of the species and the phyla (or taxa). IUCLID and EAT mention the Latin name and the trophic level but not the phyla, EAT simply makes a discrimination between the Daphnia genus and the other invertebrates. AQUIRE mentions the latin name and if the species belong to the animal or plant kingdom. Compared to others, ATD is more complex to analyse since only the English name of the species is mentioned. Due to these differences, it becomes quite complex to work with data from all these databases. Things are even more complex since different Latin names can concern the same species since databases cover nearly 4 decades and the taxonomy has made strong progress during that period, leading to changes in the Latin name of several species. Details concerning the development stage, and the weight, sex, or the size of the species are missing for about 20 percent of the tests results in the AQUIRE, PED and IUCLID databases. These criteria are missing for only 10 percent of the data for EAT. ATD describes either the weight of the species tested or its development stage for all the tests, and FMD has restricted the results to one development stage only with juvenile fish. It must also be noticed that the electronic format of IUCLID contains some errors, for example, several arthropods and echinoderms can be found in the “fishtox” database where organisms should only belong to the fish group.

Description of the tests conditions

To be useful for the calculation of effect factors, the *description of the test conditions* has to mention at least the ecotoxicological measure (e.g.: NOEC, EC50, etc.), the ecotoxicological endpoint (reproduction, lethality …), the time duration of the test, the type of test (acute or chronic; static or flow though), and the scientific reference of the test. Several other points can strongly influence the test result and are sometimes mentioned in the database like the temperature, pH, Dissolved Organic Carbon, solubility limit, etc. The ecotoxicological measure is most of the time clearly identified in the databases (except for AQUIRE where this information is missing in 35% of the tests results), nevertheless, the type of concentration measured (nominal or measured concentration) is most of the time lacking. The endpoint is always associated with the ecotoxicological measure. The time duration is an essential aspect of the ecotoxicological test and is most of the time mentioned for all the small databases (always mentioned in ATD and FMD; missing for 1% of the tests for PED and EAT3). Things are different for the biggest databases. The time duration of the tests is missing in 15% of the data in AQUIRE, and respectively in 11% and 48% of the tests results for acute and chronic data of IUCLID and PED. Concerning the distinction between acute and chronic tests this distinction is made only in IUCLID. FMD and ATD databases concern only acute tests for fish. PED mentions for each test a corresponding US guideline and it is necessary to refer to this guideline for information about the tests conditions or the acute or chronic exposure. For AQUIRE and EAT, the distinction is not made between acute and chronic. For the test documentation, PED and IUCLID do not make a link with a scientific reviewed publication (except for a limited number of data in IUCLID). These two databases refer to guidelines or standard test documents (for example from the International Standard Organisation (ISO)). ATD and FMD are based on tests performed within US state laboratories that are not reviewed in scientific articles. AQUIRE is based on data from reviewed scientific publications and from reports. The EAT database is the only one that always refers to scientifically reviewed publications.

Concerning the level of details in the test medium descriptions, AQUIRE clearly puts the emphasis on a detailed presentation of the tests, providing information on the pH, the temperature, the hardness, the Dissolved Organic Carbon (DOC), the salinity, the dissolved oxygen, etc. The media conditions are also well described in ATD (pH, temperature, hardness), and for EAT (salinity, dissolved oxygen, temperature, pH, alkalinity, hardness and organic compounds). Concerning PED and IUCLID, it is most of the time necessary to refer to the mentioned guideline, while FMD refers to scientific handbooks.

Quality control of the databases

The *quality control of the database* is the fourth crucial point. It is necessary to rely on the database for the calculation of effect factors. The review process of each database can be more or less exacting. The most reliable database is the Fathead Minnow Database. This database has been developed on data from one laboratory only to ensure consistency, with a strict control of the test conditions in order to reduce as far as possible the variability of the data. Indeed, the results have been used for building a QSAR model based on the Toxic Mode of Action of chemicals and it was necessary to select the most reliable data for that purpose.

The ATD database presentation does not mention review process for the quality insurance of the data. Nevertheless, this database is developed on data from one laboratory only, and has been used for an analysis of acute data variability sources (Mayer and Ellersieck 1986). This work has probably enabled an improvement of the database, leading to a good quality level of ATD.

The PED database has put the emphasis on the quality assurance procedure. First some data from reliable document or laboratories are selected. A reviewer enters the selected data in the database and writes a data evaluation report at the same time. Then a biologist reviews the study and controls the data evaluation report. Afterwards another reviewer compares the data contained in the database and the data evaluation report. The final stage of data control consists in selecting and analysing randomly some entries of the database, detecting inconsistencies and correcting data.

The status of IUCLID is slightly different since the database is mainly based on non-reviewed data provided by industry. This is a limitation for the use of the database, but at the same time, there is only a small number of data errors at least in the pdf format of IUCLID. Concerning the table format of IUCLID, several errors have been observed probably due to the interface between the two data format.

As what is done in PED, EAT relies at first on the selection of the publication used for developing the database. Nevertheless, no quality control procedure has been done concerning the database itself. Consequently, in spite of the strict selection of the most reliable publications, many input errors have been made in the database development (erroneous CAS, time duration, etc), and an intensive

data review is therefore necessary before making the database usable for analyses.
The Quality control of AQUIRE is based firstly on the quality of the reviewer's training and secondly on a replicate review of one independent reviewer for 10% of the publication. Regarding the number of publications (nearly 17,500 articles or reports in 2001), a simplified review process was required. Nevertheless, since many data are extracted from each publication, an error in the analysis on one publication can lead to numerous errors in the final databases. This can explain that we have met about 10% of erroneous tests description or tests results in the database. Apart from the missing data, the main sources of errors are the double counting of data (the same original data reported in several publications presented several times under different references) and the unit errors (for example confusion between concentrations in ppb or ppm).

Description of the Dataset for the calculation of Effect Factors

As mentioned in the previous section, most of the existing databases contain some mistakes. At the same time, the calculation of Effect Factors for LCIA on Aquatic Ecosystems requires the use of reliable data. Thus we have created a dataset used for the estimation of the maximum number of Effect Factors that can be calculated by eliminating the unreliable data from existing databases. The process for the data selection is presented below, and the data included is described.

Distinction between acute, subchronic and chronic tests results

The criteria for distinguishing between Acute, Chronic and Sub-chronic tests results are defined according to guidelines from ISO, OECD, US-EPA, FIFRA, ASTM, UBA, and publication from (Heger, Jung et al. 1995). Table 16 summarizes the retained exposure durations

Table 16: Exposure duration of the acute, sub-chronic and chronic tests

	Acute	Sub-chronic	Chronic
Vertebrates	< 7 days	7 days; < 32 days	32 days

Invertebrates	< 7 days	7 days ; < 21 days	21 days
Plants	< 7 days	-	7 days
Algae	< 3 days	-	3 days

For chronic tests, the endpoint addresses a whole Life Cycle or a sensitive life stage (ex: larvae, young, etc); the endpoint can be biochemical or histo-pathological effects, growth (length and/or weight), hatching, reproduction, larval development or mortality, juvenile development or mortality, emergence, behaviour.
The exposure duration indicated for the distinction between acute and subchronic is from Heger et al. (1995). Concerning the distinction between sub-chronic and chronic, things are much more complex since the relevant exposure duration for a chronic test depends on the generation time of the species and the life stage tested. For that reason, we have presented in the table above the exposure duration of chronic test that is mostly represented in the available databases. Nevertheless, in some cases, some chronic tests can be done with shorter exposure duration both for fishes and invertebrates. It is the case for example for fishes with the ASTM (American Society for Testing methods) 7 days test on larvae, or for crustacean with the 7 days test on Ceriodaphnia dubia. Therefore, the Table 16 just presents general indications but is not valid for all the tests. For some test, it is necessary to refer to the original guidelines.

In the data selection for the current study, we have referred to specific guidelines when the information was specified in the database. When the information was not available, we have classified data on the basis of Table 16.

Data selection and improvement

Among the different characteristics analyzed above, the data are selected on the basis of the following points: (1) the identification of the chemical, with a clear mention of the chemical name, the CAS number and the fraction of active ingredient; (2) the identification of the organism tested, with the species name and the phyla (for animals), or the taxa (algae or vascular for plants). (3) the test conditions with the exposure duration, the concentration of effect for active ingredients, the ecotoxicological measure (NOEC, LOEC, or EC50), and the original reference.
Among the databases presented above, the test results that satisfy these requirements have been extracted and combined in a single dataset. The

indications related to the organisms were often missing (latin name, phyla or taxa), therefore an intensive work has been done in the data implementation with the identification of the phyla for all animal species, and correction of erroneous data. Only the EC50s, the NOECs, and the LOECs have been kept in the data selection since they are the measures typically used in the calculation of effect factors in LCIA. For each dataset, the description of the tests has been analysed (chemical description; organism identification; tests conditions). When part of the data was missing an effort was made to fill in the gap of the data whenever it was possible. When the test description was insufficient or if some tests results presented some inconsistencies, they were eliminated. On the basis of the 6 databases (FMD, ATD, IUCLID, PED, AQUIRE and EAT), 128,864 tests results have been selected. A distinction between acute, chronic and sub-chronic data has been done on the basis of the indications mentionned above. Due to the limited number of chronic and sub-chronic data, the two groups have been gathered in one. By this way we finally have 113,031 acute data on the one hand and 15,833 chronic and sub-chronic data on the other hand, covering 4,959 different chemicals.

Table 17: Acute data availability from each database after selection

	AQUIRE	IUCLID	PED	EAT	ATD	FMD
Number of substances	3755	1491	600	470	327	617
Number of tests	80477	13382	6478	3721	8220	753

Table 18: Chronic data availability fromeach database after tests selection

	AQUIRE	IUCLID	PED	EAT
Number of substances	1090	638	254	270
Number of tests	11653	2208	581	1391

Data description

Figure 26 and Figure 27 present the content of the databases according to the type of chemical covered. The term “general” concerns chemicals that are used or emitted in industrial processes. Compared to pesticides, the substances

concerned by industrial processes represent the major part of the covered substances.

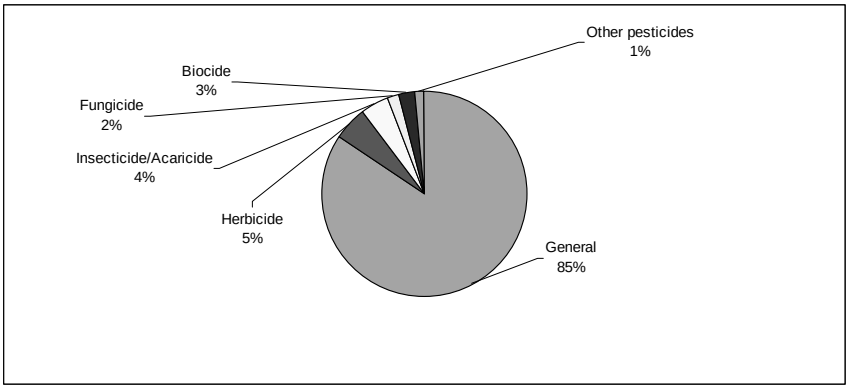

Figure 26: Description of the 4,838 chemicals after the selection procedure of the acute tests.

Comparing acute and chronic tests, we can observe a considerably higher representation of pesticides for Chronic and sub-chronic data.

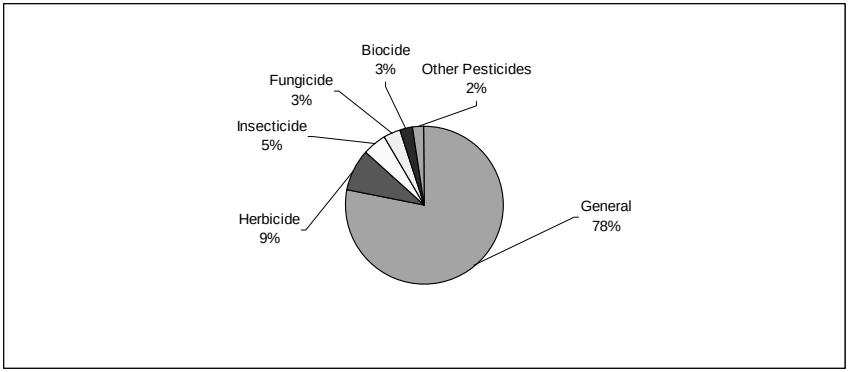

Figure 27 : Description of the 1,664 chemicals after the selection procedure of the chronic and sub-chronic tests.

Table 19 presents the number of species used in the ecotoxicological tests and the corresponding phyla. 13 animal phyla are covered in the 128,864 tests. Data concerning vascular plants and algae are gathered under the denomination “plant taxa”. For acute toxicity test results, arthropods, chordata (such as fish) and plants represent 29, 56% and 5% of the data available respectively, that is a total of 90%, while for the chronic and subchronic tests, arthropods, chordata and the plant taxa represent 93% of the data available (nearly 30% each).

Table 19: Representation of the phyla (or the plant taxa) among the acute and chronic/sub-chronic toxicity tests results and the number of species tested per phyla (or plant taxa).

	Acute toxicity tests		Chronic and sub-chronic tox. tests	
Phyla	Tests per phyla	Species per phyla	Tests per Phyla	Species per phyla
Annelida	1,797	75	337	26
Arthropod	33,342	769	4539	182
Chaetognatha	12	1	0	0
Chordata	63,426	541	5247	140
Cnidaria	123	6	34	7
Ctenophora	4	2	0	0
Echinodermata	239	17	7	2
Ectoprocta	0	0	13	4
Gastrotricha	4	1	0	0
Mollusca	5,746	208	587	55
Nematoda	455	28	29	6
Plathyhelminte	253	16	20	6
Protozoa	1,278	37	0	0
Rotifera	878	14	78	8
Algae	5,006	137	4198	126
Vascular plants	468	21	744	64
Total	**113031**	**1873**	**15833**	**626**

Concerning the ecotoxicological measures, Table 20 presents the review of the data available. For acute and chronic data, the EC50s are dominating (95% of the acute data and 52% of the chronic data). The NOECs is well represented in the chronic data with nearly 30% of the data available.

Table 20: Number of ecotoxicological tests (EC50, NOEC, LOEC) regarding the phyla (or taxa) for acute and chronictoxicity tests.

	Acute toxicity tests			Chronic and sub-chronic tox. tests		
Phyla	EC50	LOEC	NOEC	EC50	LOEC	NOEC
Annelida	1771	11	15	267	19	51
Arthropod	32150	369	823	2075	755	1709
Chaetognatha	12	0	0	0	0	0
Chordata	61536	722	1168	2413	995	1839
Cnidaria	100	15	8	13	16	5
Ctenophora	4	0	0	0	0	0
Echinodermata	213	15	11	3	2	2
Ectoprocta	0	0	0	13	0	0
Gastrotricha	4	0	0	0	0	0
Mollusca	5561	53	132	474	30	83
Nematoda	367	60	28	12	9	8
Plathyhelminte	252	0	1	13	1	6
Protozoa	1131	19	128	0	0	0
Rotifera	799	28	51	24	27	27
Algae	4640	163	203	3363	357	478
Vascular plants	454	12	2	624	48	72
Total	**108994**	**1467**	**2570**	**9294**	**2259**	**4280**

LCIA Effect Factors are typically based on minimum requirements regarding the number of species and/or phyla. Figure 28 and Figure 29 present the number of chemicals available regarding the number of phyla covered per chemical or regarding the number of species covered per chemical. In both cases, data for acute and chronic exposures are considered.

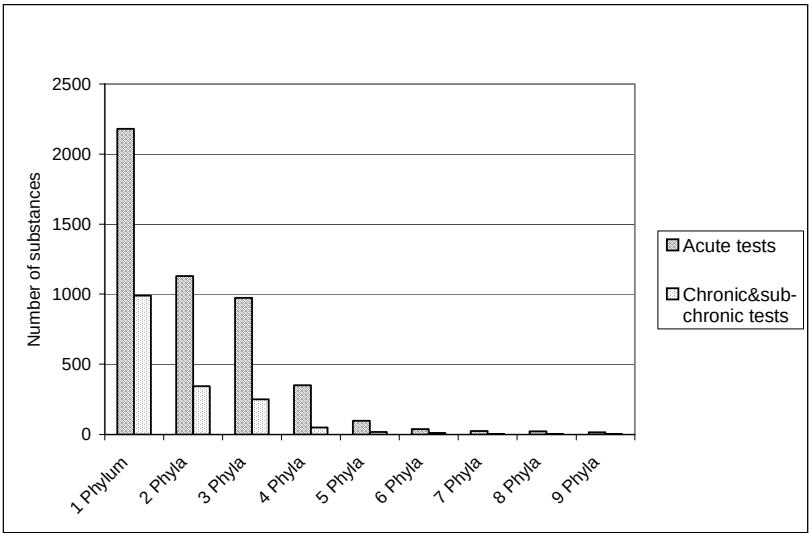

Figure 28 : Number of chemicals tested with 1 to 9 phyla

Both for chronic and acute tests, nearly half of the substances are tested for one phylum only, and less than one third are tested with at least three phyla. This is a considerable limitation for comparative assessment. Indeed, the comparative purpose requires to estimate the variability of the response of species exposed to one chemical. A minimum requirement of three species covering three phyla is generally necessary for giving an estimate of the spread of the species response. Therefore, data availability is limited and this will considerably affect the number of calculable Effect Factors.

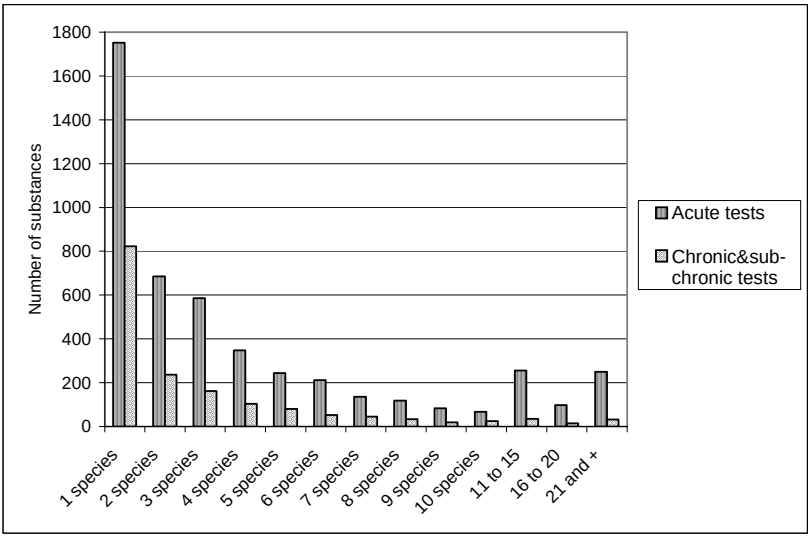

Figure 29: Number of chemicals tested depending on the number of species

On the opposite, some methods require data concerning more species but do not put requirements on the phyla representation. Nevertheless, calculating the cumulative number of chemicals tested for more than 4 species indicates that a method requiring this number of species (Sloof 1992) will not be able to calculate more than 1,800 Effect Factors using acute data and less than 500 Effect Factors if chronic data are necessary. If the limit is 8 species (Host, Regal et al. 1991), it immediately comes down to 800 substances with acute tests and less than 100 substances based on chronic data.

Estimation of the maximum number of possible Effect Factor

On the basis of the data presented, we estimate the maximum number of Effect Factors potentially calculated with 4 different methods used for the LCIA on aquatic ecosystems.
The EDIP method, based on the PNEC, is currently providing 76 Effect Factors (Wenzel, Hauschild et al. 1998). Nevertheless, the method can calculate effect factors with only one ecotoxicity data, an EC50 or a NOEC, and a procedure for extrapolation from acute to chronic based on conservative safety factors is

provided. On the one hand, this does not give satisfaction for a comparative assessment (no indication concerning the data variability and conservative extrapolation factors), but on the other hand is offers the possibility to calculate an important number of Effect Factors. Considering the databases presented here, the calculation of 4,959 effect factors is possible.
The AMI method (Payet and Jolliet 2004), based on the $HC50_{EC50}$ requires at least three species tested from three different phyla (acute or chronic). An extrapolation procedure from acute to chronic exposure using best-estimate extrapolation factors is provided. Therefore, 1,554 Effect Factors can be calculated. The calculation of 4,959 Effect Factors would be potentially feasible if substances tested for a single or for two species would be considered, but this will be associated with a significantly higher confidence interval.
In the Eco-Indicator report (Goedkoop, Effting et al. 2000), where the method based on the $HC50_{NOEC}$ is presented, the minimum data requirement for the calculation of Effect Factors is not specified, it is simply mentioned that Effect Factors are based on chronic NOECs. Assuming that a minimum number of three different NOECs from three different phyla is necessary to express the potential variability of the species response for one chemical, we have estimated that 117 Effect Factors can be calculated.
For USES-LCA (Huijbregts, Thissen et al. 2000), no indication for the minimum data requirement is provided. An extrapolation procedure for the assessment of the $HC5_{NOEC}$ based on acute data is proposed by Huijbregts (Huijbregts, VandeMeent et al. 2002), but this is based on the Toxic Mode of Action (TMoA) of the substances and this information is rarely provided in the databases. For the assessment of the number of $HC5_{NOEC}$ calculable, we have therefore referred to two well documented publications related to the $HC5_{NOEC}$ requiring at least 8 chronic NOECs (Host, Regal et al. 1991), or 10 chronic NOECs (EU-Commission 2002) covering generally 8 phyla in both cases. The number of Effect Factors potentially calculable would be 32 Effect Factors on the basis of the databases considered here. Nevertheless, in the USES-LCA database, Huijbregts (2000) has included Effect Factors based on the most sensitive species (PNEC) extending the database to 181 substances.
The results of the analysis are provided in Table 21.

Table 21: Maximum number of effect factors that can be calculated with selected data for 4 LCIA methods

	Acute only	Acute &Chronic	Chronic only	**Total**
PNEC (EDIP)	3,295	1,543	121	**4,959**
$HC50_{EC50}$ (AMI)	1,224	304	26	**1,554**
$HC50_{NOEC}$ (EcoIndicator)	-	-	123	**123**
$HC5_{NOEC}$ (USES-LCA) with 10 species covering 8 phyla	-	-	32	**32**

Conclusion

The first observation following this large analysis of available data is the huge gap between the number of available tests results –128,864 tests after selection- and the limited number of chemicals that can satisfy requirements for a comparative assessment –about 1,550 substances represented with three phyla minimum. Using the existing databases, it will not be possible to provide Effect Factors for Life Cycle Assessment, giving an exhaustive estimate on the impacts of the substances emitted during the Life Cycle of a product. Indeed, tens of thousands of substances can be potentially emitted by industrial processes, and the assessment of effect factors for all these substances will require other sources of data. The use of QSAR is possible for some substances; nevertheless, more research is required for comparative assessment since the estimation of the toxicity based on the structure properties of substances tends to underestimate the variability of the species response. Furthermore, the development of reliable QSAR requires substantial databases for some groups of chemicals (based on the chemical structure or the TMoA) and the lack of data also affects QSAR developments.

Nevertheless, the current number of Effect Factors available in different methods is far from the maximum number of effect factors calculable. In this respect, the AQUIRE database can be the most useful for the calculation of new effect factors. This is limited by its current design and the low level of data reliability which does not allow its use without strong modification, especially concerning the improvement of the species identification at the phyla level, and the control of data with the elimination of the unreliable tests results and double counted data. At the same time, the strength of this database is the update

frequency (two or three times per year) and the identification of the tests conditions like salt or fresh water enabling assessment of Effect Factors for the two types of ecosystems.

The second observation concerns the description of the media of the test and the speciation of substances. Indeed, for most of the inorganic and part of organic substances, the toxicity is partially determined by the medium conditions (pH, percent of Organic carbon, etc). Effect Factors relating the toxicity of substances to the media conditions will therefore be required, for example for metals that often plays a major role in the LCA results while the actual toxicity is strongly determined by the medium conditions. At this time, this type of information is generally omitted in the databases and therefore the influence of the medium on the toxicity cannot be easily considered in the Effect Factor.

Regarding the data available, two points can be highlighted:

1) the number of data available (more thatn 128,000 tests results in this studey) appears to be quite important, nevertheless, as presented here, this amount of data allows a PNEC estimation for less than 5'000 substances and a comparative assessment for about 1'500 substances. Compared to the 106,000 substances currently comercialized in the EU and the 16 millions of substances currently registered (Allanou et al, 1999), this is very problematic for assessing potential impact of existing substances;
2) furthermore, all databases considered provide an acceptable description of the test conditions, but each database has its specificities. One focuses on the medium conditions, another on the substance description, but in many cases, important information is missing which requires to complete the data or to ignore the test.

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CHAPTER 7

General Conclusions

Main findings

This section aims at concluding on the key points presented in the introduction.

Feasibility of the comparative impact assessment on ecosystems

The comparison of impacts on ecosystems has been considered in several studies. In most of these studies, the PNEC method based on the most sensitive species was used in a comparative purpose. Nevertheless, no validation was made for applying this method in a comparative framework. In the thesis, results indicate that the PNEC method is not well-suited for a comparative purpose. At the same time, it is also demonstrated that the comparative assessment of toxics on ecosystems is feasible but that the statistical aspects must be considered carefully, especially regarding the specific constraints of comparative assessment (e.g. discrimination between chemicals, and data availability) and regarding the underlying assumptions of the statistical estimator used (e.g. distribution assumption).
The constraints associated with a comparative assessment in the LCA framework need to be restated here:

- *Compatibility with the LCI*: typically, numerous substances are considered in the LCI, and the comparative method must be able to provide numerous effect factors.
- *Compatibility with fate modelling*: several assumptions are associated with fate modelling like the use of a linear model, and the integration of the exposures over time and space. The effect model used for the comparative assessment must be compatible with these assumptions or lead to the definition of new requirements on fate.
- *An unbiased estimate of the toxicity:* three sources of bias have been identified; the use of conservative extrapolation factors; the mix between different methods, like the PNEC based on the most sensitive species and the HC5, and the bias associated with the use of different databases which is due to the difference in the number of tests performed per substance (e.g., Effect Factors based on the US database present a difference of two order of magnitude with those based on European database for certain substances).
- *Ability to discriminate between substances:* Discrimination between chemicals: all methods have almost the same range of variability of

Effect Factors, but only AMI currently provides 95% Confidence Intervals for the Effect Factors. USES-LCA can possibly provide a 90% Confidence Interval on the $HC5_{NOEC}$ but it can be up to 10 orders of magnitude larger than the 95% Confidence Interval on the $HC50_{EC50}$.

- *A practical unit for expressing the impact:* the PAF of species calculated in AMI provides a practical basis for ecotoxicological impact aggregation in LCA. This indicator is interpreted in terms of the fraction of species experiencing an increase in exposure above the chronic EC50.

Choice of the most relevant ecotoxicity measure (ECxs, NOECs and LOECs) used in comparative assessment

The choice of the relevant ecotoxicity measure among available data (ECxs, NOECs and LOECs) is mainly driven by four requirements:

- The *need for a stable indicator* for the impact assessment: the LOECs and the NOECs are strongly dependent on the experimental design (Laskowski 1995; OECD 1998). Depending on whether the number of concentrations tested is high or low, the NOEC or LOEC may vary. This is not the case for the EC50 value.
- The *optimisation of the confidence interval*: For most ecotoxicological studies, the use of EC50 modelling recommends interpolating the EC50 level among concentrations tested. Consequently, the concentration-effect ratio presents minimum variability at the 50% or mean effects level or close to that level of effect (Forbes and Forbes 1993; Riviere 1998).
- Compatibility with the assumption of a linear model: an impact estimated on the basis of NOEC in LCA implicitly assumes a linear relation between the concentration and the effect under the level of No Effect. This is disputable and an indicator based on an EC50 avoids this assumption.
- Compatibility with endpoint modelling: in LCIA, it is particularly relevant to explicitly link an impact like ecotoxicity to the damage it causes to exposed ecosystems, to enable comparison with damage caused by other impacts considered in LCA, such as land use or eutrophication. The link with other causes of ecosystem damage can be established through measuring the reduction of biodiversity (e.g., quantification of disappeared species). It is for example possible to define a connection between an ECx value and probability of disappearance (Tanaka and

Nakanishi 2000). This link could not be established if the endpoint was a no-effect level like the NEC (No Effect Concentration) or NOEC.

Development of best-estimate extrapolation factors for assessing chronic effects based on acute data

With the purpose of using all the available data, best estimate extrapolation factors are presented in this thesis.
The extrapolation of chronic effects from acute data is based on the relation between the acute and the chronic $HC50_{EC50}$, that provides more reliable results than a species-by-species extrapolation. The best estimate extrapolation factors are also provided for the calculation of the upper and lower limits of the confidence interval of the chronic $HC50_{EC50}$ based on the limits of the acute $HC50_{EC50}$. In order to use more accurate extrapolation factors, separate factors are calculated for inorganics, non-pesticide organics, and pesticide organics. It turned out to be possible to use all the EC50s available for the calculation of the Effect Factors.
Best estimate extrapolation factors are also calculated for the estimation of the EC50 based on NOEC or LOEC data. The extrapolation factor depends upon whether an acute and chronic NOEC is considered, and different factors are provided for LOECs toxicity data.
Using these indications, the $HC50_{EC50}$ -required for the calculation of the Effect Factors in LCIA- can be estimated using most of the ecotoxicity data available.

Development of a reliable statistical estimator for comparing impact on ecosystems

The reliability of the assessment depends on the compatibility between the statistical estimator and the purpose of the comparative assessment. In spite of the scarcity of aquatic toxicological data and the variability of the responses of species exposed to a substance, it is necessary to find a robust enough statistical estimator that allows ranking of substances on the basis of their toxicity. Furthermore, this estimator must also provides an indication on the variability of the species response, associating an uncertainty to the estimate of the toxicity.
Finally, the statistical tool provided for the comparative impact assessment has to satisfy several requirements:

- A stable estimator must be used, which does not vary from one database to another;
- The selected statistical estimator must be associated with a confidence interval;
- Data are scarce for most of the substances used in industrial processes, therefore the estimator must provide the most reliable results possible with small samples;
- The statistical estimator must be robust regarding the assumption of lognormal distribution of ecotoxicity data,.

A statistical estimator satisfying all these requirements does not exist. Therefore, two versions of the AMI method for impacts assessment have been developed and can be used together.

Parametric version of AMI:
This version is based on the geometric mean of the EC50s of species tested for each substance, and the confidence interval is calculated using the Student table. This method is especially suitable for small samples, indeed, the geometric mean is a quite stable estimator also with 3 or 4 EC50s for one substance. Furthermore, the geometric mean is applicable with log-normally distributed data (which is most of the time the case for ecotoxicity data), and this estimator is robust against outliers. The Student based confidence interval on the geometric mean is a well known method that has been proven to give acceptable results for small datasets.
Furthermore, for an extrapolation between acute and chronic EC50s, it is more relevant at this time to use at first a method based on the geometric mean and Student. Indeed, the feasibility of an extrapolation based on a non-parametric statistical estimator has not been explored and therefore, some errors can be made in the extrapolation of a confidence interval based on a distribution-free method applied to acute data.

Non parametric version of AMI:
The non parametric version of the AMI method offers interesting new insights for the comparative assessment of chemicals, for LCIA. Indeed, being close to the mode, the median is a good representative of the responses of the greater number of species. Furthermore, this estimator is not influenced by outliers and is a stable statistical estimator if sufficient data are considered, but this is not true when the median is based on less than 5 EC50s. With only three or four

data points for example, the gap between consecutive EC50s can reach several orders of magnitude and the median, as a breakdown point indicator, would become quite unstable. Considering the confidence interval of the median, the bootstrap is a distribution-free method that fits the data spread. Therefore, the asymmetry of the confidence interval follows the asymmetry of the EC50s spread. When a very sensitive species is tested while all other species have a good resistance to the substance, the confidence interval is skewed in favour of the lowest concentration. On the other hand, if the substance is very toxic for most species while only a small number are resistant, the confidence interval is skewed in favour of the high concentrations. The skewness of the distribution is therefore important in the description of the substances toxicity, and the only way to express this information is the use of a distribution-free method for the assessment of confidence intervals. Nevertheless, this is also likely to be a problem for small dataset or when a biological group of species is over-represented in the EC50s dataset for a substance. For the first point, the bootstrap based confidence interval requires at least 5 data points and the calculation is not feasible for only 3 or 4 species. For the second point, the over-representation of one phyla or taxa could lead to a biased estimate of the confidence interval by excluding one whole phylum from the confidence interval of the median. In order to avoid these problems, three rules can be applied: (1) To require a minimum number of EC50s for the calculation of the Effect Factor (e.g.: a minimum of 5 EC50s or NOECs); (2) To fix a minimum diversity representation (e.g.: data covering at least three species from three different phyla or taxa); (3) To propose a flexible application of the confidence interval with some alternatives to the bootstrap (e.g. if the Geometric mean of one phyla is out of the range of the confidence interval, the confidence limit can be expanded to contain the geometric mean of this phyla).

The consequences of the non-lognormal distributions can sometimes affect a LCA study if the concerned substance has a major influence on the LCA results. Therefore, the relative influence of the non-lognormal substances on the LCA results must not be underestimated, and we would therefore suggest the following strategy. As a first approach, we suggest the use of the geometric mean with the Student confidence interval as a baseline, calculating the non parametric median and the bootstrap confidence interval as a sensitivity study, to test skewness and its consequences when non-parametric $HC50_{EC50}$s are available. If the result significantly differs and this influences the preferred decision, special care in the analysis should be taken.

Review of the data availability for the calculation of Effect Factors

In terms of database content, most of the available databases provide an acceptable description of the test conditions, but each database has its specificities. One focuses on the medium conditions, another on the substance description, but in many cases, important information is missing which requires to complete the data or to ignore the test.
Concerning the description of the media of the test and the speciation of substances, for most of inorganic and some organic substances, the toxicity is partially determined by the medium conditions (pH, percent of Organic carbon, etc). At this time, this type of information is generally omitted from the databases and therefore the influence of the medium on the toxicity cannot be easily considered in the Effect Factor.
Two interesting results can be highlighted: first, among the data reviewed, the most reported ecotoxicity measure is the EC50 both for acute and chronic data; and the estimation of the maximum number of Effect Factors calculable underlines the huge gap observed between the number of available tests results –128,864 tests after selection- and the limited number of chemicals that can satisfy requirements for a comparative assessment –about 1,550 substances represented with three phyla minimum. Using the existing databases, it will not be possible to provide effect factors for Life Cycle Assessment, giving an exhaustive estimate on the impacts of the substances emitted during the Life Cycle of a product. Indeed, tens of thousands of substances can be potentially emitted by industrial processes, and the assessment of effect factors for all these substances will require other sources of data. The use of QSAR is possible for some substances; nevertheless, more research is required for comparative assessment since the estimation of the toxicity based on the structural properties of substances tends to underestimate the variability of the species response.

Analysis of the ecological realism of the comparative assessment method

The question of ecological realism is mainly considered in terms of three issues, the choice of the mixture model, the ability to account for multiple stressors, and the distinction between function and structure of the ecosystems.
1- *Choice of the mixture model*: influence of concentration addition or effect addition modelling of mixtures have been explored in LCA (Roelofs, Huijbregts

et al. 2003; Pennington, Payet et al. 2004), and results indicate a small variability from one model to another in the LCA framework. Furthermore, other studies (e.g. Pedersen, Kristensen et al. 1994) conclude that complex mixtures of toxics tend to fit the concentration-additive model for mixtures. In LCA, where the substance is released to a medium in which numerous substances are already present, the concentration addition model appears to be the most relevant.

2- *Multiple stressors*: LCIA deals with multiple environmental stressors, such as ecotoxicity, eutrophication, acidification, etc. and the model selected for ecotoxicity assessment must be interpretable in the same unit as other impact categories. This unit could be a change in biodiversity for example. Using EC50 data as basis for calculation of Effect Factors offers the possibility of making a link with the endpoint indicator, such as biodiversity losses. The question of the working point (which is the actual level of damage on the ecosystems) is often discussed in LCA (Goedkoop, Effting et al. 2000; Pennington, Payet et al. 2004). On the one hand, the monitoring of chemicals in the environment generally identifies very small concentrations of individual chemicals in the environmental medium, and this exposure is thus considered environmentally relevant. This corresponds to concentrations ranging typically in the same order of magnitude as the $HC5_{NOEC}$ or PNEC. It would make better sense to take into account that 10 to 50% of species are already affected (Kleeper, Bakker et al. 1998) and consider the toxic impact on the present species. This can be explained by the joint effect of stressors. Species are exposed to several stressors simultaneously and therefore stressor effects are assumed to be additive (possibly synergistic) (Payet, Margand et al. 2004). For example, a species already exposed to a lack of dissolved oxygen in water can be much more sensitive to toxic stress (Stuijfzand, Helms et al. 2000). Furthermore, in terms of biodiversity, the species sensitive to the reduction in oxygen are also likely to be sensitive to toxic stress. On this basis, the Eco-indicator and Ami methods present a better ecological realism.

3- *Structure and function of the ecosystem*: The structure typically corresponds to the biodiversity of the ecosystem while the function can be for example characterised by the energy flows. Some keystone species may represent only a very small part of the biodiversity but can at the same time play a major role in energy transfer. LCIA models do not consider thresholds but work with linear dose-effect relations, therefore, LCIA aims at comparing the levels of stress to ecosystems, but does not aim at ensuring the protection of ecosystem species. The underlying assumption is therefore that all species in ecosystems have the

same probability to be keystone species, and the potential reduction of the number of species affected by toxic stress is likely to have a repercussion on both the structure and function of the ecosystem.

Key features of the AMI method:

The AMI method satisfies most of the requirements presented above. The AMI method calculates Effect Factors on the basis of the $HC50_{EC50}$ and associates a confidence interval to the Effect Factor. A parametric version based on the geometric mean for the assessment of the $HC50_{EC50}$ and a confidence interval based on Student, and a non-parametric version –using the median estimate of the $HC50_{EC50}$ with a confidence interval based on bootstrap- are each provided in this thesis. The method expresses results as an emission in a given compartment in terms of the fraction of affected species in the aquatic ecosystem. This result can therefore be linked with most of the fate modelling provided that it translates chemical emissions calculated in the Life Cycle Inventory into an increase in concentration in the relevant medium for a defined time period. In order to increase the number of Effect Factors that can be calculated, the method also provides best-estimate extrapolation factors for assessing chronic effects on the basis of acute data, and also for estimating chronic EC50s on the basis of acute or chronic LOECs and NOECs. Both acute and chronic Effect Factors are provided in the AMI Effect Factors Database. This database currently cover 522 substances but will be extended to more than 1,500 substances in the coming months. Furthermore, the Effect Factors based on the non-parametric will also be included for the substances characterised by at least 5 chronic EC50s.
At the same time, an important limit of the AMI method concerns the restriction of the method to the impact of toxics on aquatic ecosystems and does not consider terrestrial ecosystem. At the same time, the method does not distinguish so far between freshwater and saltwater ecosystems, as most data are available for freshwater only.

Perspectives

The development of a core method for comparative assessment of the toxic impacts on aquatic ecosystems for comparative assessment is opening numerous perspectives. These perspectives are presented below:

1- Considering metals speciation: the toxicity of metals depends on the media properties since the pH, Dissolved Organic Carbon (DOC) and hardness influence the speciation of metals and simultaneously determine the speciation and the toxicity of metals. This has been already explored in relation with AMI and some promising first results have been obtained concerning the speciation of copper with the calculation of LCIA Effect Factors for copper for freshwater ecosystems using the Bio-Ligand Model (BLM). As a general rule, the tests concerning copper present a huge EC50s variability (about 8 orders of magnitude). We have proved that we can expect a reduction of the variability by 4 to 6 orders of magnitude in considering the copper speciation with the BLM (Simonnin et al, 2004). These results have to be extended to other metals and other ecosystems.
2- Spatial differentiation of the impacts: as illustrated by the example of metals above, for some substances, the level of toxicity depends on the media quality (pH, DOC, hardness, temperature, etc). Using existing databases mapping the media quality depending on the location of the aquatic or terrestrial ecosystems, it is possible to map the potential effect of substances at the regional, continental or global level.
3- Extension to other ecosystems: the method for the assessment of toxics on ecosystems is available for freshwater ecosystems, and it should be extended to other ecosystems concerning US or EU like saltwater and terrestrial ecosystems, or to other ecosystems like tropical ecosystems.
4- Link with other stressors: the AMI method focuses on the impact of toxic substances, nevertheless several other stressors affect ecosystems. A first analysis has been done in order to explore the compatibility of AMI with the eutrophication impact. For that purpose, we have compared the variability of the biodiversity in a stream in relation with different stressors [Payet et al, 2004]: eutrophication, impact from pesticides (using the AMI method), and natural variability (season, temperature, light, etc). The results indicate that eutrophication explains the main part of the variability of the macro-invertebrate biodiversity, but the impact of

pesticides also appears to be important. The concentration of only 4 pesticides (among 11 tested) explains about 1/3 of the variability. These results now have to be extended to a larger scale (continental or global). Other stressors affecting ecosystems biodiversity can also be linked with the toxic stress such as acidification or the effect of ionizing radiation. This link with other stressors also offers the opportunity to validate the method for the assessment of toxic impact on ecosystems, by comparing the predicted change due to a toxic stress with the actual change in biodiversity observed. In this way, the study of the biodiversity change in the stream has highlighted the ecological realism of the AMI method for the prediction of the impact of toxics on ecosystems.

5- Endpoint modelling of the impacts: at this time, the AMI method expresses impacts in terms of species affected, which is considered as a midpoint analysis in the LCA terminology [Bare et al, 2000]. In order to improve consistency with the LCA framework, it is necessary to translate the midpoint assessment to an endpoint like a change in biodiversity. For that purpose, the existing model linking the fraction of affected species to the fraction of disappeared species must be checked (several models have been developed in the field of biodiversity conservation). Therefore, they must be applied in the LCA framework in order to test their influence on the final results of LCA studies concerning impact on ecosystems.

6- Altough AMI provides the largest database among the existing methods, it does not cover enough substances for LCA, and therefore further extension of the AMI Effect Factors database need to be performed considering several tens of thousands of substances, possibly based on QSAR or using large confidence interval on the $HC50_{EC50}$.

Output of the thesis

Proposing for the first time the $HC50_{EC50}$ as the most adapted basis for the comparative assessment of impacts on ecosystems, the AMI method is used as a basis for the development of the ecotoxicity indicator in several LCA tools and projects.

For facilitating the application of the AMI method in LCA, the AMI method has also been implemented in the IMPACT 2002 model. Thus, AMI can now be used in several LCA softwares tools like SIMAPRO and GABY. Furthermore,

enabling its use also for North-American LCA practitioners, the AMI Effect Factors are provided with the TRACI method by the US-EPA, and the method is also used for the development of the Canadian approach for the impact assessment of products EICV.

The European Project "OMNIITOX" has retained the basis of the $HC50_{EC50}$ for the impact assessment of toxics in LCA in the European framework. Nevertheless, the method differs for accounting of the confidence interval. Instead of a parametric or non-parmetric based confidence interval, the OMNIITOX project retains a rank of variability of the $HC50_{EC50}$ defined by the geometric mean of the most sensitive and the least sensitive phyla.

Considering researches developed for AMI, the UNEP-SETAC Life-Cycle-Initiative has also selected the $HC50_{EC50}$ for the calculation of Effect Factors for Life Cycle Assessment. But it was also decided to put the emphasis on more focused topics like the accounting of the metals speciation in the fate and effect modelling, the endpoint modelling, the extension to tropical ecosystems, etc.

Beyond the application of AMI in the Life Cycle Impact Assessment framework, the comparative basis is also an asset for answering questions in Ecological Risk Assessment, especially concerning the comparison of impacts between stressors, or between toxics. Such prospects will be explored in the European Project "NOMIRACLE" that will start in the coming months. At this time, beyond the LCA framework two examples from chapter 2 and chapter 4 of the thesis highlight the applicability of the AMI method for a Substance-to-substance comparison. In the first example, the parametric version of AMI is used for comparing the potential aquatic toxicological impact of two substitutable fungicides: the Chlorothalonil on the one hand and the Propiconazole on the other hand. In chapter 4, two herbicides are compared using the parametric and the non-parametric version of AMI. This comparison concerns the Prosulfuron and the Sulfosulfuron. In both cases, the assessment indicates if the two substances present differences in terms of toxicity on aquatic ecosystems. For the comparison between the two herbicides, the AMI method identifies a difference in toxicity in spite of similar levels of PNECs for the herbicides. These two examples present promising perspectives for the comparative assessment of ecological impacts of substances.

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AMI Database

Acute and Chronic $HC50_{EC50}$ for 522 Substances for Calculation of AMI Effect Factors

Acute and Chronic HC50EC50 data calculated with the parametric version of AMI with the confidence interval and the average EC50s of the most sensitive phyla (when not included in the Student confidence interval)

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
1,1'-(2,2,2-Trichloroethylidene)bis(4-chlorobenzene)	50-29-3	4.90E-02	3.38E-02	2.33E-02	1.54E-02	No factor	1.60E+00	2.49E-01	3.87E-02	5.04E-03
2,2,2-Trichloro-1-hydroxyethyl phosphonic acid, Dimethyl ester	52-68-6	1.93E+00	9.57E-01	4.75E-01	9.76E-02	No factor	1.05E+00	1.08E-01	1.11E-02	Included
fenthion	55-38-9	1.71E-01	9.73E-02	5.55E-02	9.65E-03	No factor	1.35E+00	8.45E-02	5.30E-03	Included
bis(tributyltin) oxide	56-35-9	3.82E-02	1.78E-02	8.32E-03	1.82E-03	No factor	6.27E-03	3.28E-03	1.71E-03	2.50E-04
Paraoxon (Parathion Degrad)	56-38-2	1.27E-01	7.41E-02	4.30E-02	7.05E-03	No factor	1.43E-02	2.70E-03	5.11E-04	Included
Coumaphos	56-72-4	1.57E+00	3.46E-01	7.66E-02	3.46E-03	No factor	1.18E+02	7.90E-02	5.29E-05	Included
1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene	57-74-9	1.18E-01	6.42E-02	3.49E-02	Included	No factor	1.96E+01	8.78E-02	3.94E-04	Included
(1alpha,2alpha,3beta,4alpha,5alpha,6beta)-1,2,3,4,5,6-Hexachlorocyclohexane	58-89-9	2.66E-01	1.75E-01	1.15E-01	4.25E-02	No factor	2.60E-01	7.69E-02	2.28E-02	6.36E-03
4-Chloro-3-methylphenol	59-50-7	1.01E+01	5.02E+00	2.51E+00	Included	No factor	1.26E+01	7.44E+00	4.40E+00	Included
dimethoate	60-51-5	3.04E+00	1.15E+00	4.36E-01	1.08E-01	No factor	4.23E+01	1.06E+01	2.65E+00	2.62E-01
(1a alpha, 2 beta, 2a alpha, 3 beta, 6 beta, 6a alpha, 7 beta, 7a alpha)-3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene	60-57-1	4.86E-02	3.26E-02	2.19E-02	2.01E-02	No factor	6.52E-02	2.34E-02	8.36E-03	3.93E-03
1H,1,2,4-Triazol-3-amine	61-82-5	1.72E+02	5.21E+01	1.58E+01	7.50E-01	No factor	5.39E+01	9.58E+00	1.70E+00	1.00E+00
DICHLORVOS	62-73-7	1.27E+00	6.77E-01	3.61E-01	3.36E-02	No factor	1.42E+00	2.55E-02	4.58E-04	4.08E-04
1-Naphthalenol, Methylcarbamate	63-25-2	1.44E+00	9.34E-01	6.07E-01	5.38E-02	No factor	3.85E+00	1.31E+00	4.45E-01	3.95E-02
2-Propanone	67-64-1	8.04E+03	4.81E+03	2.88E+03	5.10E+01	No factor	1.27E+04	2.97E+03	6.97E+02	1.00E+02
chloroform	67-66-3	1.58E+02	8.35E+01	4.42E+01	Included	No factor	2.58E+02	5.77E+01	1.29E+01	Included
1,1,1-trichloroethane	71-55-6	2.82E+02	1.20E+02	5.09E+01	Included	No factor	1.04E+03	1.15E+02	1.28E+01	6.21E+00
3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-[2,7:3,6-dimethanonaphth[2,3-b]oxirene, [1a alpha,2 beta,2a beta,3 alpha,6 alpha,6a beta,7 beta,7a alpha]	72-20-8	6.44E-03	4.02E-03	2.51E-03	Included	No factor	1.51E-02	1.24E-03	1.02E-04	Included
1,1'-(2,2,2-Trichloroethylidene)bis[4-methoxybenzene]	72-43-5	3.98E-02	2.71E-02	1.85E-02	7.81E-03	No factor	3.35E-01	5.07E-02	7.66E-03	1.13E-03
1,1'-(2,2-Dichloroethylidene)bis(4-chlorobenzene)	72-54-8	9.21E-02	3.62E-02	1.42E-02	8.29E-03	No factor	2.65E+02	3.04E-03	3.48E-08	Included
1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene	76-44-8	7.77E-02	4.89E-02	3.08E-02	2.35E-02	No factor	1.24E-01	2.65E-02	5.69E-03	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
Hydroxytriphenylstannane	76-87-9	4.16E-01	1.05E-01	2.66E-02	1.60E-03	No factor	3.54E-02	3.12E-04	2.75E-06	1.31E-06
1,2,3,4,5,5-Hexachloro-1,3-cyclopentadiene	77-47-4	3.09E-01	1.14E-01	4.22E-02	3.50E-02	No factor	3.13E+03	1.74E-02	9.62E-08	Included
1,1,2-trichloroethane	79-00-5	1.13E+02	8.72E+01	6.71E+01	5.60E+01	No factor	9.69E+01	5.87E+01	3.56E+01	2.45E+01
Trichloroethene	79-01-6	7.51E+01	5.47E+01	3.99E+01	8.45E+00	No factor	5.68E+02	1.59E+02	4.43E+01	4.05E+01
2-Propenamide	79-06-1	2.21E+02	1.44E+02	9.34E+01	5.13E+01	No factor	1.01E+03	7.88E+01	6.12E+00	Included
1,1,2,2-tetrachloroethane	79-34-5	2.92E+01	1.76E+01	1.06E+01	9.44E+00	No factor	6.45E+01	2.24E+01	7.75E+00	Included
PCNB	82-68-8	1.96E+00	2.65E-01	3.58E-02	2.69E-02	No factor	1.48E+01	3.53E-01	8.42E-03	Included
ACENAPHTHENE	83-32-9	2.02E+00	1.20E+00	7.16E-01	7.14E-01	No factor	1.56E+00	7.44E-01	3.55E-01	Included
1,2-Benzenedicarboxylic acid, Dibutyl ester	84-74-2	2.76E+00	1.75E+00	1.11E+00	4.65E-01	No factor	4.26E+00	8.42E-01	1.66E-01	Included
6,7-Dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium, Dibromide	85-00-7	2.09E+01	8.83E+00	3.73E+00	5.09E-01	No factor	3.46E+00	1.13E-01	3.67E-03	Included
1,2-Benzenedicarboxylic acid, Butyl phenylmethyl ester	85-68-7	3.70E+00	2.20E+00	1.31E+00	1.18E+00	No factor	5.60E+00	1.46E+00	3.78E-01	Included
AZINPHOSMETHYL	86-50-0	8.06E-02	3.79E-02	1.78E-02	8.42E-03	No factor	2.37E-01	3.61E-02	5.52E-03	4.99E-03
1,2,3-Trichlorobenzene	87-61-6	3.29E+00	1.85E+00	1.04E+00	Included	No factor	2.11E+00	3.71E-01	6.52E-02	Included
PENTACHLOROPHENOL	87-86-5	7.66E-01	5.55E-01	4.02E-01	1.41E-01	No factor	9.07E-01	5.02E-01	2.78E-01	1.52E-01
4-Nitro-3-(trifluoromethyl)phenol	88-30-2	1.36E+01	1.05E+01	8.11E+00	4.35E+00	No factor	8.77E+00	5.70E+00	3.71E+00	3.38E+00
2-(4-Chloro-2-methylphenoxy)propanoic acid	93-65-2	3.41E+02	6.53E+01	1.25E+01	Included	No factor	3.59E+02	2.27E+01	1.43E+00	Included
(4-chloro-2-methylphenoxy)acetic acid	94-74-6	7.71E+01	2.66E+01	9.20E+00	5.84E-01	No factor	4.79E+01	4.98E+00	5.18E-01	Included
(2,4-Dichlorophenoxy)acetic acid	94-75-7	7.07E+01	3.49E+01	1.73E+01	1.99E+00	No factor	8.31E+01	2.27E+01	6.20E+00	Included
1,2-dichlorobenzene	95-50-1	1.59E+01	8.12E+00	4.15E+00	Included	No factor	1.09E+02	2.21E+01	4.47E+00	1.76E+00
3,4-dichloroaniline	95-76-1	5.17E+00	3.02E+00	1.77E+00	1.27E+00	No factor	1.63E+00	7.84E-01	3.77E-01	2.06E-01
4,6-Dichloro-N-(2-chlorophenyl)-1,3,5-triazin-2-amine	101-05-3	4.93E-01	2.42E-01	1.19E-01	Included	No factor	2.98E+08	3.26E-02	3.56E-12	Included
1,4-Dichlorobenzene	106-46-7	1.12E+01	6.55E+00	3.82E+00	3.74E+00	No factor	9.90E+00	2.54E+00	6.51E-01	4.49E-01
4-chlorophenol	106-48-9	1.86E+01	9.82E+00	5.18E+00	4.74E+00	No factor	1.63E+01	3.99E+00	9.77E-01	1.53E-01
2-Propenal	107-02-8	1.84E-01	1.26E-01	8.67E-02	7.57E-02	No factor	1.23E-01	6.99E-02	3.96E-02	Included
1,2-Dichloroethane	107-06-2	3.12E+02	1.95E+02	1.22E+02	1.21E+02	No factor	9.12E+02	1.55E+02	2.64E+01	Included
Methylbenzene	108-88-3	8.47E+01	5.53E+01	3.62E+01	5.04E+00	No factor	1.65E+02	3.53E+01	7.55E+00	Included
CHLOROBENZENE	108-90-7	2.56E+01	1.00E+01	3.90E+00	Included	No factor	7.40E+01	1.19E+01	1.92E+00	Included
PHENOL	108-95-2	7.88E+01	6.05E+01	4.64E+01	1.39E+01	No factor	2.66E+01	9.83E+00	3.63E+00	2.77E+00
2-(1-Methylethoxy)phenol, Methylcarbamate	114-26-1	3.14E+00	1.56E+00	7.73E-01	3.19E-01	No factor	4.94E+02	1.48E+00	4.42E-03	Included
6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	115-29-7	3.11E-02	1.89E-02	1.15E-02	7.17E-03	No factor	1.38E-01	2.05E-02	3.05E-03	2.45E-04
4-Chloro-alpha-(4-chlorophenyl)-alpha-(trichloromethyl)benzenemethanol	115-32-2	8.92E-01	4.86E-01	2.64E-01	7.30E-02	No factor	7.74E-01	7.89E-02	8.05E-03	Included
1,2-Benzenedicarboxylic acid, bis(2-Ethylhexyl)ester	117-81-7	1.76E+01	6.07E+00	2.09E+00	Included	No factor	7.77E+00	2.00E+00	5.16E-01	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
1,2-Benzenedicarboxylic acid, Dioctyl ester	117-84-0	5.07E+02	3.34E+02	2.20E+02	Included	No factor	5.90E+03	2.23E+01	8.39E-02	Included
HEXACHLOROBENZENE	118-74-1	1.20E+00	2.31E-01	4.46E-02	7.75E-03	No factor	7.63E-02	3.03E-02	1.20E-02	Included
anthracene	120-12-7	7.61E-01	1.28E-01	2.14E-02	7.83E-03	No factor	9.86E-02	1.72E-02	3.00E-03	Included
1,2,4-trichlorobenzene	120-82-1	3.45E+00	2.37E+00	1.63E+00	9.10E-01	No factor	1.08E+01	2.45E+00	5.56E-01	Included
[(Dimethoxyphosphinothioyl)thio]butanedioic acid, Diethyl ester	121-75-5	7.16E-01	4.29E-01	2.57E-01	1.32E-02	No factor	6.70E-01	1.50E-01	3.37E-02	3.67E-03
FENITROTHION	122-14-5	2.97E-01	1.71E-01	9.86E-02	1.19E-02	No factor	3.74E+00	6.53E-01	1.14E-01	1.23E-03
6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine	122-34-9	2.98E+01	1.59E+01	8.46E+00	2.48E-01	No factor	9.50E-01	3.44E-01	1.25E-01	1.00E-02
Tetrachloroethene	127-18-4	1.54E+01	8.15E+00	4.33E+00	1.12E+00	No factor	1.51E+02	2.05E+01	2.77E+00	2.35E+00
Dimethyldithiocarbamic acid, Sodium salt	128-04-1	5.19E+00	1.16E+00	2.60E-01	Included	No factor	1.29E+00	2.52E-02	4.92E-04	Included
1,2-Benzenedicarboxylic acid, Dimethyl ester	131-11-3	1.71E+02	1.21E+02	8.51E+01	8.42E+01	No factor	1.07E+02	5.56E+01	2.90E+01	Included
3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3-(2H)-dione	133-06-2	1.49E+00	6.93E-01	3.22E-01	1.91E-01	No factor	7.95E+00	1.50E+00	2.85E-01	Included
2-[(Trichloromethyl)thio]-1H-isoindole-1,3(2H)dione	133-07-3	1.43E+00	6.19E-01	2.67E-01	8.56E-02	No factor	1.53E+00	2.37E-02	3.68E-04	Included
Tetramethylthioperoxydicarbonic diamide	137-26-8	4.54E-01	1.25E-01	3.44E-02	4.70E-03	No factor	2.98E+00	3.27E-02	3.58E-04	Included
Zinc dimethyldithiocarbamate	137-30-4	6.46E-01	3.00E-01	1.40E-01	3.05E-02	No factor	1.23E+00	5.95E-02	2.88E-03	2.82E-03
Propazine	139-40-2	4.52E+00	8.69E-01	1.67E-01	5.04E-02	No factor	4.69E-01	1.04E-01	2.32E-02	Included
Acetic acid ethyl ester	141-78-6	1.02E+03	6.74E+02	4.45E+02	8.96E+01	No factor	6.16E+03	4.35E+02	3.06E+01	1.93E+01
1,2-Ethanediylbiscarbamodithioic acid, Disodium salt	142-59-6	4.34E+00	1.67E+00	6.39E-01	5.09E-01	No factor	8.95E+00	7.65E-01	6.53E-02	Included
1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one	143-50-0	1.46E-01	8.80E-02	5.32E-02	3.33E-02	No factor	2.15E-01	2.60E-02	3.15E-03	Included
7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid	145-73-3	5.20E+01	1.92E+01	7.06E+00	Included	No factor	6.17E+01	1.08E+01	1.90E+00	Included
2-(4-Thiazolyl)-1H-benzimidazole	148-79-8	5.47E+00	1.42E+00	3.71E-01	1.00E-01	No factor	2.33E+01	2.70E-01	3.11E-03	Included
SODIUM DODECYL SULPHATE	151-21-3	1.33E+01	9.22E+00	6.38E+00	2.54E+00	No factor	2.07E+01	1.06E+01	5.47E+00	1.40E+00
METHYL PARATHION	298-00-0	1.19E+00	6.43E-01	3.46E-01	1.44E-02	No factor	7.73E+00	1.54E+00	3.08E-01	1.77E-03
O,O-Diethyl S-[(ethylthio)methyl])ester, Phosphorodithioic acid	298-02-2	1.45E-01	4.04E-02	1.12E-02	8.48E-03	No factor	6.67E+00	4.60E-02	3.17E-04	2.78E-04
DISULFOTON	298-04-4	9.92E-01	3.84E-01	1.48E-01	3.73E-02	No factor	3.87E-01	2.37E-02	1.45E-03	Included
1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-(1a,4a,4ab,5a,8a,8ab)-1,4:5,8-dimethanonaphthalene	309-00-2	1.06E-01	6.05E-02	3.43E-02	Included	No factor	1.71E-01	1.93E-02	2.18E-03	Included
DIURON	330-54-1	1.67E+00	7.80E-01	3.65E-01	2.32E-02	No factor	1.57E+00	3.25E-01	6.69E-02	1.29E-02
LINURON	330-55-2	3.96E+00	1.57E+00	6.21E-01	4.46E-02	No factor	2.86E-01	1.34E-01	6.26E-02	2.74E-02
DIAZINON	333-41-5	4.11E-01	2.29E-01	1.28E-01	1.71E-02	No factor	5.18E-01	1.03E-01	2.05E-02	1.91E-02

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
cyanamide	420-04-2	4.48E+01	1.53E+01	5.23E+00	2.30E+00	No factor	2.09E+02	2.70E+00	3.51E-02	Included
PENTACHLOROBENZENE	608-93-5	9.90E-01	3.86E-01	1.51E-01	Included	No factor	6.48E-01	1.94E-01	5.81E-02	5.67E-02
1,2,3,4-Tetrachlorobenzene	634-66-2	1.23E+00	6.21E-01	3.12E-01	Included	No factor	1.78E+00	2.30E-01	2.97E-02	Included
TIN	688-73-3	6.98E-02	5.70E-03	4.66E-04	Included	No factor	3.40E-02	2.13E-03	1.34E-04	5.48E-05
N-(3,4-Dichlorophenyl)propanamide	709-98-8	7.26E+00	4.06E+00	2.27E+00	4.62E-02	No factor	1.65E-01	6.48E-02	2.54E-02	Included
PHOSMET	732-11-6	1.39E+00	4.85E-01	1.70E-01	4.16E-02	No factor	1.78E-01	1.41E-02	1.11E-03	Included
BENSULIDE	741-58-2	1.03E+00	5.48E-01	2.92E-01	Included	No factor	8.00E+00	4.56E-01	2.60E-02	1.38E-02
(Acetyloxy)triphenylstannane	900-95-8	1.89E-01	6.52E-02	2.25E-02	1.15E-02	No factor	1.01E+00	5.59E-04	3.10E-07	Included
Ethylphosphonodithioic acid, O-Ethyl S-phenyl ester	944-22-9	3.21E-01	7.35E-02	1.68E-02	1.26E-02	No factor	2.55E-02	3.83E-03	5.76E-04	Included
(2-Chloroethyl)trimethyl ammonium, Chloride	999-81-5	6.43E+02	2.44E+02	9.27E+01	3.20E+01	No factor	3.61E+05	4.16E+02	4.79E-01	Included
N-(Phosphonomethyl)-glycine	1071-83-6	7.91E+01	3.25E+01	1.33E+01	1.30E+01	No factor	1.01E+02	2.93E+01	8.49E+00	Included
Dichlobenil	1194-65-6	1.59E+01	9.57E+00	5.75E+00	4.85E+00	No factor	4.92E+00	1.89E+00	7.29E-01	Included
5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydrozybenzamide, compd. with 2-aminoethanol (1:1)	1420-04-8	9.03E-01	5.46E-01	3.30E-01	4.80E-02	No factor	3.80E-01	1.73E-01	7.83E-02	Included
Tributylchlorostannane	1461-22-9	1.69E-02	7.47E-03	3.31E-03	6.90E-04	No factor	2.26E-03	5.59E-04	1.38E-04	1.19E-04
2,3-Dihydro-2,2-dimethyl-7-benzofuranol, Methylcarbamate	1563-66-2	6.86E-01	3.43E-01	1.71E-01	5.33E-02	No factor	3.35E+00	1.74E-01	9.07E-03	Included
2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine	1582-09-8	7.82E-01	4.42E-01	2.50E-01	1.73E-01	No factor	1.42E-01	4.33E-02	1.33E-02	9.26E-03
3,5-Dibromo-4-hydroxybenzonitrile	1689-84-5	8.38E+00	2.61E+00	8.11E-01	7.36E-01	No factor	8.06E+01	6.62E+00	5.45E-01	8.66E-02
5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone	1698-60-8	5.67E+01	1.06E+01	2.00E+00	9.83E-01	No factor	1.02E+01	2.10E+00	4.32E-01	Included
MONOLINURON	1746-81-2	4.65E+01	1.56E+01	5.24E+00	3.14E-01	No factor	4.23E+00	1.01E+00	2.39E-01	Included
N-Butyl-N-ethyl-2,6-dinitro-4-(trifluoromethyl)benzenamine	1861-40-1	6.58E+00	1.97E+00	5.87E-01	1.85E-01	No factor	2.19E+01	2.06E-01	1.94E-03	Included
2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile	1897-45-6	4.57E-01	2.02E-01	8.91E-02	7.61E-02	No factor	1.40E-01	2.71E-02	5.27E-03	Included
6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine	1912-24-9	3.17E+00	1.96E+00	1.21E+00	1.44E-01	No factor	5.06E-01	3.06E-01	1.86E-01	1.60E-01
4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid	1918-02-1	6.11E+01	3.04E+01	1.51E+01	Included	No factor	5.00E+01	8.42E+00	1.42E+00	Included
Tributylfluorostannane	1983-10-4	1.28E-02	4.17E-03	1.36E-03	4.01E-04	No factor	1.27E-01	3.74E-04	1.11E-06	Included
(2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine (1:1)	2008-39-1	1.89E+02	9.48E+01	4.75E+01	2.17E+01	No factor	1.30E+02	2.10E+01	3.38E+00	Included
3,5-Dimethyl-4-(methylthio)phenol, Methylcarbamate	2032-65-7	1.09E+00	2.37E-01	5.18E-02	3.76E-02	No factor	3.24E+17	1.15E-02	4.10E-22	Included
EPN	2104-64-5	2.65E-01	9.42E-02	3.35E-02	2.29E-03	No factor	2.64E+00	1.07E-02	4.34E-05	Included
Hexahydro-1H-azepine-1-carbothioic acid, S-Ethyl ester	2212-67-1	1.04E+01	6.37E+00	3.92E+00	2.17E+00	No factor	5.35E+00	2.26E+00	9.59E-01	6.16E-01
S-(2,3,3-Trichloro-2-propenyl)ester bis(1-methylethyl), carbamothioic acid	2303-17-5	1.72E+00	8.60E-01	4.28E-01	Included	No factor	1.65E+01	6.03E-01	2.21E-02	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
2-[4-(1,1-Dimethylethyl)phenoxy]cyclohexyl-2-propynyl ester, Sulfurous acid	2312-35-8	1.85E+00	4.28E-01	9.89E-02	8.00E-02	No factor	5.73E+00	1.29E-01	2.90E-03	1.75E-03
Morestan	2439-01-2	3.97E+00	4.18E-01	4.40E-02	2.05E-02	No factor	5.53E-02	7.80E-03	1.10E-03	Included
Dodecylguanidine, Monoacetate	2439-10-3	5.35E+00	1.09E+00	2.23E-01	6.40E-04	No factor	1.05E+08	7.64E-02	5.58E-11	Included
CHLORPYRIFOS	2921-88-2	1.43E-02	9.26E-03	5.99E-03	1.22E-03	No factor	2.64E-02	1.03E-02	4.03E-03	Included
O,O'-(Thiodi-4,1-phenylene) O,O,O',O',-tetramethyl ester, Phosphorothioic acid	3383-96-8	1.53E-01	7.58E-02	3.75E-02	7.53E-03	No factor	1.08E+03	3.47E-02	1.11E-06	Included
5,6-Dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide	5234-68-4	2.74E+01	4.97E+00	9.01E-01	Included	No factor	1.23E+00	7.91E-01	5.09E-01	Included
METHYLENE THIOCYANATE	6317-18-6	8.32E-01	2.05E-01	5.05E-02	3.40E-03	No factor	9.56E-02	4.30E-02	1.93E-02	Included
2,4-Bis(isopropylamino)-6-methylthio-S-triazine	7287-19-6	1.27E+00	3.34E-01	8.82E-02	5.82E-03	No factor	2.82E-01	4.51E-02	7.21E-03	Included
Lead	7439-92-1	1.00E+01	4.23E+00	1.79E+00	6.00E-01	No factor	9.57E+00	1.88E+00	3.68E-01	7.24E-02
Nickel	7440-02-0	1.81E+01	8.66E+00	4.15E+00	8.42E-01	No factor	1.03E+01	8.10E-01	6.40E-02	5.00E-02
silver	7440-22-4	1.49E-01	5.37E-02	1.94E-02	Included	No factor	2.78E+01	1.92E-01	1.32E-03	Included
cadmium	7440-43-9	2.51E+00	1.42E+00	8.04E-01	4.88E-01	No factor	1.39E+00	4.31E-01	1.33E-01	1.28E-01
Chromium	7440-47-3	1.09E+01	5.00E+00	2.30E+00	2.47E-01	No factor	8.00E+00	2.12E+00	5.62E-01	1.70E-01
Copper	7440-50-8	6.11E-01	3.40E-01	1.89E-01	3.00E-02	No factor	3.11E-01	1.68E-01	9.08E-02	4.01E-02
Zinc	7440-66-6	5.60E+00	3.28E+00	1.92E+00	3.11E-01	No factor	2.72E+00	1.05E+00	4.08E-01	7.50E-02
Sulfuric acid, Zinc salt (1:1), Heptahydrate	7446-20-0	4.08E+00	9.73E-01	2.32E-01	1.95E-01	No factor	5.58E+00	5.03E-01	4.55E-02	1.58E-02
OPPER II CHLORIDE	7447-39-4	2.25E-01	1.54E-01	1.05E-01	3.01E-02	No factor	2.70E-01	1.19E-01	5.29E-02	2.18E-03
Potassium chloride	7447-40-7	1.60E+03	1.08E+03	7.25E+02	3.02E+02	No factor	1.61E+03	6.25E+02	2.43E+02	2.09E+02
Mercuric chloride	7487-94-7	1.77E-01	1.35E-01	1.03E-01	3.60E-03	No factor	6.02E-02	3.47E-02	2.00E-02	1.55E-03
Zinc chloride	7646-85-7	3.64E+00	2.47E+00	1.68E+00	4.61E-01	No factor	2.39E+00	1.10E+00	5.07E-01	4.47E-01
SODIUM CHLORIDE	7647-14-5	9.56E+03	6.40E+03	4.29E+03	2.43E+03	No factor	8.66E+03	2.80E+03	9.04E+02	2.31E+02
Ammonia	7664-41-7	5.18E+00	3.37E+00	2.19E+00	7.32E-01	No factor	4.95E+01	7.44E+00	1.12E+00	Included
FLUORIDE	7681-49-4	3.12E+02	1.62E+02	8.43E+01	7.62E+01	No factor	3.05E+02	1.17E+02	4.48E+01	Included
Nickelous chloride	7718-54-9	1.93E+01	1.16E+01	6.94E+00	4.35E+00	No factor	2.88E+00	8.48E-01	2.50E-01	1.09E-01
Elemental phosphorus	7723-14-0	3.53E-01	9.39E-02	2.50E-02	1.20E-02	No factor	2.96E-02	6.95E-03	1.63E-03	Included
Lead chloride	7758-95-4	6.73E+00	3.63E+00	1.96E+00	3.50E-02	No factor	4.77E+00	1.43E+00	4.26E-01	1.20E-01
Copper sulfate, pentahydrate	7758-99-8	5.46E-01	2.57E-01	1.21E-01	7.87E-02	No factor	1.99E-01	7.79E-02	3.04E-02	Included
Nitric acid, silver (1+) salt	7761-88-8	7.05E-02	4.44E-02	2.80E-02	9.31E-03	No factor	5.01E-02	2.49E-02	1.24E-02	8.00E-03
Selenium	7782-49-2	1.11E+01	3.60E+00	1.17E+00	2.00E-01	No factor	7.31E+00	1.59E+00	3.48E-01	Included
TOXAPHENE	8001-35-2	3.94E-02	2.29E-02	1.33E-02	Included	No factor	4.93E-02	8.68E-03	1.53E-03	Included
LEAD NITRATE	10099-74-8	1.44E+01	8.05E+00	4.51E+00	2.10E-01	No factor	2.46E+00	6.63E-01	1.78E-01	Included
2,2-Dibromo-3-nitrilopropionamide	10222-01-2	3.30E+00	1.64E+00	8.16E-01	6.75E-01	No factor	4.61E+06	5.60E-01	6.81E-08	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
methamidophos	10265-92-6	5.79E+01	2.08E+00	7.44E-02	1.25E-03	No factor	8.06E+13	1.56E+00	3.02E-14	Included
Bis(8-quinolinolato-N1,08)copper	10380-28-6	9.28E-01	1.73E-01	3.23E-02	1.14E-02	No factor	2.84E-02	8.83E-03	2.75E-03	Included
[5-(Phenylmethyl)-3-furanyl]methylester-2,2-dimethyl-3-(2-methyl-1-propanyl)-cyclopropenecarboxylic acid	10453-86-8	6.12E-03	3.53E-03	2.03E-03	Included	No factor	2.64E-01	2.26E-03	1.93E-05	Included
1H-Benzimidazol-2-yl carbamic acid, Methyl ester	10605-21-7	6.32E+00	1.75E+00	4.85E-01	4.45E-01	No factor	3.00E-01	2.63E-02	2.31E-03	Included
Ammonium chloride	12125-02-9	1.19E+01	8.48E+00	6.06E+00	1.10E+00	No factor	5.65E+00	2.84E+00	1.43E+00	Included
[[1,2-Ethanediylbis[carbamodithioato]](2-)]manganese	12427-38-2	5.18E+00	1.89E+00	6.88E-01	1.40E-02	No factor	6.07E+00	2.84E-01	1.33E-02	Included
PHOSPHAMIDON	13171-21-6	8.32E+00	3.36E+00	1.36E+00	2.00E-01	No factor	8.98E+00	9.73E-02	1.05E-03	Included
Ethoprop	13194-48-4	9.58E-01	2.54E-01	6.71E-02	4.93E-02	No factor	1.11E+01	4.37E-02	1.72E-04	Included
(3-Methylphenyl)carbamic acid 3-[(methoxycarbonyl)amino]phenyl ester	13684-63-4	2.91E+01	8.04E+00	2.22E+00	1.30E-01	No factor	1.39E+01	4.37E-01	1.37E-02	Included
(OC-6-11)-tris(Dimethylcarbamodithioato-(S,S')iron	14484-64-1	1.67E+00	6.25E-01	2.34E-01	Included	No factor	2.95E+02	4.61E-02	7.20E-06	Included
2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	15972-60-8	3.30E+00	1.11E+00	3.71E-01	7.72E-03	No factor	2.13E+00	3.51E-01	5.77E-02	Included
METHOMYL	16752-77-5	1.30E+00	7.13E-01	3.90E-01	1.47E-01	No factor	4.39E+10	5.37E-02	6.56E-14	Included
[1-[(Butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamic acid, Methyl ester	17804-35-2	1.16E+01	3.81E+00	1.25E+00	6.13E-01	No factor	3.00E+00	3.87E-01	4.99E-02	1.32E-02
1-(2-Benzothiazolyl)-1,3-dimethylurea	18691-97-9	1.28E+02	2.36E+00	4.38E-02	Included	No factor	9.67E+06	3.08E+00	9.82E-07	Included
3,5-Dinitro-N4, N4-dipropyl-sulfanilamide	19044-88-3	1.03E+00	3.03E-01	8.86E-02	3.41E-02	No factor	5.43E-01	1.20E-01	2.67E-02	Included
3-[2,4-Dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2(3H)-one	19666-30-9	1.18E+00	4.68E-01	1.86E-01	2.10E-02	No factor	1.97E-01	3.81E-02	7.37E-03	Included
(1-Methylethyl)phosphoramidic acid, Ethyl-3-methyl-4-(methylthio)phenyl ester	22224-92-6	4.60E-01	8.22E-02	1.47E-02	1.20E-02	No factor	7.74E+06	2.67E-03	9.18E-13	Included
2,2-Dimethyl-1,3-benzodioxal-4-ol methylcarbamate	22781-23-3	4.59E-01	1.52E-01	5.05E-02	Included	No factor	5.03E+14	5.31E-02	5.61E-18	Included
2-(Dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-ethanimidothioic acid, Methyl ester	23135-22-0	7.39E+00	2.66E+00	9.60E-01	4.00E-01	No factor	3.93E+01	4.09E+00	4.26E-01	Included
Formetanate Hydrochloride	23422-53-9	1.33E+01	2.27E+00	3.86E-01	Included	No factor	2.55E+16	6.79E-02	1.81E-19	Included
1,1-Dimethylpiperidinium chloride	24307-26-4	7.95E+01	8.84E+00	9.83E-01	2.00E-01	No factor	1.55E+01	1.05E+00	7.16E-02	Included
2-[[Ethoxy[(1-methylethyl)amino]phosphinothioyl]oxy]benzoic acid 1-methylethyl ester	25311-71-1	3.26E+00	2.67E-01	2.19E-02	2.14E-03	No factor	8.04E+01	4.69E-02	2.74E-05	Included
2-Ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranol methane sulfonate	26225-79-6	2.54E+01	1.15E+01	5.19E+00	2.23E+00	No factor	1.93E+01	4.93E+00	1.26E+00	Included

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4-Chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazinone	27314-13-2	1.81E+01	2.31E+00	2.93E-01	2.27E-02	No factor	1.87E+01	5.08E-01	1.37E-02	Included
2-(tert-Butylamino)-4-(cyclopropylamino)-6-(methylthio)-s-triazine	28159-98-0	5.86E-01	6.42E-02	7.03E-03	1.52E-03	No factor	1.90E-01	1.22E-02	7.90E-04	Included
Diethylcarbamothioic acid, S-[(4-Chlorophenyl)methyl]ester	28249-77-6	2.00E+00	1.37E+00	9.41E-01	3.20E-01	No factor	4.83E-01	1.89E-01	7.38E-02	Included
2,6-Dinitro-N,N'-dipropyl-4-(trifluoromethyl)-1,3-benzenamine	29091-21-2	5.60E+00	1.22E+00	2.66E-01	Included	No factor	8.86E+00	3.21E-02	1.16E-04	Included
Amitraz	33089-61-1	2.14E+01	6.17E+00	1.78E+00	Included	No factor	1.56E-01	4.70E-03	1.41E-04	Included
TEBUTHIURON	34014-18-1	1.35E+02	1.64E+01	2.00E+00	1.18E-01	No factor	2.38E+01	1.54E+00	9.95E-02	Included
3-(4-isopropylphenyl)-1,1-dimethylurea	34123-59-6	4.80E+00	4.36E-01	3.96E-02	3.65E-02	No factor	2.25E+00	1.67E-01	1.24E-02	Included
2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	34256-82-1	6.15E+00	1.19E+00	2.29E-01	Included	No factor	5.65E+00	3.93E-01	2.74E-02	Included
DIFLUBENZURON	35367-38-5	8.93E-01	1.99E-01	4.45E-02	1.56E-02	No factor	1.08E-02	3.34E-03	1.03E-03	Included
2-Bromo-2-(bromomethyl)pentanedinitrile	35691-65-7	1.36E+01	5.98E+00	2.63E+00	Included	No factor	1.40E+03	3.22E+00	7.44E-03	Included
3-(3,5-dichlorophenyl)-2,4-dioxo-N-isopropylimidazolidine-1-carboxamide	36734-19-7	2.83E+00	9.96E-01	3.51E-01	2.91E-01	No factor	1.16E+00	4.26E-01	1.56E-01	Included
2,2,3,3-Tetramethylcyclopropane carboxylic acid, Cyano(3-phenoxyphenyl)methyl ester	39515-41-8	1.71E-02	4.03E-03	9.52E-04	5.47E-04	No factor	7.41E-03	9.56E-05	1.23E-06	Included
N-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine	40487-42-1	1.44E+00	4.38E-01	1.33E-01	Included	No factor	6.65E-01	4.59E-02	3.17E-03	Included
Phosphorothioic acid, O-(4-Bromo-2-chlorophenyl)-O-ethyl-S-propyl ester	41198-08-7	6.53E-02	2.21E-02	7.48E-03	4.00E-03	No factor	6.29E-02	1.60E-03	4.05E-05	Included
4-Amino-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one	41394-05-2	9.74E+02	3.76E+01	1.45E+00	Included	No factor	5.48E+03	1.88E+01	6.47E-02	Included
3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide, sodium salt	50723-80-3	1.92E+02	7.24E+01	2.73E+01	1.68E+01	No factor	1.55E+04	3.38E+02	7.39E+00	Included
2-chloro-2'-ethyl-N-(2-methoxy-1-methylethyl)-6'-methylacetanilide	51218-45-2	5.68E+00	2.39E+00	1.00E+00	4.21E-01	No factor	1.06E+00	3.98E-01	1.49E-01	Included
3-Cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione	51235-04-2	5.01E+01	1.08E+01	2.32E+00	1.45E-01	No factor	9.36E-01	1.66E-01	2.93E-02	Included
2-[4-(2,4-Dichlorophenoxy)phenoxy]propionic acid, Methyl ester	51338-27-3	1.13E+00	5.53E-01	2.72E-01	Included	No factor	9.88E-01	1.31E-01	1.73E-02	Included
Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	51630-58-1	9.94E-03	4.42E-03	1.97E-03	7.87E-04	No factor	4.61E-03	4.76E-04	4.91E-05	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid, Cyano(3-phenoxyphenyl)methyl ester	52315-07-8	1.55E-03	7.73E-04	3.85E-04	1.91E-04	No factor	2.37E-03	1.80E-04	1.36E-05	1.29E-05
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid, (3-Phenoxyphenyl)methyl ester	52645-53-1	2.59E-02	1.33E-02	6.85E-03	3.97E-03	No factor	2.54E+00	3.43E-02	4.63E-04	2.45E-04
Chlorethoxyfos	54593-83-8	7.57E-03	7.23E-04	6.90E-05	Included	No factor	6.19E-02	2.71E-04	1.18E-06	Included
alpha-tert-butyl-beta-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol	55219-65-3	3.64E+01	1.37E+01	5.17E+00	3.44E+00	No factor	1.13E+03	4.66E+00	1.93E-02	Included
Ethalfluralin	55283-68-6	2.17E-01	1.10E-01	5.54E-02	2.53E-02	No factor	1.12E+00	1.74E-02	2.72E-04	Included
Carbonothioic acid, O-(6-Chloro-3-phenyl-4-pyridazinyl) S-octyl ester	55512-33-9	1.67E+01	4.08E+00	9.99E-01	1.45E-01	No factor	1.13E+12	5.79E+00	2.97E-11	Included
((3,5,6-Trichloro-2-pyridinyl)oxy)-acetic acid, Cmpd. with N,N-Diethylethanamine (1:1)	57213-69-1	3.43E+02	1.55E+02	7.01E+01	1.63E+01	No factor	2.57E+02	5.64E+01	1.24E+01	Included
2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	57966-95-7	2.98E+01	8.43E+00	2.39E+00	9.06E-01	No factor	1.05E+01	1.78E+00	3.03E-01	Included
N,N'-[Thiobis[(methylimino)carbonyloxy]]bisethanimidothioic acid, Dipentyl ester	59669-26-0	1.63E+00	4.36E-01	1.17E-01	3.33E-02	No factor	2.03E+01	6.27E-01	1.94E-02	Included
1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone	59756-60-4	1.36E+01	8.84E+00	5.74E+00	3.30E+00	No factor	2.86E+01	2.02E+00	1.43E-01	Included
1-[[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole	60207-90-1	3.48E+00	1.16E+00	3.88E-01	Included	No factor	3.42E+00	1.15E+00	3.84E-01	Included
2-Chloro-N-[2,6-dinitro-4-(trifluoromethyl)phenyl]-N-ethyl-6-fluorobenzene methanamine	62924-70-3	1.14E-01	4.25E-02	1.59E-02	Included	No factor	5.23E+00	1.63E-02	5.09E-05	Included
2-Chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide	64902-72-3	1.91E+02	2.37E+01	2.95E+00	3.37E-01	No factor	3.18E+01	2.20E+00	1.52E-01	Included
5-O-Demethyl-antibiotic C 067A1a	65195-55-3	6.53E-01	5.44E-02	4.53E-03	1.92E-03	No factor	1.34E+04	1.06E+00	8.38E-05	Included
2,2-Dimethyl-3-(1,1,2,2-tetrabromoethyl)cyclopropane carboxylic acid, Cyano(3-phenoxyphenyl)methyl ester	66841-25-6	6.32E-03	2.07E-03	6.79E-04	5.71E-04	No factor	1.37E+08	1.91E-05	2.67E-18	Included
N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]-1H-imidazole-1-carboxamide	67747-09-5	2.95E+00	7.24E-01	1.78E-01	5.40E-02	No factor	4.49E-01	1.12E-01	2.80E-02	Included

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
3-(2,2-Dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylic acid, Cyano(4-fluoro-3-phenoxy phenyl)methyl ester	68359-37-5	3.32E-02	2.12E-03	1.36E-04	Included	No factor	1.59E-03	2.50E-05	3.92E-07	Included
ADBAC/Octyl decyl dimethyl ammonium chloride	68424-85-1	1.38E+00	7.00E-01	3.54E-01	5.44E-02	No factor	2.43E+01	9.45E-02	3.68E-04	Included
Fluvalinate	69409-94-5	9.21E-03	1.75E-03	3.34E-04	2.01E-04	No factor	9.07E-04	2.24E-04	5.55E-05	Included
4-(Difluoromethoxy)-alpha-(1-methylethyl)benzeneacetic acid, (3-Phenoxyphenyl)methyl ester	70124-77-5	1.90E-03	6.25E-04	2.05E-04	Included	No factor	6.58E-03	2.27E-04	7.85E-06	Included
(2-(4-phenoxyphenoxy)ethyl)carbamic acid, Ethyl ester	72490-01-8	3.42E+00	1.27E+00	4.70E-01	1.50E-01	No factor	7.43E+25	8.69E-04	1.02E-32	Included
2-[1-(Ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one	74051-80-2	1.56E+01	6.20E+00	2.46E+00	2.75E-01	No factor	3.85E+01	1.75E+00	8.00E-02	Included
2-[[[[(4,6-Dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid, Methylester	74222-97-2	2.77E+02	2.07E+01	1.55E+00	1.40E-02	No factor	1.05E+01	1.27E-01	1.54E-03	Included
3-(2-Chloro-3,3,3-trifluoro-1-propenyl-2,2-dimethylcyclopropanecarboxylic acid, cis-(+-)-(2,3,5,6-Tetrafluoro-4-methylphenyl)methyl ester	79538-32-2	1.19E-02	4.77E-04	1.91E-05	Included	No factor	3.62E-02	2.82E-05	2.19E-08	Included
2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid	81334-34-1	1.26E+02	3.04E+01	7.36E+00	Included	No factor	4.16E+02	2.24E+01	1.20E+00	Included
2-[(2-Chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone	81777-89-1	2.20E+01	5.48E+00	1.36E+00	1.19E+00	No factor	1.65E+01	7.36E+00	3.28E+00	Included
Isoxaben	82558-50-7	1.23E+00	1.05E+00	8.95E-01	Included	No factor	3.99E+00	1.33E+00	4.46E-01	Included
Bifenthrin	82657-04-3	3.54E-03	7.96E-04	1.79E-04	Included	No factor	4.94E+06	4.44E-05	3.99E-16	Included
2-[[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoic acid methyl ester	83055-99-6	2.67E+02	9.42E+01	3.32E+01	8.00E-01	No factor	3.43E+03	4.33E+01	5.46E-01	Included
2-[[[[[4,6-Bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl] benzoic acid, Methyl ester	86209-51-0	3.89E+01	2.60E+00	1.73E-01	3.18E-02	No factor	3.70E+00	1.39E-01	5.25E-03	Included
2-Chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide	87674-68-8	3.67E+00	7.31E-01	1.46E-01	6.52E-02	No factor	1.99E+00	1.89E-01	1.80E-02	Included
3-(2-Chloro-3,3,3-trifluoro-1-propenyl)2,2-dimethyl cyclopropane carboxylic acid, [1alpha (S*), 3 alpha(z)-(+-)-Cyano(3-phenoxyphenyl)methyl ester	91465-08-6	3.15E-03	1.86E-04	1.09E-05	Included	No factor	3.36E-03	3.43E-05	3.49E-07	Included

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N-[[(4-Methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-2-(3,3,3-trifluoropropyl)benzenesulfonamide	94125-34-5	5.01E+01	1.46E+00	4.26E-02	1.50E-02	No factor	2.57E+01	2.53E-01	2.49E-03	Included
(R*,R*)-(+-)-alpha-(4-Chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol	94361-06-5	2.66E+01	1.27E+01	6.03E+00	2.60E+00	No factor	1.35E+03	1.91E+00	2.71E-03	Included
4-Chloro-2-(1,1-dimethylethyl)-5-[[[4-(1,1-dimethylethyl)phenyl]methyl]thio]-3(2H)-pyridazinene	96489-71-3	1.67E-02	6.63E-03	2.64E-03	1.18E-03	No factor	2.23E-02	4.22E-03	7.98E-04	2.11E-04
2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioic acid, S,S-Dimethyl ester	97886-45-8	2.45E+00	5.28E-01	1.14E-01	2.00E-02	No factor	8.82E-01	1.09E-01	1.35E-02	Included
Flumetsulam	98967-40-9	2.89E+02	1.63E+01	9.16E-01	3.25E-01	No factor	5.04E+02	1.48E+00	4.34E-03	Included
3-Chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1H-pyrazole-4-carboxylic acid, Mether ester	100784-20-1	5.69E+01	2.52E+00	1.11E-01	1.93E-02	No factor	1.69E+01	1.69E-01	1.68E-03	Included
Flumioxazin (V-53482)	103361-09-7	2.58E+00	1.15E-01	5.11E-03	8.73E-04	No factor	5.60E+02	5.66E-01	5.72E-04	Included
Clodinafop-propargyl(CGA-184927/CGA-185072)	105511-96-4	1.36E+01	3.49E+00	8.93E-01	2.87E-01	No factor	3.35E+09	4.10E+00	5.01E-09	Included
N-Methylneodecanamide	105726-67-8	3.72E+02	5.13E+01	7.06E+00	Included	No factor	2.13E+03	4.64E+01	1.01E+00	Included
1-[(6-Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine	105827-78-9	3.59E+01	2.35E+00	1.54E-01	Included	No factor	4.48E+03	4.16E+00	3.86E-03	Included
alpha-[2-(4-Chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	107534-96-3	4.22E+00	1.73E+00	7.06E-01	Included	No factor	1.68E+00	3.81E-01	8.64E-02	Included
4-[3-(4-Chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]morpholine	110488-70-5	3.59E+01	1.80E+01	9.02E+00	Included	No factor	2.19E+05	9.76E+00	4.34E-04	Included
4-Chlorobenzoic acid, 2-Benzoyl-2-(1,1-dimethylethyl)hydrazide	112226-61-6	7.19E+00	3.23E+00	1.45E+00	7.80E-01	No factor	5.96E+00	7.49E-01	9.40E-02	Included
aplha-[2-(4-Chlorophenyl)ethyl]-alpha-phenyl-1H-1,2,4-triazole-1-propanenitrile	114369-43-6	1.45E+00	8.91E-01	5.47E-01	4.10E-01	No factor	1.63E+00	2.23E-01	3.05E-02	Included
1-[[4-Bromo-2-(2,4-dichlorophenyl)tetrahydro-2-furaanyl]methyl]-1H-1,2,4-triazole	116255-48-2	2.35E+00	5.96E-01	1.51E-01	Included	No factor	6.97E-01	2.53E-01	9.21E-02	Included
[[2-Chloro-4-fluoro-5-[(tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidine)amino]phenyl]thioacetic acid methyl ester	117337-19-6	2.28E-01	5.27E-02	1.22E-02	5.19E-03	No factor	3.48E-02	9.37E-03	2.52E-03	Included

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5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile	120068-37-3	2.65E-01	5.12E-02	9.88E-03	1.18E-03	No factor	2.35E-01	2.02E-02	1.74E-03	6.24E-04
N-[2,4-Dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl] phenyl]methanesulfonamide	122836-35-5	2.67E+01	3.32E+00	4.14E-01	2.95E-01	No factor	8.95E+00	5.96E-01	3.97E-02	Included
Pymetrozine	123312-89-0	1.63E+02	5.34E+01	1.74E+01	3.05E+00	No factor	1.46E+15	1.55E+00	1.65E-15	Included
2-Chloro-6-[4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid sodium salt	123343-16-8	3.84E+02	3.05E+01	2.43E+00	5.81E-01	No factor	3.88E+02	7.08E+00	1.29E-01	Included
Bispyribac-sodium	125401-92-5	7.67E+01	9.41E+00	1.16E+00	4.93E-01	No factor	7.08E+06	4.69E+02	3.11E-02	Included
Fenhexamid	126833-17-8	9.17E+00	4.57E+00	2.28E+00	Included	No factor	1.09E+01	1.97E+00	3.56E-01	Included
Fludioxonil(Maxim 4FS)	131341-86-1	1.05E+00	5.69E-01	3.08E-01	Included	No factor	1.96E+00	8.69E-02	3.86E-03	Included
2-[[6-(2-Cyanophenoxy)-4-pyrimidinyl]oxy-alpha-(methoxymethylene)benzeneacetic acid, E-Methyl ester	131860-33-8	3.41E+00	8.15E-01	1.95E-01	Included	No factor	1.96E+00	3.48E-01	6.19E-02	Included
Acibenzolar-s-methyl	135158-54-2	8.21E+00	2.34E+00	6.64E-01	5.90E-01	No factor	2.84E+00	1.37E-01	6.62E-03	Included
4"-Deoxy-4"-(methylamino)-avermectin B1 benzote (salt)	137512-74-4	3.48E-01	2.41E-02	1.68E-03	3.51E-04	No factor	1.21E-01	2.75E-03	6.28E-05	Included
alpha-(Methoxyimino)-2-[[[(E)-[1-[3-(trifluoromethyl)phenyl]ethylidene]amino] oxy]methylbenzene acetic acid, (E,E)-Methyl ester	141517-21-7	1.24E+00	1.80E-01	2.63E-02	Included	No factor	2.58E+00	6.02E-02	1.41E-03	Included
alpha-(Methoxyimino)-2-[[[(E)-[1-[3-(trifluoromethyl)phenyl]ethylidene]amino] oxy]methyl]benzeneacetic acid, (alpha,E)-Methyl ester	141776-32-1	1.06E+02	9.00E+00	7.63E-01	Included	No factor	3.68E+02	5.18E+00	7.28E-02	Included
3-Methoxy-2-methylbenzoic acid, 2-(3,5-Dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide	161050-58-4	3.92E+00	2.45E+00	1.53E+00	1.20E+00	No factor	4.13E+00	5.25E-01	6.67E-02	Included
Indoxacarb(DPX-MP062)	173584-44-6	8.39E-01	4.18E-01	2.09E-01	1.42E-01	No factor	8.85E-01	1.90E-01	4.06E-02	Included
Flucarbazone sodium(MKH 6562)metabolite	181274-17-9	1.19E+02	4.22E+01	1.49E+01	Included	Best est. Factors	1.49E+02	1.92E+01	2.45E+00	N.A.
formaldehyde	50-00-0	1.02E+02	6.74E+01	4.47E+01	9.77E+00	Best est. Factors	1.27E+02	3.06E+01	7.33E+00	N.A.
Benzo(a)pyrene	50-32-8	1.76E+00	2.20E-01	2.76E-02	1.00E-02	Best est. Factors	2.20E+00	1.16E-01	6.58E-03	N.A.
Piperonyl butoxide	51-03-6	1.48E+00	8.74E-01	5.18E-01	Included	Best est. Factors	1.85E+00	3.97E-01	8.49E-02	N.A.

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N-Ethyl-N-nitrosoethanamine	55-18-5	3.26E+03	8.33E+02	2.13E+02	Included	Best est. Factors	4.08E+03	4.38E+02	5.06E+01	N.A.
carbon tetrachloride	56-23-5	2.60E+02	7.43E+01	2.12E+01	5.48E-01	Best est. Factors	3.26E+02	3.91E+01	5.04E+00	N.A.
Cyanide	57-12-5	5.16E+00	7.71E-01	1.15E-01	Included	Best est. Factors	4.69E+00	2.75E-01	1.56E-02	N.A.
ETHANOL	64-17-5	6.55E+03	2.84E+03	1.23E+03	1.00E+02	Best est. Factors	8.19E+03	1.49E+03	2.93E+02	N.A.
acetic acid	64-19-7	3.71E+02	2.18E+02	1.29E+02	Included	Best est. Factors	4.63E+02	1.15E+02	3.06E+01	N.A.
METHANOL	67-56-1	7.98E+03	3.34E+03	1.40E+03	1.00E+02	Best est. Factors	9.97E+03	1.76E+03	3.33E+02	N.A.
HEXACHLOROETHANE	67-72-1	5.07E+00	3.03E+00	1.80E+00	1.65E+00	Best est. Factors	6.34E+00	1.59E+00	4.30E-01	N.A.
BENZENE	71-43-2	9.11E+01	6.17E+01	4.17E+01	1.00E+01	Best est. Factors	1.14E+02	3.25E+01	9.94E+00	N.A.
bromomethane	74-83-9	1.16E+01	2.77E+00	6.63E-01	Included	Best est. Factors	1.44E+01	1.26E+00	1.09E-01	N.A.
acetaldehyde	75-07-0	3.82E+02	1.06E+02	2.92E+01	Included	Best est. Factors	4.78E+02	5.57E+01	6.96E+00	N.A.
DICHLOROMETHANE	75-09-2	4.86E+02	2.87E+02	1.69E+02	Included	Best est. Factors	6.08E+02	1.51E+02	4.03E+01	N.A.
Cacodylic acid	75-60-5	1.89E+02	6.69E+01	2.37E+01	2.30E+01	Best est. Factors	2.36E+02	3.04E+01	3.89E+00	N.A.
DEF	78-48-8	7.20E-01	2.47E-01	8.48E-02	8.22E-02	Best est. Factors	9.00E-01	1.12E-01	1.39E-02	N.A.
1,2-Benzenedicarboxylic acid, Diethyl ester	84-66-2	6.66E+01	4.09E+01	2.51E+01	1.60E+01	Best est. Factors	8.33E+01	2.15E+01	5.98E+00	N.A.
HEXACHLOROBUTADIENE	87-68-3	2.38E+00	8.59E-01	3.10E-01	Included	Best est. Factors	2.98E+00	3.91E-01	5.08E-02	N.A.
DINITRO-6-SEC-BUTYLPHENOL, 2,4-	88-85-7	5.07E-01	2.45E-01	1.18E-01	2.38E-02	Best est. Factors	6.34E-01	1.11E-01	1.93E-02	N.A.
(2,4-Dichlorophenoxy)acetic acid, 1-Methylethyl ester	94-11-1	4.11E+00	5.66E-01	7.80E-02	Included	Best est. Factors	5.13E+00	2.57E-01	1.28E-02	N.A.

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1,2-Dimethylbenzene	95-47-6	2.96E+01	1.75E+01	1.03E+01	4.10E+00	Best est. Factors	3.70E+01	9.21E+00	2.46E+00	N.A.
ETHYL BENZENE	100-41-4	3.64E+01	2.20E+01	1.33E+01	1.04E+01	Best est. Factors	4.55E+01	1.16E+01	3.17E+00	N.A.
Ethenylbenzene	100-42-5	4.60E+01	2.09E+01	9.46E+00	Included	Best est. Factors	5.75E+01	1.10E+01	2.25E+00	N.A.
1,3-Dimethylbenzene	108-38-3	2.70E+01	1.31E+01	6.39E+00	4.13E+00	Best est. Factors	3.37E+01	6.91E+00	1.52E+00	N.A.
Hexane	110-54-3	8.17E+02	1.77E+02	3.85E+01	Included	Best est. Factors	1.02E+03	9.33E+01	9.16E+00	N.A.
glutaral	111-30-8	1.76E+01	7.07E+00	2.84E+00	7.16E-01	Best est. Factors	2.20E+01	3.21E+00	4.65E-01	N.A.
Dodecylguanidine HCL	112-65-2	5.67E+01	4.66E+00	3.83E-01	Included	Best est. Factors	7.09E+01	2.12E+00	6.27E-02	N.A.
2-Methyl-2-(methylthio)propanol O-[(methylamino)carbonyl]oxime	116-06-3	2.19E+00	7.91E-01	2.87E-01	1.46E-01	Best est. Factors	2.19E+01	2.20E-01	1.80E-03	N.A.
1,2-Dihydro-3,6-pyridazinedione	123-33-1	2.61E+02	1.13E+02	4.91E+01	Included	Best est. Factors	3.27E+02	5.15E+01	8.05E+00	N.A.
Propanal	123-38-6	1.36E+02	5.98E+01	2.63E+01	Included	Best est. Factors	1.70E+02	3.15E+01	6.26E+00	N.A.
2-(Hydroxymethyl)-2-nitro-1,3-propanediol	126-11-4	3.54E+02	1.50E+02	6.34E+01	2.28E+01	Best est. Factors	4.43E+02	6.82E+01	1.04E+01	N.A.
2,2-Dichloropropanoic acid, Sodium salt	127-20-8	4.72E+02	4.43E+01	4.16E+00	1.00E+00	Best est. Factors	5.90E+02	2.01E+01	6.82E-01	N.A.
Methylcarbamodithioic acid, Monopotassium salt	137-41-7	1.93E+01	7.15E+00	2.65E+00	Included	Best est. Factors	1.93E+02	1.99E+00	1.66E-02	N.A.
DCDIC	138-93-2	1.97E+02	1.15E+01	6.66E-01	Included	Best est. Factors	2.46E+02	5.21E+00	1.09E-01	N.A.
Dicrotophos	141-66-2	4.28E+00	1.55E+00	5.60E-01	4.79E-01	Best est. Factors	5.35E+00	7.04E-01	9.18E-02	N.A.
heptane	142-82-5	3.12E+03	6.14E+02	1.21E+02	Included	Best est. Factors	3.89E+03	3.23E+02	2.88E+01	N.A.
Disodium methanearsonate	144-21-8	1.78E+02	4.90E+01	1.35E+01	1.00E+00	Best est. Factors	2.22E+02	2.23E+01	2.21E+00	N.A.

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
1,2-Dibromo-2,2-dichloroethyldimethyl ester phosphoric acid	300-76-5	4.94E-01	1.99E-01	8.03E-02	3.71E-02	Best est. Factors	6.17E-01	9.05E-02	1.32E-02	N.A.
HYDRAZINE	302-01-2	1.80E+00	6.36E-01	2.25E-01	4.79E-03	Best est. Factors	2.25E+00	3.35E-01	5.35E-02	N.A.
HCFC-123	306-83-2	2.59E+02	4.02E+01	6.22E+00	Included	Best est. Factors	3.24E+02	2.11E+01	1.48E+00	N.A.
5-Bromo-6-methyl-3-(1-methylpropyl)-2,4(1H,3H)-pyrimidinedione	314-40-9	9.60E+01	1.10E+01	1.27E+00	2.53E-02	Best est. Factors	1.20E+02	5.01E+00	2.08E-01	N.A.
2-Methyl-5-nitro-1H-imidazole-1-ethanol	443-48-1	7.19E+02	2.67E+02	9.92E+01	3.33E+01	Best est. Factors	8.99E+02	1.21E+02	1.63E+01	N.A.
dazomet	533-74-4	2.17E+00	1.02E+00	4.81E-01	3.06E-01	Best est. Factors	2.71E+00	4.64E-01	7.88E-02	N.A.
Isothiocyanatomethane	556-61-6	2.82E-01	1.34E-01	6.35E-02	Included	Best est. Factors	3.53E-01	6.08E-02	1.04E-02	N.A.
ETHION	563-12-2	5.26E-01	1.59E-01	4.84E-02	4.60E-03	Best est. Factors	6.57E-01	7.25E-02	7.93E-03	N.A.
2,2'-Oxybisethanol, Dinitrate	693-21-0	3.76E+02	2.17E+02	1.26E+02	3.91E+01	Best est. Factors	4.70E+02	1.14E+02	2.99E+01	N.A.
Dipropylcarbamothioic acid, S-Ethyl ester	759-94-4	1.97E+01	1.04E+01	5.53E+00	5.00E+00	Best est. Factors	2.47E+01	4.75E+00	9.07E-01	N.A.
AMETRYN	834-12-8	1.91E+00	7.72E-01	3.12E-01	2.71E-02	Best est. Factors	2.39E+00	3.51E-01	5.12E-02	N.A.
METHIDATHION	950-37-8	1.18E+00	2.69E-01	6.11E-02	1.02E-02	Best est. Factors	1.48E+00	1.22E-01	1.00E-02	N.A.
2,3,4,5,6,7,7-Heptachloro-1a,1b,5,5a,6,6a,-hexahydro-(2a alpha, 1b beta, 2 alpha, 5 alpha, 5a beta, 6 beta, 6a alpha-2,5-methano-2H-indeno[1,2-b]oxirene	1024-57-3	2.34E+00	3.01E-02	3.88E-04	Included	Best est. Factors	2.93E+00	1.59E-02	9.24E-05	N.A.
Butylethylcarbamothioic acid, S-Propyl ester	1114-71-2	1.35E+01	6.26E+00	2.91E+00	1.20E+00	Best est. Factors	1.68E+01	2.85E+00	4.77E-01	N.A.
(2,4-Dichlorophenoxy)acetic acid, Monoester with 1,2-propanediol, Butyl ether	1320-18-9	2.67E+00	1.33E+00	6.61E-01	5.50E-02	Best est. Factors	3.34E+00	6.04E-01	1.08E-01	N.A.
Arsenic oxide (As203)	1327-53-3	1.62E+01	4.26E+00	1.12E+00	4.28E-01	Best est. Factors	1.47E+01	1.52E+00	1.51E-01	N.A.

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
XYLENE	1330-20-7	1.01E+02	4.99E+01	2.47E+01	2.47E+01	Best est. Factors	1.26E+02	2.62E+01	5.89E+00	N.A.
Calcium polysulfide	1344-81-6	4.63E+00	5.32E-01	6.12E-02	1.70E-03	Best est. Factors	4.21E+00	1.90E-01	8.27E-03	N.A.
Antimycin A	1397-94-0	9.13E-04	4.18E-04	1.92E-04	1.80E-04	Best est. Factors	1.14E-03	2.20E-04	4.56E-05	N.A.
6-Methoxy-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine	1610-18-0	4.94E+01	2.07E+01	8.70E+00	9.80E-02	Best est. Factors	6.18E+01	9.43E+00	1.43E+00	N.A.
2-Methoxy-2-methylpropane	1634-04-4	1.15E+03	5.98E+02	3.10E+02	Included	Best est. Factors	1.44E+03	3.15E+02	7.38E+01	N.A.
2,6-Dibromo-4-cyanophenyl ester, Octanoic acid	1689-99-2	1.29E-01	7.46E-02	4.32E-02	Included	Best est. Factors	1.61E-01	3.39E-02	7.08E-03	N.A.
2,3,5,6-Tetrachloro-1,4-benzenedicarboxylic acid, Dimethyl ester	1861-32-1	2.72E+01	1.21E+01	5.35E+00	3.94E-01	Best est. Factors	3.40E+01	5.48E+00	8.78E-01	N.A.
1,1'-Dimethyl-4,4'-bipyridinium, Dichloride	1910-42-5	1.73E+01	6.38E+00	2.35E+00	3.21E-01	Best est. Factors	2.17E+01	2.90E+00	3.85E-01	N.A.
3,6-Dichloro-2-methyoxybenzoic acid	1918-00-9	5.73E+01	2.28E+01	9.09E+00	9.63E-01	Best est. Factors	7.16E+01	1.04E+01	1.49E+00	N.A.
(2,4-Dichlorophenoxy)acetic acid, 2-Butoxyethyl ester	1929-73-3	5.89E+00	2.65E+00	1.19E+00	Included	Best est. Factors	7.37E+00	1.21E+00	1.96E-01	N.A.
Dipropylcarbamothioic acid S-propylester	1929-77-7	6.00E+00	3.03E+00	1.53E+00	1.42E+00	Best est. Factors	7.51E+00	1.38E+00	2.50E-01	N.A.
Nitrapyrin	1929-82-4	5.45E+00	2.63E+00	1.27E+00	3.26E-01	Best est. Factors	6.81E+00	1.20E+00	2.08E-01	N.A.
N-(2-Methylcyclohexyl)-N'-phenylurea	1982-49-6	2.82E+01	5.15E+00	9.41E-01	2.10E-01	Best est. Factors	3.53E+01	2.34E+00	1.54E-01	N.A.
(4-Chloro-2-methylphenoxy)acetic acid compd. with N-Methylmethanamine (1:1)	2039-46-5	9.94E+01	2.41E+01	5.83E+00	Included	Best est. Factors	1.24E+02	1.09E+01	9.55E-01	N.A.
Tributyl[(2-methyl-1-oxo-2-propenyl)oxy]stannane	2155-70-6	1.49E-01	3.15E-02	6.65E-03	9.14E-04	Best est. Factors	1.65E-01	2.22E-03	2.84E-05	N.A.
Monosodium methane arsonate	2163-80-6	2.61E+02	1.41E+02	7.57E+01	2.42E+01	Best est. Factors	3.26E+02	6.39E+01	1.24E+01	N.A.
7-Oxabicyclo(2.2.1)heptane-2,3-dicarboxylic acid, Dipotassium salt	2164-07-0	2.93E+02	1.41E+02	6.76E+01	Included	Best est. Factors	3.66E+02	6.40E+01	1.11E+01	N.A.

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Fluometuron	2164-17-2	7.17E+00	1.49E+00	3.10E-01	3.58E-02	Best est. Factors	8.97E+00	6.78E-01	5.09E-02	N.A.
1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachloroactahydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene	2385-85-5	4.42E+00	1.50E+00	5.09E-01	2.98E-01	Best est. Factors	5.53E+00	6.82E-01	8.35E-02	N.A.
3a,4,7,7a-Tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1H-isoindole-1,3(2H)-dione	2425-06-1	1.73E+00	5.17E-01	1.55E-01	1.28E-01	Best est. Factors	2.16E+00	2.35E-01	2.54E-02	N.A.
4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid, Monopotassium salt	2545-60-0	1.33E+02	5.46E+01	2.25E+01	Included	Best est. Factors	1.66E+02	2.48E+01	3.69E+00	N.A.
5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole	2593-15-9	4.25E+00	1.15E+00	3.12E-01	2.81E-01	Best est. Factors	5.31E+00	5.23E-01	5.12E-02	N.A.
1,2-Benzisothiazol-3(2H)-one	2634-33-5	1.28E+01	2.61E+00	5.34E-01	6.20E-02	Best est. Factors	1.59E+01	1.19E+00	8.76E-02	N.A.
[(4-Aminophenyl)sulfonyl]carbamic acid, Methyl ester	3337-71-1	2.13E+03	2.00E+02	1.87E+01	1.40E-01	Best est. Factors	2.67E+03	9.07E+01	3.06E+00	N.A.
1-(3-Chloro-2-propenyl)-3,5,7-triaza-1-azoniatricyclo[3.3.1.1 3,7]decane chloride	4080-31-3	1.40E+02	6.72E+01	3.22E+01	Included	Best est. Factors	1.75E+02	3.05E+01	5.28E+00	N.A.
1,3,5-Triazine-1,3,5(2H,4H,6H)triethanol	4719-04-4	5.85E+02	3.03E+01	1.57E+00	Included	Best est. Factors	7.31E+02	1.38E+01	2.57E-01	N.A.
(2,4-Dichlorophenoxy)acetic acid compd. with isopropylamine (1:1)	5742-17-6	1.76E+03	5.13E+02	1.50E+02	4.34E+01	Best est. Factors	2.20E+03	2.33E+02	2.45E+01	N.A.
(2,4-Dichlorophenoxy)acetic acid compd. with 2,2-iminobis[ethanol] (1:1)	5742-19-8	1.67E+02	5.24E+01	1.64E+01	Included	Best est. Factors	2.09E+02	2.38E+01	2.69E+00	N.A.
5-Chloro-3-(1,1-dimethylethyl)-6-methyl-2,4(1H,3H)pyrimidinedione	5902-51-2	4.53E+01	3.65E+00	2.94E-01	5.42E-02	Best est. Factors	5.67E+01	1.66E+00	4.83E-02	N.A.
6-Chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine	5915-41-3	8.09E-01	1.24E-01	1.89E-02	7.39E-03	Best est. Factors	1.01E+00	5.63E-02	3.11E-03	N.A.
MCPB Sodium Salt	6062-26-6	3.39E+01	8.29E+00	2.03E+00	8.47E-01	Best est. Factors	4.24E+01	3.77E+00	3.32E-01	N.A.
(2-Bromo-2-nitroethenyl)benzene	7166-19-0	7.27E-02	5.54E-02	4.22E-02	3.30E-02	Best est. Factors	9.09E-02	2.52E-02	6.92E-03	N.A.
DDAC	7173-51-5	8.52E-01	3.60E-01	1.52E-01	Included	Best est. Factors	1.06E+00	1.63E-01	2.49E-02	N.A.
Aluminum	7429-90-5	1.02E+01	3.36E+00	1.10E+00	5.43E-01	Best est. Factors	9.29E+00	1.20E+00	1.49E-01	N.A.

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iron	7439-89-6	7.89E+01	2.76E+01	9.62E+00	Included	Best est. Factors	7.17E+01	9.84E+00	1.30E+00	N.A.
Mercury	7439-97-6	3.86E-01	2.16E-01	1.21E-01	1.08E-01	Best est. Factors	3.51E-01	7.72E-02	1.64E-02	N.A.
Thallium	7440-28-0	7.76E+01	4.08E+00	2.14E-01	Included	Best est. Factors	7.06E+01	1.46E+00	2.89E-02	N.A.
MOLYBDENUM	7631-95-0	2.40E+03	6.22E+02	1.61E+02	4.39E+01	Best est. Factors	2.18E+03	2.22E+02	2.18E+01	N.A.
NITRATE	7631-99-4	3.90E+03	2.54E+03	1.65E+03	8.09E+02	Best est. Factors	3.55E+03	9.07E+02	2.23E+02	N.A.
Hydrochloric acid	7647-01-0	3.70E+03	7.83E+01	1.65E+00	4.60E-02	Best est. Factors	3.37E+03	2.80E+01	2.24E-01	N.A.
Crotoxyphos	7700-17-6	5.68E-01	1.32E-01	3.08E-02	1.72E-02	Best est. Factors	7.10E-01	6.01E-02	5.05E-03	N.A.
Sulfur	7704-34-9	3.96E+03	4.14E+02	4.33E+01	4.96E-01	Best est. Factors	3.60E+03	1.48E+02	5.86E+00	N.A.
7a-Ethyldihydro-1H,3H,5H-oxazolo[3,4-c]oxazole	7747-35-5	2.44E+02	8.98E+01	3.30E+01	Included	Best est. Factors	3.05E+02	4.08E+01	5.41E+00	N.A.
sodium chlorite	7758-19-2	1.14E+02	1.05E+01	9.76E-01	3.50E-01	Best est. Factors	1.03E+02	3.76E+00	1.32E-01	N.A.
Chloric acid, Sodium salt	7775-09-9	3.27E+03	1.32E+03	5.30E+02	2.82E+01	Best est. Factors	2.97E+03	4.70E+02	7.16E+01	N.A.
arsenic acid	7778-39-4	1.61E+01	4.27E+00	1.13E+00	3.80E-01	Best est. Factors	1.47E+01	1.53E+00	1.53E-01	N.A.
3-[(Dimethoxyphosphinyl)oxy]-2-butenoic acid, Methyl ester	7786-34-7	2.32E-01	8.57E-02	3.17E-02	1.12E-02	Best est. Factors	2.90E-01	3.90E-02	5.19E-03	N.A.
Coal Tar Creosote	8001-58-9	1.41E+00	2.66E-01	5.00E-02	Included	Best est. Factors	1.76E+00	1.40E-01	1.19E-02	N.A.
Pyrethrin	8003-34-7	3.24E-02	1.98E-02	1.21E-02	8.80E-03	Best est. Factors	4.05E-02	9.01E-03	1.99E-03	N.A.
[[1,2-Ethanediyl bis[carbamodithioato]](2-)]manganese, Mixt. with [[1,2-Ethanediyl bis[carbamodithioato]](2-)]zinc	8018-01-7	2.94E+00	8.17E-01	2.27E-01	4.70E-02	Best est. Factors	2.94E+01	2.27E-01	1.43E-03	N.A.
DEMETON	8065-48-3	1.73E+00	6.00E-01	2.09E-01	6.52E-02	Best est. Factors	2.16E+00	2.73E-01	3.42E-02	N.A.

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Copper salts of fatty acids & rosin acids	9007-39-0	3.54E+01	2.07E+00	1.21E-01	Included	Best est. Factors	4.42E+01	9.40E-01	1.98E-02	N.A.
5-Methyl-3(2H)-isoxazolone	10004-44-1	2.08E+02	5.10E+01	1.25E+01	4.12E+00	Best est. Factors	2.60E+02	2.32E+01	2.06E+00	N.A.
Cadmium nitrate Tetrahydrate	10022-68-1	4.09E+01	1.43E+00	5.03E-02	Included	Best est. Factors	3.72E+01	5.12E-01	6.79E-03	N.A.
COPPER II NITRATE	10031-43-3	5.76E+00	2.02E-01	7.07E-03	Included	Best est. Factors	5.24E+00	7.21E-02	9.55E-04	N.A.
Cadmium dichloride	10108-64-2	2.02E+00	1.52E+00	1.15E+00	8.08E-02	Best est. Factors	1.83E+00	5.44E-01	1.56E-01	N.A.
alpha-Cyclopropyl-alpha-(4-methoxyphenyl)-5-pyrimidinemethanol	12771-68-5	1.38E+03	1.67E+01	2.03E-01	Included	Best est. Factors	1.72E+03	7.60E+00	3.33E-02	N.A.
S-[[(1,1-Dimethylethyl)thio]O,O-diethylmethyl]ester phosphorodithioic acid	13071-79-9	1.78E-02	6.16E-03	2.13E-03	Included	Best est. Factors	2.23E-02	2.80E-03	3.49E-04	N.A.
CYHEXATIN	13121-70-5	8.89E-03	3.71E-03	1.55E-03	2.38E-04	Best est. Factors	9.87E-03	2.61E-04	6.63E-06	N.A.
FENBUTATIN OXIDE	13356-08-6	2.16E-02	6.63E-03	2.04E-03	3.70E-04	Best est. Factors	2.40E-02	4.67E-04	8.72E-06	N.A.
(T-4)-Bis(1-hydroxy-2(1H0-pyridinethionato-O,S) zinc	13463-41-7	6.87E-02	1.43E-02	2.96E-03	Included	Best est. Factors	8.58E-02	6.49E-03	4.86E-04	N.A.
[3-[[(Phenylamino)carbonyl]oxy]phenyl]carbamic acid, Ethyl ester	13684-56-5	1.84E+00	4.93E-01	1.32E-01	Included	Best est. Factors	2.30E+00	2.24E-01	2.16E-02	N.A.
Ammonium	14798-03-9	8.21E+02	5.55E+01	3.75E+00	Included	Best est. Factors	7.46E+02	1.98E+01	5.06E-01	N.A.
Napropamide	15299-99-7	2.06E+01	1.08E+01	5.71E+00	3.88E+00	Best est. Factors	2.57E+01	4.93E+00	9.35E-01	N.A.
1-Bromo-3-chloro-5,5-dimethyl-2,4-imidazolidinedione	16079-88-2	2.00E+02	3.43E+01	5.88E+00	Included	Best est. Factors	2.50E+02	1.56E+01	9.63E-01	N.A.
(2-Chloroethyl)phosphonic acid	16672-87-0	1.26E+02	3.17E+01	7.94E+00	1.61E+00	Best est. Factors	1.58E+02	1.44E+01	1.30E+00	N.A.
4-Amino-6-(1,1-dimethylethyl)-3-methylthio)-1,2,4-triazin-5(4H)-one	21087-64-9	3.54E+01	4.54E+00	5.84E-01	2.06E-02	Best est. Factors	4.42E+01	2.07E+00	9.57E-02	N.A.
(2-Benzothiazolylthio)methyl ester, Thiocyanic acid	21564-17-0	2.29E-01	5.27E-02	1.22E-02	Included	Best est. Factors	2.86E-01	2.40E-02	1.99E-03	N.A.

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
2-[(4-Chloro-6-(ethylamino)-S-triazin-2-yl)amino]-2-methylpropionitrile	21725-46-2	6.26E+00	1.18E+00	2.21E-01	1.81E-02	Best est. Factors	7.83E+00	5.35E-01	3.62E-02	N.A.
Tetrachlorvinphos	22248-79-9	4.20E+00	3.85E-01	3.52E-02	2.31E-02	Best est. Factors	5.25E+00	1.75E-01	5.77E-03	N.A.
[1,2-Phenylene bis(iminocarbonothioyl)]bis carbamic acid, Diethyl ester	23564-05-8	2.13E+01	7.84E+00	2.88E+00	2.52E+00	Best est. Factors	2.13E+02	2.18E+00	1.81E-02	N.A.
Propyzamide	23950-58-5	4.40E+01	1.37E+01	4.26E+00	2.18E+00	Best est. Factors	5.49E+01	6.22E+00	6.98E-01	N.A.
Propamocarb	24579-73-5	2.32E+02	1.28E+02	7.02E+01	Included	Best est. Factors	2.32E+03	3.55E+01	4.41E-01	N.A.
3-(1-Methylethyl)-1H,2,1,3-benzothiadiazin-4(3H)-one, 2,2-Dioxide	25057-89-0	4.67E+02	1.86E+02	7.37E+01	4.25E+01	Best est. Factors	5.84E+02	8.44E+01	1.21E+01	N.A.
2,4-D ISOOCTYL ESTER	25168-26-7	7.60E+00	1.45E+00	2.77E-01	6.93E-03	Best est. Factors	9.49E+00	6.59E-01	4.54E-02	N.A.
(Amino carbonyl)phosphonic acid, Monoethyl ester, Monoammonium salt	25954-13-6	1.61E+02	9.02E+01	5.06E+01	2.67E+01	Best est. Factors	2.01E+02	4.10E+01	8.30E+00	N.A.
2-Octyl-3(2H)-isothiazolone	26530-20-1	1.73E-01	9.43E-02	5.14E-02	1.30E-02	Best est. Factors	2.16E-01	4.28E-02	8.42E-03	N.A.
(4-Chloro-2-methylphenoxy)acetic acid isooctyl ester	26544-20-7	9.12E+00	7.57E-01	6.28E-02	Included	Best est. Factors	1.14E+01	3.44E-01	1.03E-02	N.A.
2-(2,4-Dichlorophenoxy)propionic acid, Isooctyl ester	28631-35-8	6.94E+02	2.16E+00	6.74E-03	Included	Best est. Factors	8.67E+02	9.83E-01	1.11E-03	N.A.
Acephate	30560-19-1	1.69E+02	6.88E+01	2.81E+01	2.34E+01	Best est. Factors	2.11E+02	3.13E+01	4.60E+00	N.A.
Busan 77	31512-74-0	4.98E+00	1.37E+00	3.77E-01	8.60E-02	Best est. Factors	6.23E+00	6.23E-01	6.18E-02	N.A.
(2,4-Dichlorophenoxy)acetic acid compd. with 1,1',1''-nitrilotris[2-propanol]	32341-80-3	4.88E+02	1.60E+02	5.28E+01	4.48E+01	Best est. Factors	6.09E+02	7.29E+01	8.65E+00	N.A.
FLUCHLORALIN	33245-39-5	2.26E-01	3.67E-02	5.93E-03	Included	Best est. Factors	2.83E-01	1.67E-02	9.73E-04	N.A.
Butralin	33629-47-9	3.44E-01	1.63E-01	7.73E-02	Included	Best est. Factors	4.31E-01	7.42E-02	1.27E-02	N.A.
N-(Phosphonomethyl)glycine compd with 2-Propanamine (1:1)	38641-94-0	7.38E+01	3.79E+01	1.94E+01	9.26E+00	Best est. Factors	9.23E+01	1.72E+01	3.18E+00	N.A.

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
aluminium triethyl triphosphonate	39148-24-8	8.51E+01	2.89E+01	9.82E+00	1.90E+00	Best est. Factors	1.06E+02	1.31E+01	1.61E+00	N.A.
5-(2,4-Dichlorophenoxy)-2-nitrobenzoic acid, Methyl ester	42576-02-3	9.05E-01	2.17E-01	5.22E-02	Included	Best est. Factors	1.13E+00	9.88E-02	8.56E-03	N.A.
2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene	42874-03-3	3.70E+00	4.40E-01	5.23E-02	Included	Best est. Factors	4.63E+00	2.00E-01	8.58E-03	N.A.
1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butanone	43121-43-3	2.72E+01	1.01E+01	3.76E+00	1.52E+00	Best est. Factors	3.40E+01	4.59E+00	6.16E-01	N.A.
3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione	50471-44-8	6.99E+00	3.08E+00	1.36E+00	9.69E-01	Best est. Factors	8.74E+00	1.40E+00	2.22E-01	N.A.
4,4-Dimethyloxazolidine	51200-87-4	2.45E+02	6.99E+01	1.99E+01	9.20E+00	Best est. Factors	3.06E+02	3.18E+01	3.27E+00	N.A.
N-Phenyl-N'-1,2,3-thiadiazol-5-ylurea	51707-55-2	2.30E+01	6.34E+00	1.75E+00	1.50E-01	Best est. Factors	2.87E+01	2.88E+00	2.86E-01	N.A.
alpha-Isooctadecyl-omega-hydroxypoly(oxy-1,2-ethanediyl)	52292-17-8	1.70E+02	2.21E+01	2.87E+00	1.94E+00	Best est. Factors	2.12E+02	1.00E+01	4.70E-01	N.A.
[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	52918-63-5	3.93E-02	6.30E-03	1.01E-03	9.26E-05	Best est. Factors	4.92E-02	2.86E-03	1.65E-04	N.A.
Mefluidide, diethanolamine salt	53780-36-2	1.29E+02	1.04E+02	8.41E+01	7.02E+01	Best est. Factors	1.61E+02	4.73E+01	1.38E+01	N.A.
1,1,4,4-Tetraoxide-2,3-dihydro-5,6-dimethyl-1,4-dithiin	55290-64-7	3.40E+01	1.78E+01	9.37E+00	Included	Best est. Factors	4.25E+01	8.11E+00	1.54E+00	N.A.
3-Iodo-2-propynyl butylcarbamate	55406-53-6	4.48E-01	2.10E-01	9.86E-02	2.34E-02	Best est. Factors	4.48E+00	5.84E-02	6.19E-04	N.A.
Bromoxynil heptanoate	56634-95-8	2.86E-01	6.30E-02	1.39E-02	Included	Best est. Factors	3.58E-01	2.86E-02	2.27E-03	N.A.
3,6-Dichloro-2-pyridinecarboxylic acid compd. with 2-aminoethanol (1:1)	57754-85-5	5.42E+03	3.23E+02	1.92E+01	6.90E+00	Best est. Factors	6.78E+03	1.47E+02	3.15E+00	N.A.
Metalaxyl	57837-19-1	1.10E+02	4.50E+01	1.84E+01	2.83E+00	Best est. Factors	1.38E+02	2.05E+01	3.02E+00	N.A.
2-Iodo-6-(methylthio)pyrazine	58138-08-2	1.82E+01	8.52E-01	3.98E-02	3.60E-02	Best est. Factors	2.28E+01	3.87E-01	6.52E-03	N.A.

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Azadioxabicyclooctane	59720-42-2	3.18E+02	1.30E+02	5.34E+01	4.20E+01	Best est. Factors	3.98E+02	5.92E+01	8.75E+00	N.A.
Alkyl* amino)-3-aminopropane	61791-63-7	3.19E+01	1.25E+00	4.88E-02	1.89E-02	Best est. Factors	3.99E+01	5.67E-01	8.00E-03	N.A.
1-(Alkyl* amino)-3-aminopropane diacetate	61791-64-8	1.17E+01	1.02E+00	8.97E-02	Included	Best est. Factors	1.46E+01	4.65E-01	1.47E-02	N.A.
5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid, Sodium salt	62476-59-9	2.39E+02	7.80E+01	2.55E+01	Included	Best est. Factors	2.98E+02	3.55E+01	4.18E+00	N.A.
4,5-Dichloro-2-octyl-3(2H)-isothiazolone	64359-81-5	9.25E-02	3.38E-02	1.23E-02	Included	Best est. Factors	1.16E-01	1.54E-02	2.02E-03	N.A.
[3,5,6-Trichloro-2-pyridinyloxy]acetic acid, 2-Butoxyethyl ester	64700-56-7	1.98E+00	1.24E+00	7.73E-01	3.84E-01	Best est. Factors	2.47E+00	5.62E-01	1.27E-01	N.A.
Endothall, dimethylalkylamine	66330-88-9	2.00E+00	1.04E+00	5.44E-01	Included	Best est. Factors	2.50E+00	4.74E-01	8.92E-02	N.A.
Flutolanil	66332-96-5	1.49E+01	7.35E+00	3.62E+00	3.29E+00	Best est. Factors	1.86E+01	3.34E+00	5.93E-01	N.A.
2-[4-[(6-Chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid ethyl ester	66441-23-4	2.19E+00	9.91E-01	4.48E-01	4.00E-01	Best est. Factors	2.74E+00	4.50E-01	7.34E-02	N.A.
AMDRO	67485-29-4	9.78E-02	1.54E-02	2.44E-03	8.98E-04	Best est. Factors	1.22E-01	7.02E-03	4.00E-04	N.A.
Alkyl amino 3, adipate/Isopropyl alcohol	68155-42-0	2.11E+02	3.88E+00	7.16E-02	Included	Best est. Factors	2.63E+02	1.76E+00	1.17E-02	N.A.
[(4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid	69377-81-7	4.92E+01	2.03E+01	8.39E+00	6.66E+00	Best est. Factors	6.15E+01	9.23E+00	1.38E+00	N.A.
2-[4-[[5-(Trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-propanoic acid, Butyl ester	69806-50-4	1.06E+02	2.56E+00	6.17E-02	Included	Best est. Factors	1.33E+02	1.16E+00	1.01E-02	N.A.
Mefenoxam	70630-17-0	4.71E+02	2.00E+01	8.45E-01	Included	Best est. Factors	5.89E+02	9.07E+00	1.39E-01	N.A.
2-[4[(6-Chloro-2-quinoxalinyl)oxy]phenoxy]propanoic acid ethyl ester	76578-14-8	1.26E+00	4.65E-01	1.72E-01	Included	Best est. Factors	1.57E+00	2.11E-01	2.82E-02	N.A.
Glufosinate-ammonium	77182-82-2	1.16E+02	3.55E+01	1.09E+01	3.39E+00	Best est. Factors	1.45E+02	1.62E+01	1.79E+00	N.A.

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3-[[[[(4-Methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid methyl ester	79277-27-3	3.59E+01	4.60E-01	5.88E-03	Included	Best est. Factors	4.49E+01	2.09E-01	9.65E-04	N.A.
2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid	81335-77-5	5.02E+03	5.06E+01	5.10E-01	Included	Best est. Factors	6.27E+03	2.30E+01	8.36E-02	N.A.
Dowco 433	81406-37-3	3.71E+00	1.21E+00	3.96E-01	6.80E-02	Best est. Factors	4.63E+00	5.50E-01	6.48E-02	N.A.
N-(Phosphonomethyl)-ion(1-) glycine, Trimethyl sulfonium	81591-81-3	1.71E+02	6.03E+01	2.12E+01	2.08E+01	Best est. Factors	2.14E+02	2.74E+01	3.47E+00	N.A.
2-(2-Chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide	82097-50-5	5.28E+01	5.28E+00	5.27E-01	4.62E-01	Best est. Factors	6.61E+01	2.40E+00	8.64E-02	N.A.
3,7-Dichloro-8-quinolinecarboxylic acid	84087-01-4	4.58E+01	1.12E+01	2.76E+00	5.00E-01	Best est. Factors	5.72E+01	5.10E+00	4.52E-01	N.A.
(S)-2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methyl ester)acetamide	87392-12-9	1.86E+01	5.13E-01	1.41E-02	Included	Best est. Factors	2.33E+01	2.33E-01	2.32E-03	N.A.
[2-Chloro-4-fluoro-5-(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)phenoxy]acetic acid pentylester	87546-18-7	8.30E+00	9.59E-01	1.11E-01	3.55E-02	Best est. Factors	1.04E+01	4.36E-01	1.82E-02	N.A.
alpha-Butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile	88671-89-0	4.69E+00	1.42E+00	4.32E-01	Included	Best est. Factors	5.86E+00	6.47E-01	7.09E-02	N.A.
4-((Cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylic acid, Ethyl ester	95266-40-3	4.84E+01	1.45E+01	4.33E+00	2.48E+00	Best est. Factors	6.05E+01	6.58E+00	7.10E-01	N.A.
2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine	95737-68-1	3.33E-01	1.63E-01	7.94E-02	Included	Best est. Factors	4.17E-01	7.39E-02	1.30E-02	N.A.
(+-)-2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl, 3-Pyridine carboxylic acid, Monoammonium salt	104098-49-9	2.28E+01	6.48E-01	1.84E-02	Included	Best est. Factors	2.85E+01	2.95E-01	3.02E-03	N.A.
3,5-Dimethylbenzoic acid, 1-(1,1-Dimethylethyl)-2-(4-ethylbenzoyl)hydrazide	112410-23-8	2.76E+00	1.25E+00	5.67E-01	1.90E-01	Best est. Factors	3.45E+00	5.69E-01	9.30E-02	N.A.
1-[[(2,4-Dichlorophenyl)amino]carbonyl]cyclopropane carboxylic acid	113136-77-9	7.46E+00	2.07E+00	5.75E-01	2.41E-01	Best est. Factors	9.32E+00	9.41E-01	9.43E-02	N.A.

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2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid	114311-32-9	1.94E+04	1.18E+01	7.20E-03	Included	Best est. Factors	2.42E+04	5.37E+00	1.18E-03	N.A.
2-(Difluoromethyl)-5-(4,5-dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3-pyridinecarboxylic acid methylester	117718-60-2	2.34E+00	7.01E-01	2.10E-01	1.19E-01	Best est. Factors	2.92E+00	3.19E-01	3.44E-02	N.A.
1-[[2-[2-Chloro-4-(4-chlorophenyl)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole	119446-68-3	6.69E+00	7.48E-01	8.36E-02	Included	Best est. Factors	8.37E+00	3.40E-01	1.37E-02	N.A.
4-Cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine	121552-61-2	5.40E+00	1.47E+00	4.01E-01	Included	Best est. Factors	6.75E+00	6.69E-01	6.58E-02	N.A.
N-[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridine sulfonamide	122931-48-0	7.07E+01	4.16E+00	2.45E-01	2.79E-02	Best est. Factors	8.83E+01	1.89E+00	4.01E-02	N.A.
2-[[[[[4-(Dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]-3-methylbenzoic acid, Methyl ester	126535-15-7	4.05E+02	2.47E+00	1.51E-02	Included	Best est. Factors	5.06E+02	1.12E+00	2.47E-03	N.A.
alpha,2-Dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, Ethyl ester	128639-02-1	3.01E+00	4.27E-01	6.05E-02	2.14E-02	Best est. Factors	3.77E+00	1.94E-01	9.91E-03	N.A.
(5-Cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone	141112-29-0	4.04E+00	1.19E+00	3.48E-01	3.05E-01	Best est. Factors	5.05E+00	5.39E-01	5.71E-02	N.A.
Flufenacet	142459-58-3	1.05E+01	5.74E-01	3.15E-02	Included	Best est. Factors	1.31E+01	2.61E-01	5.16E-03	N.A.
alpha-(Methoxyimino)-2-[(2-methylphenoxy)methyl]benzeneacetic acid, (alpha,E)-Methyl ester	143390-89-0	7.68E-01	2.59E-01	8.73E-02	1.47E-02	Best est. Factors	9.60E-01	1.18E-01	1.43E-02	N.A.
Diclosulam(ASTP metabolite)	145701-21-9	1.59E+02	3.65E+01	8.37E+00	Included	Best est. Factors	1.99E+02	1.66E+01	1.37E+00	N.A.
Didecyl Dimethyl Ammonium Carbonate	148788-55-0	8.01E-01	2.13E-01	5.68E-02	Included	Best est. Factors	1.00E+00	9.70E-02	9.31E-03	N.A.
Tepraloxydim	149979-41-9	1.35E+02	1.06E+02	8.27E+01	Included	Best est. Factors	1.69E+02	4.81E+01	1.36E+01	N.A.
(S)-2-Chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl) acetamide	163515-14-8	5.05E+00	8.87E-01	1.56E-01	7.80E-02	Best est. Factors	6.32E+00	4.03E-01	2.55E-02	N.A.
2,2'-(ethylenedioxy)diethanol	112-27-6	N.A.	N.A.	N.A.	N.A.	N.A.	3.06E+05	6.30E+03	1.30E+02	Included
2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1746-01-6	N.A.	N.A.	N.A.	N.A.	N.A.	4.70E-02	1.32E-04	3.72E-07	Included

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Arsenic	7440-38-2	N.A.	N.A.	N.A.	N.A.	N.A.	5.65E+01	3.94E+00	2.75E-01	1.70E-01
Ethin	*74-86-2*	*1.27E+03*	*5.80E+02*	*1.05E+03*	*N.A.*	*Best est. Factors*	*1.59E+03*	*3.05E+02*	*2.50E+02*	*N.A.*
2 3 4-tm-Pentane	*565-75-3*	*4.60E+00*	*5.18E-01*	*5.85E-02*	*N.A.*	*Best est. Factors*	*5.75E+00*	*2.73E-01*	*1.39E-02*	*N.A.*
2 3-Dimethylbutane	*79-29-8*	*1.00E+01*	*1.66E+00*	*2.74E-01*	*N.A.*	*Best est. Factors*	*1.25E+01*	*8.73E-01*	*6.53E-02*	*N.A.*
2 3 3-tm-Pentane	*560-21-4*	*4.29E+00*	*4.74E-01*	*5.23E-02*	*N.A.*	*Best est. Factors*	*5.36E+00*	*2.49E-01*	*1.25E-02*	*N.A.*
3-Methylpentane	*96-14-0*	*7.41E+00*	*1.10E+00*	*1.63E-01*	*N.A.*	*Best est. Factors*	*9.26E+00*	*5.77E-01*	*3.87E-02*	*N.A.*
2-Methylpentane	*107-83-5*	*1.43E+01*	*2.68E+00*	*5.04E-01*	*N.A.*	*Best est. Factors*	*1.79E+01*	*1.41E+00*	*1.20E-01*	*N.A.*
3-Methylhexane	*589-34-4*	*7.15E+00*	*9.91E-01*	*1.37E-01*	*N.A.*	*Best est. Factors*	*8.94E+00*	*5.22E-01*	*3.27E-02*	*N.A.*
2-Methylhexane	*591-76-4*	*7.15E+00*	*9.91E-01*	*1.37E-01*	*N.A.*	*Best est. Factors*	*8.94E+00*	*5.22E-01*	*3.27E-02*	*N.A.*
n-undecane	*1120-21-4*	*1.05E-01*	*2.58E-03*	*6.36E-05*	*N.A.*	*Best est. Factors*	*1.31E-01*	*1.36E-03*	*1.52E-05*	*N.A.*
n-dodecane	*112-40-3*	*2.23E-01*	*7.04E-03*	*2.23E-04*	*N.A.*	*Best est. Factors*	*2.78E-01*	*3.71E-03*	*5.30E-05*	*N.A.*
Acenaphthylene	*208-96-8*	*1.03E+01*	*4.44E+00*	*7.60E+00*	*N.A.*	*Best est. Factors*	*1.29E+01*	*2.34E+00*	*1.81E+00*	*N.A.*
n-tridecane	*629-50-5*	*8.27E-02*	*1.78E-03*	*3.85E-05*	*N.A.*	*Best est. Factors*	*1.03E-01*	*9.39E-04*	*9.16E-06*	*N.A.*
n-tetradecane	*629-59-4*	*3.73E-02*	*6.25E-04*	*1.05E-05*	*N.A.*	*Best est. Factors*	*4.66E-02*	*3.29E-04*	*2.49E-06*	*N.A.*
n-pentadecane	*629-62-9*	*1.86E-02*	*2.17E-04*	*2.54E-06*	*N.A.*	*Best est. Factors*	*2.33E-02*	*1.14E-04*	*6.05E-07*	*N.A.*
n-hexadecane	*544-76-3*	*8.05E-03*	*6.72E-05*	*5.61E-07*	*N.A.*	*Best est. Factors*	*1.01E-02*	*3.54E-05*	*1.34E-07*	*N.A.*
Benzo(a)anthracene	*56-55-3*	*5.20E-01*	*2.04E-02*	*8.03E-04*	*N.A.*	*Best est. Factors*	*6.50E-01*	*1.08E-02*	*1.91E-04*	*N.A.*

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
Chrysene	*218-01-9*	*4.79E-01*	*1.82E-02*	*6.94E-04*	*N.A.*	*Best est. Factors*	*5.99E-01*	*9.59E-03*	*1.65E-04*	*N.A.*
Benzo(b)fluoranthene	*205-99-2*	*5.59E-01*	*2.17E-02*	*8.41E-04*	*N.A.*	*Best est. Factors*	*6.99E-01*	*1.14E-02*	*2.00E-04*	*N.A.*
Benzo(k)fluoranthene	*207-08-9*	*3.22E-01*	*1.02E-02*	*3.20E-04*	*N.A.*	*Best est. Factors*	*4.03E-01*	*5.35E-03*	*7.63E-05*	*N.A.*
Benzene, chloromethyl-	*25168-05-2*	*2.97E+00*	*3.47E-01*	*1.61E-01*	*N.A.*	*Best est. Factors*	*3.72E+00*	*1.83E-01*	*3.83E-02*	*N.A.*
Benzo(ghi)perylene	*191-24-2*	*1.44E-01*	*3.34E-03*	*7.72E-05*	*N.A.*	*Best est. Factors*	*1.80E-01*	*1.76E-03*	*1.84E-05*	*N.A.*
Indeno(123cd)pyrene	*193-39-5*	*1.39E-01*	*2.95E-03*	*6.27E-05*	*N.A.*	*Best est. Factors*	*1.73E-01*	*1.55E-03*	*1.49E-05*	*N.A.*
Dibenz(ah)anthracene	*189-55-9*	*4.99E-02*	*7.90E-04*	*1.25E-05*	*N.A.*	*Best est. Factors*	*6.24E-02*	*4.16E-04*	*2.97E-06*	*N.A.*
Ethane, 1,2-dichloro-1,1,2,2-tetrafluoro-	*76-14-2*	*7.38E+01*	*4.56E+01*	*1.12E+02*	*N.A.*	*Best est. Factors*	*9.22E+01*	*2.40E+01*	*2.67E+01*	*N.A.*
Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	*76-13-1*	*1.27E+01*	*5.47E+00*	*9.35E+00*	*N.A.*	*Best est. Factors*	*1.59E+01*	*2.88E+00*	*2.23E+00*	*N.A.*
1,1,1,2-Tetrafluoroethane	*811-97-2*	*3.93E+02*	*2.49E+02*	*6.26E+02*	*N.A.*	*Best est. Factors*	*4.91E+02*	*1.31E+02*	*1.49E+02*	*N.A.*
Ethylen, chlor-, Vinylchlorid	*75-01-4*	*1.63E+03*	*1.08E+02*	*2.87E+01*	*N.A.*	*Best est. Factors*	*2.04E+03*	*5.71E+01*	*6.84E+00*	*N.A.*
1,1-Dichloro-1-fluoroethane	*1717-00-6*	*5.05E+01*	*3.12E+01*	*7.66E+01*	*N.A.*	*Best est. Factors*	*6.31E+01*	*1.64E+01*	*1.82E+01*	*N.A.*
Ethane, 1-chloro-1,1-difluoro-	*75-68-3*	*4.34E+01*	*2.68E+01*	*6.58E+01*	*N.A.*	*Best est. Factors*	*5.42E+01*	*1.41E+01*	*1.57E+01*	*N.A.*
Ethylen	*74-85-1*	*1.08E+02*	*6.84E+01*	*1.72E+02*	*N.A.*	*Best est. Factors*	*1.35E+02*	*3.60E+01*	*4.10E+01*	*N.A.*
Acetic acid, trifluoro-	*76-05-1*	*3.79E+04*	*2.10E+04*	*4.62E+04*	*N.A.*	*Best est. Factors*	*4.73E+04*	*1.10E+04*	*1.10E+04*	*N.A.*
1-Propene	*115-07-1*	*1.62E+02*	*1.03E+02*	*2.58E+02*	*N.A.*	*Best est. Factors*	*2.03E+02*	*5.40E+01*	*6.15E+01*	*N.A.*
Propan	*74-98-6*	*1.90E+01*	*1.18E+01*	*2.89E+01*	*N.A.*	*Best est. Factors*	*2.38E+01*	*6.19E+00*	*6.88E+00*	*N.A.*

Chemical name	CAS	Acute Max $HC50_{EC50}$ (mg/l)	Acute $HC50_{EC50}$ (mg/l)	Acute Min $HC50_{EC50}$ (mg/l)	Acute Min Phyla (mg/l)	Extrapolation	Chronic Max $HC50_{EC50}$ (mg/l)	Chronic $HC50_{EC50}$ (mg/l)	Chronic Min $HC50_{EC50}$ (mg/l)	Chronic Min Phyla (mg/l)
n-butane	*106-97-8*	*1.66E+01*	*3.77E+00*	*8.60E-01*	*N.A.*	*Best est. Factors*	*2.07E+01*	*1.99E+00*	*2.05E-01*	*N.A.*
Butene	*25167-67-3*	*2.42E+01*	*1.50E+01*	*3.68E+01*	*N.A.*	*Best est. Factors*	*3.03E+01*	*7.87E+00*	*8.75E+00*	*N.A.*
METHYL ETHYL KETONE	*78-93-3*	*2.92E+03*	*1.82E+03*	*1.14E+03*	*N.A.*	*Best est. Factors*	*3.66E+03*	*9.59E+02*	*2.70E+02*	*N.A.*
2-Methyl-2-butene	*513-35-9*	*2.90E+01*	*7.54E+00*	*1.96E+00*	*N.A.*	*Best est. Factors*	*3.63E+01*	*3.97E+00*	*4.66E-01*	*N.A.*
Isopentane	*78-78-4*	*2.74E+01*	*6.92E+00*	*1.74E+00*	*N.A.*	*Best est. Factors*	*3.43E+01*	*3.64E+00*	*4.15E-01*	*N.A.*
Pentane	*109-66-0*	*4.89E+00*	*2.10E+00*	*3.60E+00*	*N.A.*	*Best est. Factors*	*6.12E+00*	*1.11E+00*	*8.57E-01*	*N.A.*
n-octane	*111-65-9*	*6.39E-01*	*4.03E-02*	*2.54E-03*	*N.A.*	*Best est. Factors*	*7.98E-01*	*2.12E-02*	*6.05E-04*	*N.A.*
2 2 4-tm-Pentane	*540-84-1*	*4.29E+00*	*4.74E-01*	*5.23E-02*	*N.A.*	*Best est. Factors*	*5.36E+00*	*2.49E-01*	*1.25E-02*	*N.A.*
ACETOPHENONE	*98-86-2*	*3.28E+02*	*1.57E+02*	*7.56E+01*	*N.A.*	*Best est. Factors*	*4.10E+02*	*8.29E+01*	*1.80E+01*	*N.A.*
n-nonane	*111-84-2*	*1.55E+00*	*1.15E-01*	*8.55E-03*	*N.A.*	*Best est. Factors*	*1.93E+00*	*6.05E-02*	*2.03E-03*	*N.A.*
2 2 5-tm-Hexane	*1069-53-0*	*2.29E+00*	*1.88E-01*	*1.55E-02*	*N.A.*	*Best est. Factors*	*2.86E+00*	*9.90E-02*	*3.68E-03*	*N.A.*
1,1,1-Trifluoroethane	*420-46-2*	*3.24E+02*	*2.05E+02*	*5.15E+02*	*N.A.*	*Best est. Factors*	*4.05E+02*	*1.08E+02*	*1.23E+02*	*N.A.*
Methane, dichlorodifluoro-	*75-71-8*	*5.22E+01*	*3.22E+01*	*7.92E+01*	*N.A.*	*Best est. Factors*	*6.53E+01*	*1.70E+01*	*1.89E+01*	*N.A.*
Methane, trichlorofluoro-	*75-69-4*	*5.93E+01*	*3.66E+01*	*9.00E+01*	*N.A.*	*Best est. Factors*	*7.41E+01*	*1.93E+01*	*2.14E+01*	*N.A.*
CHLOROMETHANE	*74-87-3*	*4.88E+02*	*3.08E+02*	*1.94E+02*	*N.A.*	*Best est. Factors*	*6.10E+02*	*1.62E+02*	*4.62E+01*	*N.A.*
Methane	*74-82-8*	*1.08E+02*	*6.47E+01*	*3.87E+01*	*N.A.*	*Best est. Factors*	*1.35E+02*	*3.41E+01*	*9.20E+00*	*N.A.*
Methan, chlordifluor-, H-FCKW-22	*75-45-6*	*3.33E+02*	*2.11E+02*	*5.30E+02*	*N.A.*	*Best est. Factors*	*4.16E+02*	*1.11E+02*	*1.26E+02*	*N.A.*

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1-Chloro-1,2,2,2-Tetrafluoroethane	*2837-89-0*	*5.26E+02*	*3.33E+02*	*8.37E+02*	*N.A.*	*Best est. Factors*	*6.57E+02*	*1.75E+02*	*1.99E+02*	*N.A.*
CARBON DIOXIDE (BIOMASS)	*124-38-9*	*5.02E+02*	*3.22E+02*	*2.07E+02*	*N.A.*	*Best est. Factors*	*6.28E+02*	*1.70E+02*	*4.92E+01*	*N.A.*
Methan, dichlorfluor-, H-FCKW-21	*75-43-4*	*3.97E+02*	*2.51E+02*	*6.31E+02*	*N.A.*	*Best est. Factors*	*4.96E+02*	*1.32E+02*	*1.50E+02*	*N.A.*
Methane, bromochlorodifluoro-	*353-59-3*	*6.37E+02*	*4.03E+02*	*1.01E+03*	*N.A.*	*Best est. Factors*	*7.97E+02*	*2.12E+02*	*2.41E+02*	*N.A.*
Methan, bromtrifluor, Halon 1301	*75-63-8*	*5.74E+02*	*3.63E+02*	*9.13E+02*	*N.A.*	*Best est. Factors*	*7.17E+02*	*1.91E+02*	*2.17E+02*	*N.A.*
Ethane, chloropentafluoro-	*76-15-3*	*6.67E+01*	*4.12E+01*	*1.01E+02*	*N.A.*	*Best est. Factors*	*8.34E+01*	*2.17E+01*	*2.41E+01*	*N.A.*
Ethane, hexafluoro-	*76-16-4*	*5.96E+01*	*3.68E+01*	*9.04E+01*	*N.A.*	*Best est. Factors*	*7.45E+01*	*1.94E+01*	*2.15E+01*	*N.A.*
Methane, chlorotrifluoro-	*75-72-9*	*4.03E+02*	*2.55E+02*	*6.41E+02*	*N.A.*	*Best est. Factors*	*5.03E+02*	*1.34E+02*	*1.53E+02*	*N.A.*
Methane, tetrafluoro-	*75-73-0*	*3.39E+02*	*2.14E+02*	*5.40E+02*	*N.A.*	*Best est. Factors*	*4.24E+02*	*1.13E+02*	*1.29E+02*	*N.A.*
Ethan, 1,1-difluor-, FKW-152a	*75-37-6*	*3.23E+03*	*1.47E+03*	*2.67E+03*	*N.A.*	*Best est. Factors*	*4.04E+03*	*7.74E+02*	*6.35E+02*	*N.A.*
Pentafluoroethane	*354-33-6*	*4.62E+02*	*2.92E+02*	*7.36E+02*	*N.A.*	*Best est. Factors*	*5.78E+02*	*1.54E+02*	*1.75E+02*	*N.A.*
Chlorofluoromethane	*593-70-4*	*3.35E+03*	*1.53E+03*	*2.76E+03*	*N.A.*	*Best est. Factors*	*4.19E+03*	*8.03E+02*	*6.58E+02*	*N.A.*
Methan, difluor-, FKW-32	*75-10-5*	*2.55E+03*	*1.16E+03*	*2.10E+03*	*N.A.*	*Best est. Factors*	*3.18E+03*	*6.10E+02*	*5.00E+02*	*N.A.*
CARBONYL SULFIDE	*463-58-1*	*2.28E+05*	*6.24E+04*	*1.71E+04*	*N.A.*	*Best est. Factors*	*2.85E+05*	*3.29E+04*	*4.07E+03*	*N.A.*

(Data in italic are based on QSAR from ECOSAR US-EPA)

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Curriculum Vitae

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Current Position
Since 2000, Jérôme Payet is achieving a PhD at the Swiss Federal Institute of Technolgy of Lausanne (EPFL) in the Life Cycle System laboratory with the Prof. Olivier Jolliet [http://gecos.epfl.ch/lcsystems/publications/]. During this PhD, he has worked 8 months at the Technical University of Denmark with Prof. Michael Hauschild.
Research activities
He is leading researches related to the impact assessment on ecosystem in Life Cycle Assessment (LCA) and in Comparative Ecological Risk Assessment. In relation with these researches, he plays a role leading task forces for the development of innovative Life Cycle Impact Assessment (LCIA) methods in international groups like the OMNIITOX European FP5 project and the UNEP-SETAC Life Cycle Initiative.
Teaching activities
He also managed some teaching activities concerning LCA, LCIA and Comparative Ecological Risk Assessment for Diploma student at the EPF-Lausanne, and he gives lectures in LCA, LCIA at the University of Geneva (Switzerland), Architecture School of Lyon (France). He also gives lecture related to impact on ecosystems for the annual SETAC International short course on LCIA and comparative risk assessment.
Background
He graduated at the University of Lyon [http://www.univ-lyon1.fr/] with a degree in organisms biology, population dynamics and ecology. In July 1997, he graduated in Environmental Assessment (on the topic of waste management) at the National Institute of Applied Sciences (INSA) of Lyon [http://www.insa-lyon.fr/].

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